Medizinische Fakultät der Martin-Luther-Universität Halle-Wittenberg

In vitro und *in vivo* Untersuchungen zur Tumorigenität des *Insulin-like growth factor 2 mRNA-binding protein 1* (IGF2BP1)

Dissertation
zur Erlangung des akademischen Grades
Doktor der Medizin (*Dr. med.*)

vorgelegt
der Medizinischen Fakultät
der Martin-Luther-Universität Halle-Wittenberg

von *Nikolaos Pazaitis*

geboren am 22. Dezember 1986 in *Hannover*

Betreuer:

Prof. Dr. rer. nat. Stefan Hüttelmaier

Gutachter:

1. *Prof. Dr. med. Jan-Henning Klussmann*

2. *assoz. Prof. PD Dr. med. univ. Martin Pichler* (Graz)

08.01.2019

17.01.2020

gewidmet Sophia

Referat

Ribonukleinsäure(RNA)-bindende Proteine (RBPs) sind zentrale Regulatoren einer jeden Phase des RNA-Metabolismus. Der Lebenszyklus einer Boten-RNA (mRNA) kann bereits während der Transkription bis hin zu ihrem Abbau durch RBPs beeinflusst werden. Das *Insulin-like growth factor 2 mRNA-binding protein 1* (IGF2BP1) gehört zu einer Familie stark konservierter RBPs und zeigt im Menschen einen onkofetalen Expressionscharakter. Durch seine regulierenden Wirkungen auf zahlreiche m-/RNAs, die eine wichtige Funktion in entwicklungsbiologischen Vorgängen erfüllen, kann IGF2BP1 im Embryo Einfluss auf zellbiologische Prozesse wie Wachstum, Teilung und Motilität nehmen. Andererseits kann seine Dysregulation im pathologischen Kontext einen ungünstigen Krankheitsverlauf fördern. Eine stark hochregulierte Expression von IGF2BP1 ist bereits in zahlreichen malignen Neoplasien unterschiedlichster Entitäten beobachtet worden. Im hepatozellulären Karzinom (HCC) hingegen wurde bisher weder eine mutmaßlich veränderte Expression noch die funktionelle Bedeutung dieses Faktors untersucht. Im Rahmen der vorliegenden Arbeit zeigten wir, dass IGF2BP1 im humanen HCC im Vergleich zum normalen Lebergewebe das am höchsten exprimierte RBP ist. Seine Depletion führte in allen untersuchten HCC-Zelllinien zu einer Reduktion der Proliferation und zu einer Steigerung der Apoptose *in vitro* sowie zu einem signifikant reduzierten Wachstum von *xenograft*-Tumoren in thymusaplastischen Mäusen *in vivo*. Nach vorausgegangener Etablierung wurde für diesen Zweck vor der Xenotransplantation eine stabile Depletion von IGF2BP1 mittels lentiviral vermittelter RNA-Interferenz (RNAi) durch den Transfer eines shRNA(*short hairpin-RNA*)-kodierenden Plasmids in die HCC-Zelllinie Hep G2 erreicht. Die Versuchstiere wurden in regelmäßigen Abständen mit einer dafür etablierten Methode zur nichtinvasiven Fluoreszenzbildgebung überwacht. Bezüglich IGF2BP1 handelt es sich dabei um das erstbeschriebene Funktionsverlustexperiment *in vivo*. Als mögliche Erklärung fanden wir auf molekularer Ebene einen stabilisierenden Effekt von IGF2BP1 auf die mRNAs des Protoonkogens MYC (*myc proto-oncogene protein*) und des Proliferationsmarkers MKI67 (*marker of proliferation ki-67*).

In einer zweiten Arbeit konnten wir zeigen, dass IGF2BP1 essenziell für die epitheliomesenchymale-Transition (EMT) ist. Dieser Prozess ist durch ein typisches Genexpressionsprofil und einer damit verbundenen Veränderung der Zellarchitektur charakterisiert und spielt eine wesentliche Rolle in der Entwicklung sowie der Metastasierung. Die stabile Depletion von IGF2BP1 führte in den untersuchten Zelllinien zu einem Verlust des mesenchymalen Expressionsprofils und der mesenchymalen Zellmorphologie, begleitet von einem deutlich reduzierten Migrationspotenzial der Tumorzellen. Auf molekularer Ebene sahen wir eine Stabilisierung der mRNA des im Rahmen der EMT aktiven Transkriptionsfaktors LEF1 (*lymphoid enhancer binding factor 1*). Zusammenfassend lässt sich feststellen, dass IGF2BP1 aufgrund seiner proliferations- und migrationsfördernden Wirkungen *in vitro* und *in vivo*, einen von der Tumorentität unabhängigen protumorigenen Faktor darstellt und somit als vielversprechendes Ziel zukünftiger Tumortherapien anzusehen ist.

Pazaitis, Nikolaos: *In vitro* und *in vivo* Untersuchungen zur Tumorigenität des *Insulin-like growth factor 2 mRNA-binding protein 1* (IGF2BP1), Halle (Saale), Univ., Med. Fak., Diss., 44 Seiten, 2018

Abstract

Ribonucleic acid (RNA) binding proteins (RBPs) are key regulators of RNA metabolism. Every step in the life cycle of messenger RNAs (mRNAs) ranging from transcription to decay may be influenced by RBPs. The human insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) belongs to a highly conserved family of RBPs and shows an oncofetal expression pattern. During embryogenesis, the regulatory effects of IGF2BP1 on numerous RNAs, which play an important role in developmental processes, empower IGF2BP1 to influence cellular processes such as growth, division and motility. On the other hand, its dysregulation in pathologically transformed cells can promote an unfavorable course of diseases. Re-expression of IGF2BP1 has been reported in numerous malignancies of various entities. However in hepatocellular carcinoma (HCC), neither a putative expression nor the functional significance of this factor has been investigated. Here we demonstrate that IGF2BP1 is the most highly upregulated RBP in human HCCs compared to normal liver tissue. Its depletion resulted in reduced proliferation and enhanced apoptosis in all examined liver cancer cell lines *in vitro*, as well as in an impairment of tumor growth in a murine xenograft model *in vivo*. For the latter, the HCC cell line Hep G2 was injected subcutaneously into nude mice after establishing a stable depletion of IGF2BP1 by lentivirally-mediated RNA-interference (RNAi) using shRNA-encoding (short hairpin-RNA) vectors in these cells. The animals were examined periodically using a non-invasive fluorescence based imaging method. Regarding IGF2BP1, this was the first loss of function experiment described *in vivo*. The lentiviral gene transfer system, the subcutaneous injection procedure and the imaging method where established during the course of the study at hand. As an explanation for the effect observed, we found that IGF2BP1 significantly enhances the stability of the mRNAs of the proto-oncogene MYC (myc proto-oncogene protein) and the proliferation marker MKI67 (marker of proliferation ki-67).

As a second part of the present study we showed that IGF2BP1 is important for epithelial to mesenchymal transition (EMT), a process which is characterized by a specific gene expression profile associated with a typical cell morphology change and crucial for development and metastasis. Mechanistically, we observed that IGF2BP1 increased the mRNA half-life of the transcription factor LEF1 (lymphoid enhancer binding factor 1), that is an important promoter of EMT. The stable depletion of IGF2BP1 led to a loss of the mesenchymal gene expression profile and cell morphology of the investigated cancer cell lines and was associated with a significantly reduced migratory potential of the cells. In summary, the pro-proliferative and pro-migratory effects in human cancer-derived cell lines *in vitro* and *in vivo* suggest IGF2BP1 to be an important protumorigenic factor in a wide variety of tumor entities and thus a promising target for future tumor therapies.

Pazaitis, Nikolaos: *In vitro* and *in vivo* studies of the tumorigenicity of insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), Halle (Saale), Univ., Med. Fac., Doc. Thesis., 44 Pages, 2018

Inhaltsverzeichnis

Abkürzungsverzeichnis

0-9

1241 mel Melanom-Zelllinie

1F6 Melanom-Zelllinie

3'-UTR 3'-untranslatierte Region

5'-UTR 5'-untranslatierte Region

70Z/3 pre-B-ALL-Zelllinie

786-O Nierenzellkarzinom-Zelllinie

A

Abb. Abbildung

ABC *ATP binding cassette*

Abschn. Abschnitt

ACTB *actin beta* auch β-Aktin

AKT1 *KT serine/threonine kinase 1*

ALL akute lymphatische Leukämie

APC *adenomatous polyposis coli*

ASO *antisense-Oligonukleotide*

ATP Adenosintriphosphat

AUF1 *AU-rich element RNA-binding protein*

B

BCL2 *B-cell lymphoma 2, apoptosis regulator*

BE(2)-C Neuroblastom-Zelllinie

BET *bromo-and extraterminal domain family of proteins*

BIRC2 *baculoviral IAP repeat containing 2*

BRD4 *bromodomain containing 4*

BTRC *beta-transducin repeat containing E3 ubiquitin protein ligase*

βTrCP1 *beta-transducin repeat containing protein 1* (Genprodukt von BTRC)

C

C33A Zervixkarzinom-Zelllinie

CA Karzinom

CCR4-NOT *carbo-catabolite repressor 4-negative on TATA*

CD34 *cluster of differentiation 34* (ein transmembranäres Phosphoglykoprotein)

CD44 *cluster of differentiation 44 molecule – indian blood group*

cDNA *complementary DNA*

cIAP1 *cellular inhibitor of apoptosis protein 1* (Genprodukt von BIRC2)

CIS *carcinoma in situ*

CLIP *cross-linking immunoprecipitation*

CLL chronische lymphatische Leukämie

CNV Kopienzahlerhöhung

COBALT *constrain-based multiple alignment tool* (`www.ncbi.nlm.nih.gov/tools/cobalt/`)

CpG Desoxycytidin-Phosphorsäure-Desoxyguanosin

CRD-BP *coding region determinant-binding protein* (IGF2BP1-Ortholog aus *Mus musculus*)

Cre cre Rekombinase (aus Escherichia Bakteriophage P1)

CSC *cancer stem cell*

CTNNB1 *catenin beta 1*

CYFIP2 *cytoplasmic FMR1 interacting protein 2*

D

DCIS duktales *Carcinoma in situ*

dIMP *Drosophila IMP* (IGF2BP1-Ortholog aus *Drosophila melanogaster*)

DLD-1 Kolonkarzinom-Zelllinie

DNA *deoxyribonucleic acid*

E

EFS *event-free survival*

EGF *epidermal growth factor*

EJ-30 Gallenblasenkarzinom-Zelllinie

EMT epitheliomesenchymale-Transition

E

ERBB2 *erb-b2 receptor tyrosine kinase 2*

ES-2 Ovarialkarzinom-Zelllinie

et al. *et alii*

ETV6 *ETS variant 6*

F

F-Aktin filamentäres Aktin

FIGO *Fédération Internationale de Gynécologie et d'Obstétrique*

FISH Fluoreszens *in situ*-Hybridisierung

FMR1 *fragile X mental retardation 1*

FMRP *fragile X mental retardation Protein*

FN1 *Fibronectin*

G

G-Aktin globuläres Aktin

GLI1 *GLI family zinc finger 1*

GTP Guanosintriphosphat

H

HAT-144 Melanom-Zelllinie

HCC hepatozelluläres Karzinom

HCT 116 Kolonkarzinom-Zelllinie

HCV Hepatitis-C-Virus

HEK-293A, -T & -TN Embryonale Zellen (im Sinne Zelllinien)

Hela Zervixkarzinom-Zelllinie

Hep G2 HCC-Zelllinie

HER2/neu *alias* von ERBB2

Hg-Signalweg Hedgehog-Signalweg

HL HODGKIN Lymphom

HMGA2 *high mobility group AT-hook 2*

HNRNPK *heterogeneous nuclear ribonucleoprotein K*

hPSC humane pluripotente Stammzelle

HSPB1 *heat shock protein family B (small) member 1*

HULC *hepatocellular carcinoma up-regulated long non-coding RNA*

I

IDC invasives duktales Karzinom

IGF2 *insulin like growth factor 2*

IGF2BP1, -2 & -3 *insulin-like growth factor 2 mRNA-binding protein 1*, -2 & -3

IHC Immunhistochemie

ILC invasives lobuläres Karzinom

im-ncRNAs *intermediate-sized ncRNAs*

IMP1, -2 & -3 *alias* von IGF2BP1, -2 & -3

IMR-32 Neuroblastom-Zelllinie

INRG *the international neuroblastoma risk group*

IRES *internal ribosomal entry site*

ISH *in situ* Hybridisierung

ITGB5 *integrin subunit beta 5*

K

K-562 Leukämie-Zelllinie

Kelly Neuroblastom-Zelllinie

KH-Domänen -1, -2, -3 & -4 *heterogeneous nuclear ribonucleoprotein K-Homology*-Domäne 1, -2, -3 & -4

Ki-67 Antigen Ki-67 (Genprodukt von MKI67)

KOC *K homology domain containing protein overexpressed in cancer* (*alias* von IGF2BP3)

KPP klinisch-pathologischer-Parameter

KRAS *KRAS proto-oncogene, GTPase*

L

La *alias* von SSB

LEF1 *lymphoid enhancer binding factor 1*

let-7 *lethal-7* (eine heterochronie - miRNA)

LIM-2405 Kolonkarzinom-Zelllinie

LIN28B *lin-28 homolog B*

LK-Met. Lymphknotenmetastase

l-ncRNA *long-ncRNA*

LoVo Kolonkarzinom-Zelllinie

LoxP *locus of crossover in P1* (aus Escherichia Bakteriophage P1)

LS-174T Kolonkarzinom-Zelllinie

M

MA *microarray*

MAPK4 *mitogen-activated protein kinase 4*

MAPKAPK5 *mitogen-activated protein kinase-activated protein kinase 5*

MCF-7 Mammakarzinom-Zelllinie

MDA-MB-231 Mammakarzinom-Zelllinie

MDCK Nierenzellen (im Sinne Zelllinie)

MDR1 *multidrug resistance protein 1*

MEF *mouse embryo fibroblast*

MG63 Osteosarkom-Zelllinie

MHC *major histocompatibility complex*

miR *alias* von miRNA

miRISC *micro-RNA-induced silencing complex*

miRNA mikro RNA

MITF *melanogenesis associated transcription factor*

MK5 *alias* von MAPKAPK5

MKI67 *marker of proliferation ki-67*

mRNA *messenger RNA*

mRNP *messenger RNP*

MSC mesenchymale Stammzelle

MTC Mammakarzinom-Zelllinie (aus *Rattus norvegicus*)

MTLn3 Mammakarzinom-Zelllinie (aus *Rattus norvegicus*)

MYC *myc proto-oncogene protein*

N

NB *northern blot*

NCI/ADR-RES Ovarialkarzinom-Zelllinie

ncRNA *non-coding RNA*

NF-κB *nuclear factor kappa-light-chain-enhancer of activated B cells*

NIH 3T3 Fibroblasten (im Sinne Zelllinie)

NK-Zellen *natürliche Killerzelle*

nm Nanometer

NSCLC *non-small-cell lung carcinoma*

O

OS *overall survival*

P

PCR *polymerase chain reaction*

PE-CA Plattenepithelkarzinom

PPPIR9B *protein phosphatase 1 regulatory subunit 9B*

PTEN *phosphatase and tensin homolog*

PTGS2 *prostaglandin-endoperoxide synthase 2*

Q

QGY-7703 HCC-Zelllinie

qRT-PCR *quantitative real-time PCR*

R

RAC1 *Rac family small GTPase 1*

RBNS *RNA bind-n-seq*

RBP RNA-bindendes Protein

REH ALL-Zelllinie

RH36 Rhabdomyosarkom-Zelllinie

RNA *ribonucleic acid*

RNAi *RNA interference*

RNA-Seq. RNA Sequenzierung

RNP Ribonukleoprotein

Ro60 *alias* von TROVE2

RRM *RNA-recognition motif*

RUNX1 *Runt-related transcription factor 1*

S

S. Seite

SCF$^{\beta TrCP1}$ *skp1-cullin1-F-box protein* (ein E3-Ubiquitin-Ligase-Komplex mit der substraterkennenden Untereinheit βTrCP1)

SG *stress granules*

shRNA *short hairpin-RNA*

siRNA *small interfering RNA*

SIRT6 *sirtuin 6*

SMMC-7721 HCC-Zelllinie

SNAI1 & -2 *snail family transcriptional repressor 1 & -2*

S

SRC *SRC proto-oncogene, non-receptor tyrosine kinase*

sRT-PCR *semi-quantitative real-time PCR*

SSA2 *alias* von TROVE2

SSB *Sjogren syndrome antigen B*

SW-480 Kolonkarzinom-Zelllinie

T

T47D Mammakarzinom-Zelllinie

TAA tumorassoziiertes Antigen

Tab. Tabelle

TCF4 *alias* von TCF7L2

TCF7L2 *transcription factor 7 like 2*

TET1 *tet methylcytosine dioxygenase 1*

TGFβ *transforming growth factor beta*

TIN testikuläre intraepitheliale Neoplasie

TMA *tissue microarray*

TNFα *Tumornekrosefaktor α*

TROVE2 *TROVE domain family member 2*

TSU-Pr1 Harnblasenkarzinom-Zelllinie

TWIST1 & -2 *twist family bHLH transcription factor 1 & -2*

U

U2OS Osteosarkom-Zelllinie

U373 Glioblastom-Zelllinie

U87 Glioblastom-Zelllinie

Upd *unpaired* (Protein aus *Drosophila melanogaster*)

V

Vg1RBP/Vera *Vg1-mRNA binding protein* (IGF2BP3-Ortholog aus *Xenopus laevis*)

VICKZ Akronym aus Vg1RBP/Vera, IGF2BP1, -2 und -3, CRD-BP, KOC & ZBP1

W

WAP *whey acidic protein*

WB *western blot*

WHO *world health organization*

Wnt-Signalweg *wingless-related integration site*-Signalweg

X

XL177 Xenopus laevis Zellen (im Sinne Zelllinie)

Y

Y3 Y3-RNA (eine im-ncRNA)

Z

ZBP1 *zipcode-binding protein 1* (IGF2BP1-Ortholog aus *Gallus gallus*)

ZEB1 & -2 *zinc finger E-box binding homeobox 1 & -2* (ein EMT-Transkriptionsfaktor)

1 Einleitung und Zielstellung

Die regelrechte Funktion einer jeden Zelle im Gewebsverband erfordert eine komplexe Koordination und Kontrolle der zeitlichen und örtlichen Genexpression. Diese Regulationsleistung beginnt bereits vor der Transkription und endet mit dem koordinierten Abbau der funktionstragenden Proteine und Ribonukleinsäuren (RNA). Kodierende und nichtkodierende Ribonukleinsäuren (mRNAs und ncRNAs) sind nach ihrer Transkription Träger der genetischen Erbinformation und bieten im Falle von mRNAs noch vor ihrer Translation einen Angriffspunkt für regulatorische Eingriffe in die Genexpression.

Untersuchungen humaner Gewebe mittels RNA-Sequenzierung zeigten, dass bis zu 20 % des proteinkodierenden Transkriptoms aus mRNAs bestehen, die für RNA-bindende-Proteine (RBPs) kodieren, und untermalen somit eine entscheidende funktionelle Bedeutung dieser Proteinklasse [1]. Bereits während der Transkription können mRNAs Bindungen mit spezifischen RBPs eingehen. Solche aus mRNA und meist mehreren Proteinen geformte Komplexe werden mRNA-Ribonukleoproteine (mRNPs) genannt und unterliegen einem dynamischen Auf-, Um- und Abbau. Die weiteren Abschnitte im Lebenszyklus der gebundenen RNAs werden maßgeblich durch die RNPs und durch die beteiligten RBPs beeinflusst. So ermöglichen und beeinflussen sie unter anderem die Reifung, die posttranskriptionelle Modifikation, den nukleären Export, den Transport, die Lokalisation, die Translation sowie die Stabilität und den Abbau der gebundenen Transkripte und sind damit als regulatorische Elemente der posttranskriptionellen Genexpression zu verstehen [1–4]. Dabei ist nicht das RBP alleine entscheidend für die Art und Weise, in welcher die gebundene mRNA reguliert wird, sondern vielmehr die Kombination zwischen RBP und mRNA-Transkript. So kann ein und dasselbe RBP je nach gebundenem mRNA-Transkript beispielsweise dessen Abbau fördern, aber ganz im Gegenteil ein anderes vor dem Abbau schützen. Solche „mehrwertigen“ Regulationsformen sind beispielsweise für FMRP (*fragile X mental retardation Protein*) [5], AUF1 (*AU-rich element RNA-binding protein*) und seine Isoformen [6] und für Mitglieder der IGF2BP(*insulin-like growth factor 2 mRNA-binding protein*)-Proteinfamilie [7] bekannt.

Funktionsstörungen von RBPs können wie im Falle des FMRP krankheitsauslösend sein. So führt eine Triplettexpansion in der 5 ’-untranslatierten Region (5‘-UTR) des für das FMRP kodierenden Gens FMR1 (*fragile X mental retardation 1*) zu einer Hypermetylierung und einer damit verbundenen transkriptionellen Hemmung mit dem Ergebnis einer einem Funktionsverlust gleichenden Depletion von FMRP, was bei hinreichendem Funktionsverlust zum Fragilen-X-Syndrom führt [5, 8]. Konsequenterweise führen auch FMR1-Punktmutationen, die mit einem Funktionsverlust des FMRP einhergehen, zur Entwicklung desselben Krankheitsbildes [9, 10]. Abgesehen von Mutationen, die zu einem Funktionsverlust führen, sind auch krankhafte Zustände beschrieben, die in Zusammenhang mit einem Funktionsgewinn von RBPs gebracht werden konnten. So sind RBPs in zahlreichen malignen Neoplasien die am höchsten hochregulierte Protein-Klasse [11], was als Ausdruck der erhöhten Translationsleistung dieser Zellen und einer damit verbundenen erhöhten Anforderung an Regulationsmechanismen gewertet werden kann [12]. Beispiels-

weise führte eine Überexpression von AUF1 in einem transgenen Mausmodell bei über 50 % der Tiere zu einer multifokalen Entstehung sarkom-, karzinom- und lymphomartiger Tumore [13]. Auch die Bedeutung der IGF2BPs ist im Zusammenhang mit unterschiedlichen Entitäten maligner Tumorerkrankungen des Menschen häufig untersucht worden [7, 14].

1.1 Die *Insulin-like growth factor 2 mRNA-binding proteins*

Bezug zu Manuskript 1, S. 46 [7] & Manuskript 4, S. 99 [15]

Die IGF2BPs sind stark konservierte, paraloge Proteine des Menschen mit onkofetalem Expressionscharakter [7]. Ihre Fähigkeit, Interaktionen mit RNAs einzugehen, zeichnet sie als RBPs aus. Zusammen mit paralogen und orthologen Proteinen aus anderen Spezies bilden sie die Proteinfamilie der VICKZ-Proteine. Diese Bezeichnung setzt sich aus den Anfangsbuchstaben ihrer Mitglieder zusammen und ist als Akronym zu verstehen. Sie besteht aus Vg1RBP/Vera (*Vg1-mRNA binding protein*, aus *Xenopus laevis*), IGF2BP1, -2 und -3 (aus *Homo sapiens*), CRD-BP (*coding region determinant-binding protein*, aus *Mus musculus*), KOC (*K homology domain containing protein overexpressed in cancer*, aus *Homo sapiens*) und ZBP1 (*zipcode-binding protein 1*, aus *Gallus gallus*) [16, 17]. Im Rahmen der vorliegenden Arbeit wurden überwiegend Untersuchungen an humanem IGF2BP1 durchgeführt, sodass dieses Paralogon in den nachfolgenden Betrachtungen fokussiert wird.

1.2 Molekulare Struktur der IGF2BPs

Die Mitglieder der VICKZ-Proteinfamilie teilen strukturelle Gemeinsamkeiten bezüglich ihrer molekularen Primärstruktur und ihrer Proteindomänen. Der N-terminale Bereich beinhaltet zwei RRMs (*RNA-recognition motifs*), während sich am C-terminalen Ende vier HNRNPK(*heterogeneous nuclear ribonucleoprotein K*)-Homologie(KH)-Domänen befinden [7, 18]. Dabei sind jeweils zwei KH-Domänen (KH1 und -2, sowie KH3 und -4) räumlich benachbart und maßgeblich für die Interaktion der IGF2BPs mit ihren Ziel-RNAs verantwortlich [19, 20]. Ein Aminosäuresequenzvergleich der VICKZ-Proteine zeigt eine starke Ähnlichkeit der Proteine untereinander (Abb. 1A, S. 3). Besonders deutlich ist dabei die Sequenzübereinstimmung innerhalb der Proteindomänen (Abb. 1B, S. 3), sodass auch eine Ähnlichkeit der Proteine bezüglich ihrer molekularen Funktionen angenommen werden kann. Hinsichtlich der humanen IGF2BPs ist die Ähnlichkeit zwischen IGF2BP1 und -3 am höchsten, während sich IGF2BP2 stärker auch hinsichtlich der orthologen Proteine aus den anderen Spezies unterscheidet. Eine funktionelle Gleichartigkeit im Speziellen ist also besonders zwischen IGF2BP1 und -3 zu erwarten [7].

1.3 Molekulare Funktionen des IGF2BP1

Auch wenn es Hinweise für einen Kerntransport von IGF2BP1 gibt [21–24], ist es vorwiegend im Zytoplasma lokalisier [16, 18, 20, 25] und liegt dort im Komplex mit seinen Zieltranskripten und weiteren RBPs in Form von RNPs vor [26, 27]. IGF2BP1 kann mRNAs

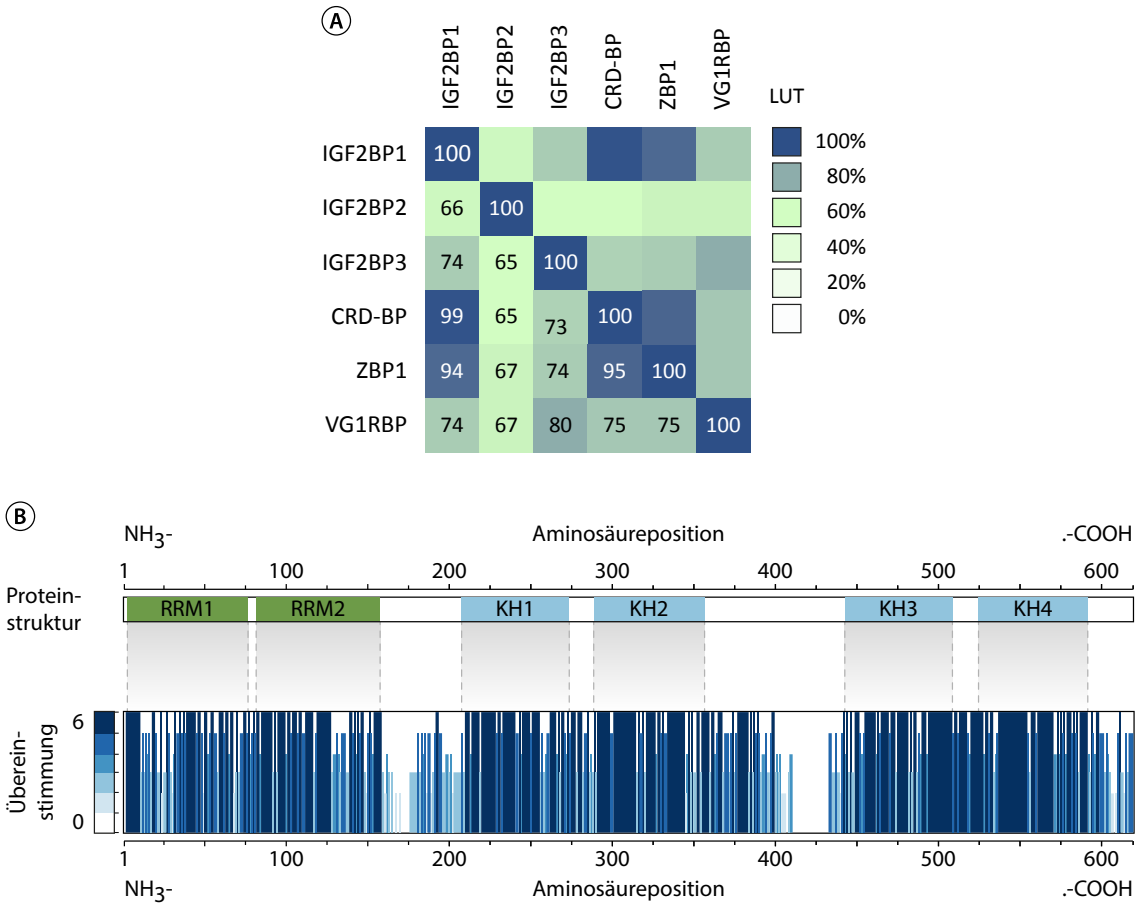


Abbildung 1 | Aminosäuresequenzvergleich und grundlegende Struktur der VICKZ-Proteine.
(A) Aminosäuresequenzvergleich zwischen IGF2BP1, -2, -3, CRD-BP, ZBP1 und VG1RBP ermittelt mittels *Protein-Blast* (`blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins`). Die Farbtafel gibt die Ähnlichkeit der Aminosäuresequenz zwischen den einzelnen VICKZ-Proteinen in Prozent an (Farbkodierung von Hell in Dunkel graduiert entsprechend dieser Ähnlichkeit, siehe LUT). CRD-BP und ZBP1 aus *Mus musculus* und *Gallus gallus* sind Orthologe des humanen IGF2BP1 und zeigen folglich die höchste Sequenzähnlichkeit, IGF2BP2 die geringste. **(B)** Schematische Darstellung der gemeinsamen Proteinstruktur der VICKZ-Proteine und ihrer Domänen maßstabsgetreu nach Sequenzausrichtung mittels COBALT (*constrain-based multiple alignment tool* - `www.ncbi.nlm.nih.gov/tools/cobalt/`). Die Abszisse gibt die Positionen der Aminosäuren an. Im unteren Diagramm ist das Ausmaß der übereinstimmenden Aminosäuren positionsgenau zu der darüber parallel dargestellten Proteinstruktur wiedergegeben. Die größte Homologie der Primärstrukturen findet sich im Bereich der konservierten RNA-Bindungsdomänen.

sowohl im Bereich der UTRs als auch in der kodierenden Sequenz binden (Manuskript 1 [7], Tab. 2, S. 51). Dafür wurden bereits unterschiedliche, experimentell eruierte und computergestützt berechnete putative RNA-Sequenzmotive vorgeschlagen [19, 26, 28–34]. In jüngster Vergangenheit kamen hierbei auch modernere Methoden wie Variationen der CLIP (*cross-linking immunoprecipitation*) [31, 33, 34] und *RNA bind-n-seq* (RBNS) [34] zur Anwendung. Außer eines Sequenzmotivs scheinen für eine molekulare Interaktion aber auch die Sekundärstruktur der RNA sowie sterische Eigenschaften von IGF2BP1 von Bedeutung zu sein [19, 35–37]. Die Bindung von RNA an IGF2BP1 ist somit nicht durch eine einzige diskrete Konsensussequenz determiniert. Die gebildeten m-/RNPs imponieren mikromorphologisch als vornehmlich zytoplasmatische, perinukleär und im Bereich von

Lamellipodien gelagerte granuläre Aggregate von 100-700 nm Durchmesser [25, 26, 29], welche aus einer distinkten Komposition von Proteinen und m-/RNAs bestehen [26]. Diese zytoplasmatischen m-/RNPs sind einer Vielzahl von regulatorischen Eingriffen ausgesetzt, von denen angenommen wird, dass sie in Abhängigkeit von der m-/RNP-Komposition den weiteren Werdegang der assoziierten m-/RNAs in unterschiedlicher Art und Weise beeinflussen. Die Abb. 2, S. 5 zeigt eine Zusammenfassung dieser Prozesse und gibt einen Überblick über bereits bekannte Zieltranskripte von IGF2BP1 und die jeweils erzielten und im Nachfolgenden einzeln aufgezählten Wirkungen.

1. RNA-Stabilisierung: Bei dem Großteil der bisher beschriebenen Zieltranskripte kommt es nach Assoziation mit IGF2BP1 zu einer Stabilisierung der RNA durch Verhinderung des Abbaus durch Exo- und Endonukleasen (Abb. 2E oben, S. 5). Dieser Mechanismus ist beispielweise für die mRNAs von CD44 (*cluster of differentiation 44 molecule – indian blood group*) [38], MYC (*myc proto-oncogene protein*) [27, 39–43] und PTEN (*phosphatase and tensin homolog*) [44] bekannt. Andererseits kann der Abbau eines Zieltranskripts auch durch die direkte oder indirekte Maskierung der Bindungsstelle einer mikro-RNA (miRNA, auch miR) verhindert werden. Ein Beispiel dafür ist die mRNA von BTRC (*beta-transducin repeat containing E3 ubiquitin protein ligase*), bei der die Bindung von miR-183 an die 3'-UTR durch IGF2BP1 inhibiert wird und damit eine miRNA vermittelte Degradation durch den miRISC (*micro-RNA-induced silencing complex*) verhindert wird [42, 45] (Abb. 2E unten, S. 5). Nach demselben Mechanismus hemmt IGF2BP1 auch die Degradation der mRNA von MITF (*melanogenesis associated transcription factor*) durch die miR-340 [46] und die Degradation der mRNAs von LIN28B (*lin-28 homolog B*), HMGA2 (*high mobility group AT-hook 2*) und sogar seiner eigenen mRNA durch die mikro-RNA let-7 (*lethal-7*) [47]. Die Inhibition des miRNA-vermittelten Abbaus von Zieltranskripten scheint im Kontext von Tumorzellen die Hauptfunktion von IGF2BP1 zu sein [47, 48]. Diesem Regulationsprinzip unterliegen offenbar insbesondere mRNAs, welche Faktoren kodieren, die beispielweise Invasivität und Proliferation von Tumorzellen begünstigen [48] und teilweise in die Kategorie der Protoonkogene einzuordnen sind.

2. Regulation des RNA-Umsatzes in Stress Granules: Bei Einwirkung von zytotoxischem Stress kommt es im Zytoplasma zur Bildung sogenannter *stress granules* (SGs), die unter anderem aus mRNPs aufgebaut sind (*Übersichtsartikel* in [49]), in denen IGF2BP1 vertreten ist [50]. Eine vermutete Funktion von SGs ist die Verhinderung einer RNA-Degradation während der zellulären Stressreaktion, um der Zelle nach Überwindung der Stressphase ein hinreichendes Reservoire von RNAs zu erhalten. Während die Aufnahme von RNAs in die SGs relativ unspezifisch zu verlaufen scheint, schützt IGF2BP1 seine Zieltranskripte während der Stressphase vor Abbau, vermutlich durch Zurückhaltung dieser in SGs (Abb. 2H, S. 5). Dies ist für die mRNAs von ACTB (*actin beta*), CD44, IGF2 (*insulin like growth factor 2*) und MYC gezeigt worden [50].

3. RNA-Degradation: Abgesehen von mRNAs, kann IGF2BP1 auch ncRNAs wie die lange ncRNA (l-ncRNA) HULC (*hepatocellular carcinoma up-regulated long non-coding RNA*) binden. Im Gegensatz zu den oben genannten Mechanismen der RNA-Stabilisierung wird HULC durch eine Interaktion von IGF2BP1 mit dem CCR4(*carbo-catabolite repres-*

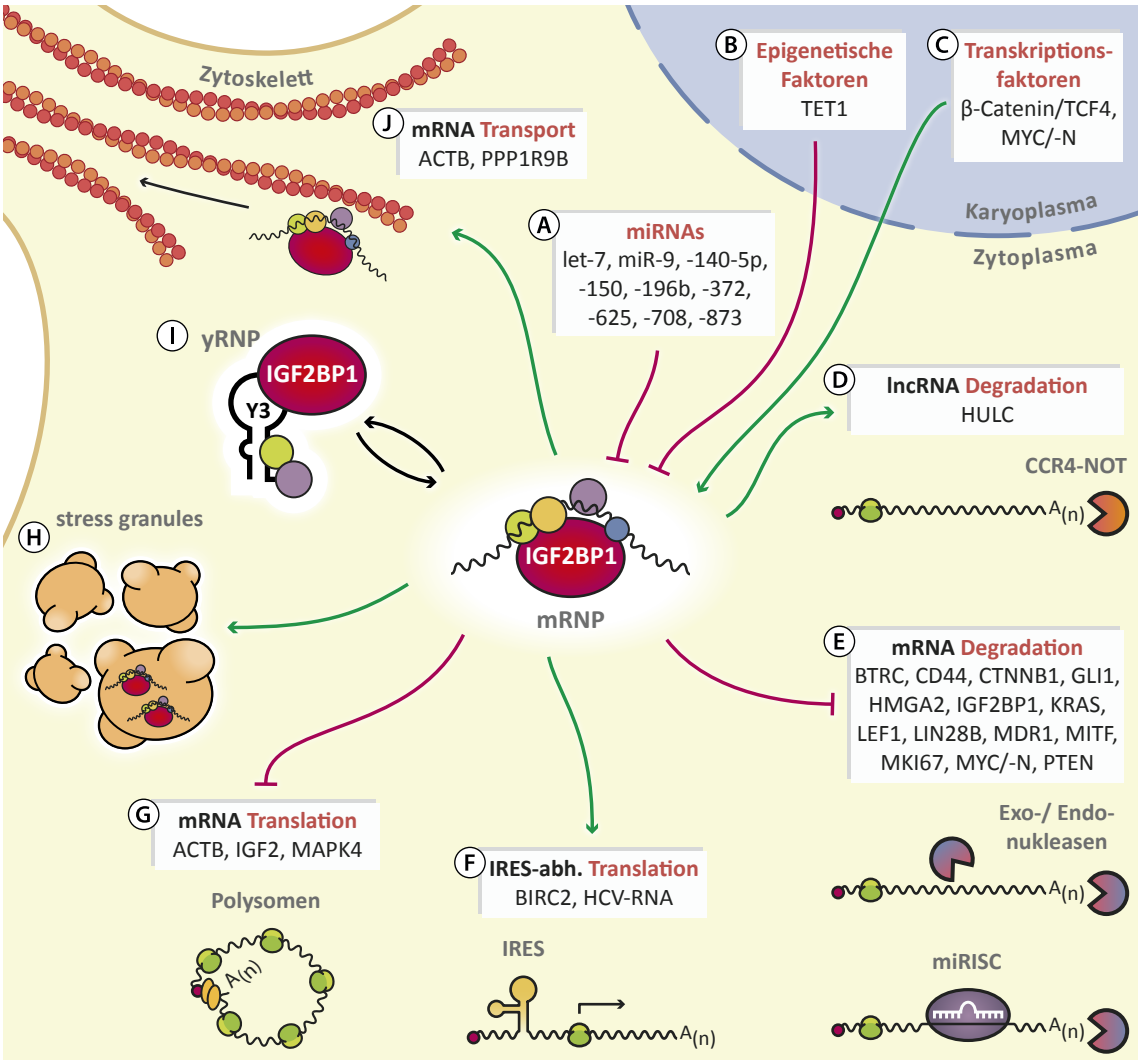


Abbildung 2 | Regulation der IGF2BP1-Expression und Synopsis über die durch IGF2BP1 wahrgenommenen Regulationsmechanismen an seinen Zieltranskripten.
(A) Die Expression von IGF2BP1 wird im Zytoplasma hauptsächlich durch miRNAs reguliert. **(B, C)** Außerdem sind epigenetische und transkriptionelle Mechanismen der Transkriptionskontrolle bekannt. Im Zytoplasma kommt es im Rahmen einer Assoziation von IGF2BP1 mit seinen Zieltranskripten zur Bildung von mRNPs. Der weitere Werdegang der jeweiligen RNA kann dann verschiedenartig beeinflusst werden. **(D)** Die nicht-kodierende RNA HULC ist das erste bekannte Zieltranskript, für das eine Destabilisierung durch eine IGF2BP1-vermittelte Rekrutierung des CCR4-NOT-Deadenylierungsapparates gezeigt ist. **(E)** Bei den meisten bekannten Zieltranskripten verhindert eine Assotiation mit IGF2BP1 einen mRNA-Abbau durch Exo- und Endonukleasen (oben). Ferner ist für eine Reihe von mRNAs eine Hemmung der miRNA vermittelten Degradation durch den miRISC gezeigt (unten). **(F)** Im Falle der BIRC2- und der HCV-RNA vermittelt IGF2BP1 eine IRES-abhängige Aktivierung der Translation. **(G)** Die mRNAs von ACTB, IGF2 und MAPK4 erfahren bei Assoziation mit IGF2BP1 eine Inhibition ihrer Translation. **(H)** Unter zytotoxischem Stress assoziiert IGF2BP1 mit mRNAs in sogenannten *stress granules* und schützt sie auf diese Weise vor ihrem Abbau. **(I)** Außer mit mRNAs kann IGF2BP1 auch mit der Y3-RNA, einer im-ncRNA assoziieren und entsprechend yRNPs formieren. **(J)** Die mRNAs von ACTB und PPP1R9B können entlang des Zytoskletts an einen kernfernen Ort transportiert werden, um einer lokalisierten Translation zugeführt zu werden. Während des Transports wird eine vorzeitige Translation verhindert (stark modifizierte Darstellung aus Manuskript 1 [7], Abb. 2, S. 50; Zieltranskripte in weißen Boxen unterliegen den entsprechend angezeigten Regulationsmechanismen; Referenzen und Abkürzungen sind im Text aufgeführt.)

sor 4)-NOT(*negative on TATA*)-Deadenylierungsapparat, welcher einen Hauptbestandteil der RNA-Abbaumaschinerie darstellt, destabilisiert und damit abgebaut [51] (Abb. 2D, S. 5).

4. Regulation der Translation: Ein weiterer durch IGF2BP1 vermittelter Mechanismus ist die Möglichkeit einer IRES (*internal ribosomal entry site*) abhängigen Aktivierung der Translation der mRNA von BIRC2 (*baculoviral IAP repeat containing 2*) [52] und der RNA des Hepatitis-C-Virus (HCV) [53] (Abb. 2F, S. 5). Auf der anderen Seite führt eine Assoziation der mRNAs von ACTB [22, 44], IGF2 [18] und MAPK4 (*mitogen-activated protein kinase 4*) [44] mit IGF2BP1 zu einer Inhibition ihrer Translation und damit in diesen Fällen zu einer negativen Regulation der Genexpression (Abb. 2G, S. 5).

5. mRNA-Transport: Jenseits dieser grundsätzlich die Expression regulierenden Funktionen wurde im Falle der mRNAs von β-Aktin (*Übersichtsartikel* in [54]) und Spinophilin (auch PPP1R9B, *protein phosphatase 1 regulatory subunit 9B*) [32] zusätzlich eine den mRNA-Transport betreffende Funktion des IGF2BP1 beschrieben (Abb. 2J, S. 5). Die β-Aktin-mRNA weist eine konservierte, 54 Nukleotide umfassende Sequenz in der 3'-UTR auf, die für den intrazellulären Transport der mRNA essentiell ist [55]. Die β-Aktin-mRNA und IGF2BP1 treten an diesem als *zipcode* bezeichneten Sequenzmotiv in Interaktion und werden in Form eines mRNPs mittels axonalen und dendritischen Transports entlang des Zytoskeletts in die Lamelli- und Filopodien des Dendriten [56] und des axonalen Wachstumskegels [57] sowie in die Lamellipodien von Fibroblasten [29] transportiert. Erreicht der Transportkomplex seinen Zielort, dissoziiert die β-Aktin-mRNA durch Phosphorylierung des IGF2BP1 (am Thyrosin 396) durch die SRC (*SRC proto-oncogene, non-receptor tyrosine kinase*), um dann für Translationsprozesse zur Verfügung zu stehen [22]. Durch diesen Mechanismus wird zum einen eine vorzeitige Translation verhindert und zum anderen eine lokalisierte Translation an geeigneten kernfernen Zielorten ermöglicht [29].

6. Bildung von yRNPs: IGF2BP1 kann neben HULC auch mit anderen ncRNAs wie dem Vertreter der Gruppe der sogenannten *intermediate-sized* ncRNAs (im-ncRNAs) Y3 (*Erstbeschreibung* in [58]) assoziieren [15, 24] (Abb. 2I, S. 5) und als akzessorisches Protein zusammen mit den ebenfalls an der Y3-RNA gebundenen Proteinen TROVE2 (*TROVE domain family member 2*, auch Ro60 oder SSA2) und SSB (*Sjogren syndrome antigen B*, auch La) die Proteinkomponente sogenannter Y RNPs bilden [59–61]. Eine IGF2BP1-Depletion führt zu einer Akkumulation von Y3 und Ro60 im Zellkern, sodass seine Anwesenheit für den nukleären Export des Y3-Ro60-Komplexes scheinbar von Nöten ist und IGF2BP1 auch in diesem Fall seine Rolle als Vehikel für den Transport und die subzelluläre Verteilung von RNAs wahrnimmt [24]. Auch wenn noch nicht vollständig verstanden, so spricht die hohe Konservierung von Y3 [62–69] für eine bedeutende biologische Funktionen dieser im-ncRNA [70].

Die vielen durch IGF2BP1 wahrgenommenen Regulationsmöglichkeiten einer hohen Anzahl sehr unterschiedlicher RNAs verdeutlichen die hohe modulierende Einflussnahme der IGF2BPs in die Genexpression und die damit verbundenen Wirkungen auf zelluläre Prozesse im physiologischen Kontext einerseits und lassen andererseits ein hohes Ausmaß einer Dysregulation der Genexpression in einem pathologischen Kontext vermuten.

1.4 Regulation der IGF2BP1-Expression

Die Kontrolle der IGF2BP1-Expression findet auf transkriptioneller und posttranskriptioneller Ebene statt und ist bisher nicht erschöpfend aufgeklärt. Außerdem werden epigenetische Regulationsmechanismen postuliert.

1. Transkriptionelle Regulation: In HEK-293T-Zellkulturen scheint die IGF2BP1-Expression durch β-Catenin (*catenin beta 1*) in Kombination mit dem Ko-Transkriptionsfaktor TCF7L2 (*transcription factor 7 like 2*, auch TCF4) verstärkt zu werden, womit anzunehmen ist, dass die Regulation der IGF2BP1-Transkription dem kanonischen Wnt(*wingless-related integration site*)-Signalweg unterliegt [42, 71, 72]. In humanen Mammakarzinom-Zelllinien konnte spezifiziert werden, dass diese Aktivierung durch eine Bindung des β-Catenin/TCF4-Komplexes an den IGF2BP1-Promotor ausgelöst wird [71, 72]. Übereinstimmend mit diesen Ergebnissen aus Zellkulturexperimenten zeigt sich in β-Catenin-defizienten Mausembryonen eine reduzierte IGF2BP1-Expression [73]. Des Weiteren scheint IGF2BP1 im Kontext von Leukämie-Zelllinien ausgerechnet in Zellen mit aktivierter Wnt-Signalkaskade exprimiert zu sein [74]. Auf der anderen Seite stabilisiert IGF2BP1 durch Bindung im kodierenden Abschnitt die mRNA der substraterkennenden Untereinheit βTrCP1 (*beta-transducin repeat containing protein 1*) des für den β-Catenin-Abbau notwendigen E3-Ubiquitin-Ligase-Komplexes $SCF^{\beta TrCP1}$ (*skp1-cullin1-F-box protein*) [42, 75] und hemmt damit seine eigene Transkription im Sinne einer negativen Rückkopplungsschleife [7, 42].

Des Weiteren konnte eine Aktivierung der IGF2BP1-Transkription durch MYC in HEK-293T- und Hela-Zellen gezeigt werden. Der IGF2BP1-Promotor beinhaltet vier MYC Bindungsstellen, die jede einzeln für sich Einfluss auf die IGF2BP1-Expression haben [75]. Da IGF2BP1 seinerseits sowohl die MYC- [39, 42, 75–79] als auch die β-Catenin-mRNA stabilisiert [71], entsteht in diesen Achsen mit beiden Proteinen eine sich verstärkende Rückkopplungsschleife mit der Konsequenz einer Erhöhung der Expression jeweils beider Proteine (Abb. 2C, S. 5).

2. Posttranskriptionelle Regulation: Im Zytoplasma wird die Expression von IGF2BP1 wesentlich durch miRNAs reguliert. Die 3‘-UTR von IGF2BP1 beinhaltet sechs putative Bindungsstellen für die let-7 miRNA [80], welche weitläufig als tumorsuppressive miRNA akzeptiert ist (*Übersichtsartikel* in [81]). In Hela-, HEK-293TN- [80] und ES-2-Zellen [47] konnte eine in der IGF2BP1-3‘-UTR-angreifende, suppressive Wirkung der let-7 miRNA auf die IGF2BP1-Expression festgestellt werden. Diese Ergebnisse konnten von Nishino *et al.* (2013) [73] sowohl *ex vivo* in primären neuronalen Zellkulturen der Maus als auch *in vivo* am Modell einer transgenen Maus mit induzierbarer let-7 miRNA rekapituliert werden [73]. In der dIMP-3‘-UTR kommt ebenfalls mindestens eine let-7 Bindungsstelle vor [82]. Gleichlautend mit den Ergebnissen aus der Maus konnte auch in den Stammzellen des Hodens von *Drosophila melanogaster* eine Repression der dIMP-Expression durch let-7 *in vivo* gezeigt werden [82]. Der Regulation durch let-7 kann IGF2BP1 interessanter Weise durch zweierlei Mechanismen entgehen. Zum einen kann IGF2BP1, wie im vorangegangenen Abschnitt erwähnt, durch Bindung an seine eigene mRNA die let-7 Bindungsstellen maskieren [47]. Zum anderen kann in humanen Tumorzellen unter Umständen eine alter-

native Polyadenylierung der IGF2BP1-mRNA einen Verlust der let-7-Bindungsstelle in der 3'-UTR herbeiführen und so let-7 wirkungslos machen [47, 83]. In neueren Untersuchungen konnte außerdem eine Fülle von miRNAs identifiziert werden, die eine Repression der IGF2BP1-Expression in einer Reihe humaner Tumorzelllinien aus unterschiedlichen Entitäten verursachen. Wie im Falle von let-7 wurden auch diese miRNAs jeweils als tumorsuppressiv bezeichnet [84–92] (Abb. 2A, S. 5).

3. Epigenetischer Regulationsmechanismus: Außer der trans- und posttranskriptionellen Regulation gibt es auch Anhaltspunkte für einen epigenetischen Regulationsmechanismus der IGF2BP1-Expression. Die Promotorregion von IGF2BP1 weist eine erhöhte CpG(Desoxycytidin-Phosphorsäure-Desoxyguanosin)-Dinukleotid-Dichte auf [93], wodurch die reduzierte IGF2BP1-Expression in adulten Zellen möglicherweise das Resultat einer Methylierung des IGF2BP1-Promotors sein könnte [94]. In einer Reihe von IGF2BP1-negativen, aber nicht in IGF2BP1-positiven Mammakarzinom-Zelllinien aus unterschiedlichen Spezies wurde eine erhöhte Methylierung des IGF2BP1-Promotors gezeigt [72]. Tatsächlich führte auch eine Behandlung sowohl von in der Regel IGF2BP1-negativen, CD34-positiven, primären hämatopoietischen Stammzellen [41] als auch von IGF2BP1-negativen MTLn3-Zellen (Mammakarzinom aus *Rattus norvegicus*) [72] mit demethylierenden Agenzien (*Vidaza* bzw. *Decitabin*) zur Induktion einer IGF2BP1-Expression. Mechanistisch betrachtet, scheint die Promotormethylierung die Bindung von β-Catenin an den IGF2BP1-Promotor zu blockieren und damit zu einer Inhibition der IGF2BP1-Transkription zu führen [72]. Der Methylierungsstatus des IGF2BP1-Promotors scheint dabei durch TET1 (*tet methylcytosine dioxygenase 1*) kontrolliert zu werden [95] (Abb. 2B, S. 5).

1.5 IGF2BP1 im physiologischen Kontext

1.5.1 Physiologische Expression des IGF2BP1

Im physiologischen Kontext ist die stärkste Expression der IGF2BPs während der pränatalen Entwicklungsperiode in verschiedenen Geweben unterschiedlichster Organismen zu verzeichnen [1, 7, 18, 96]. Während der Embryogenese der Maus (*Mus musculus*) kommt es zu einer biphasischen Expression aller IGF2BPs auf mRNA- und Proteinebene. Dabei wird nach einem frühen Expressionsmaximum im Stadium der Zygote ein zweites Expressionsmaximum 12,5 Tage *post coitum* (entspricht Embryonaltag E12.5 und THEILER-Stadium ts20/21) beschrieben [18, 35, 96] (*Übersichtsartikel* in [97, 98]). In ganzen Mausembryonen durchgeführte *in situ*-Hybridisierungen erlaubten schließlich neben der Analyse der zeitlichen auch eine topographische Auflösung der IGF2BP1-Expression. So ist die IGF2BP1-mRNA im Moment des zweiten Expressionsmaximums insbesondere im Pros- und Rhombencephalon, im Neuralrohr und in den Extremitätenknospen sowie geringer auch im Mittelgesicht, in der Schwanzknospe, in den Schlundbögen, in den Somiten und innerhalb der Organanlagen von Augen, Zunge, Herz, Lunge sowie Leber beobachtet worden [96].

Ein ebenfalls zweizeitiger Expressionscharakter und insbesondere auch eine wie im Mausembryo auffällige Distribution im sich entwickelnden zentralen Nervensystem konnte in

sehr ähnlicher Form während der Ontogenese von *Rattus norvegicus* (Wanderratte) [56, 99], *Gallus gallus* (Bankivahuhn) [57, 100], *Drosophila melanogaster* (Tau-/ Obstfliege) [30, 101–103], *Xenopus laevis* (glatter Krallenfrosch) [104–106] und *Danio rerio* (Zebrabärbling/ -fisch) beobachtet werden [105]. Die zeitliche und örtliche Expression des IGF2BP1 scheint damit speziesübergreifend auch über die Grenzen der Vertebraten hinaus konserviert zu sein. Detailliertere Untersuchungen an neuronalem Gewebe zeigten, dass IGF2BP1 insbesondere in der dorsomedialen Rinde des Telencephalons des Mausembryos [73] sowie in der Neuralplatte, im späteren Neuralrohr unter Aussparung der Bodenplatte und in Neuralleistenderivaten von *Xenopus laevis* [106] exprimiert ist.

Im Vergleich zu embryonalen Geweben zeigt IGF2BP1 in den meisten Geweben adulter Organismen eine unter die Detektionsschwelle herabregulierte und nur noch in wenigen Zelltypen in eher geringen Mengen nachweisbare Expression (Manuskript 1 [7], Abb. 3, S. 52). So bleibt IGF2BP1 in mesenchymalen Stammzellen des Kolons [107], des Knochenmarkes [108] und der Nabelschnur [41], in den Oozyten und den Spermatogonien der adulten Maus [109] und der adulten Taufliege [110] sowie im Ejakulat des Menschen nachweißbar [109]. Eine geringe Expression ist außerdem in den Neuronen des *Ganglion spinale* adulter Ratten [111] sowie ohne Angabe des genauen Zelltyps im Dünn- und Dickdarm, im Tubulussystem der Niere und in der Leber der Maus zumindest noch einige Tage nach der Geburt nachweisbar [96]. Eine Funktion des IGF2BP1 ist somit auch jenseits entwicklungsbiologischer Prozesse zu vermuten.

In Analogie zu den noch undifferenzierten embryonalen Zellen scheint IGF2BP1 im adulten Organismus insbesondere in Zellpopulationen mit Stammzellcharakter [41, 95, 108] sowie in Zellen mit noch erhaltenem Proliferationspotenzial [112] exprimiert. Dieses Expressionsprofil legt nahe, dass IGF2BP1 die Proliferation und Selbsterneuerung von Zellen begünstigen könnte und würde damit typische Eigenschaften eines Protoonkogens erkennen lassen.

1.5.2 IGF2BP1 in der Ontogenese

Neben deskriptiven Expressionsanalysen von IGF2BP1 sind in Hansen *et al.* (2004) [96] Ergebnisse einer IGF2BP1-*knockout*-Maus veröffentlicht, die zahlreiche Erkenntnisse auch bezüglich einer biologischen Bedeutung des Proteins erbrachten. Homozygot IGF2BP1-defiziente Mäuse zeigten einen geschlechtsunabhängigen pathologischen Phänotyp. Es stellte sich eine erhöhte perinatale und postnatale Mortalität von 50 % innerhalb der ersten drei Lebenstage sowie eine um durchschnittlich 18 % reduzierte Masse der Plazenta dar [96]. Des Weiteren zeigten die überlebenden Mäuse ein um 21 % reduziertes Geburtsgewicht, kombiniert mit einem disproportionierten Körperbau und einer gewissen fazialen Dysmorphie sowie eine postnatale Gedeihstörung mit resultierender Reduktion der Körpermasse um durchschnittlich 40 % im adulten Stadium [96]. Ferner konnten skelettale Wachstumsanomalien im Sinne eines reduzierten Ausmaßes der langen Röhrenknochen neben einer verzögerten Mineralisation der Knochensubstanz und einer Reduktion des Knorpelgewebes in einer Reihe von Skelettelementen gezeigt werden [96]. In Zeitreihenanalysen der Embryonen wurden die ersten Zeichen eines verzögerten Körper- und Organwachstums be-

reits intrauterin im Stadium E14.5 registriert und erreichten im Stadium E17.5 statistische Signifikanz [96]. Die intrauterine Wachstumsstörung entsteht folglich erst nach dem Zeitpunkt der höchsten IGF2BP1-Expression, sodass ein direkter Zusammenhang zwischen der Wachstumsstörung und dem IGF2BP1-Defizit wahrscheinlich ist. In der Chronologie der Embryonalentwicklung der Maus wirkt das IGF2BP1-Defizit also in der Übergangphase der von der Organogenese geprägten Embryonalperiode in die von einem Organwachstum geprägten Fetalperiode. Es lässt sich damit eine wichtige Rolle von IGF2BP1 in proliferativ aktivem fetalen Gewebe vermuten. Unterstützt wird diese Annahme durch Proliferationsexperimente mit aus Embryonen des Stadiums E17.5 isolierten Fibroblasten (*mouse embryo fibroblasts*; MEFs), in denen sich eine reduzierte Proliferationsrate der IGF2BP1-defizienten MEFs zeigte [96]. Ferner verweist auch die Tatsache, dass die reduzierte Körper- und Organmasse Ausdruck einer Hypoplasie und nicht etwa einer Hypotrophie ist [96], auf eine reduzierte Fähigkeit des embryonalen Gewebes zur Proliferation.

Die Autoren unterscheiden die Mechanismen die eine intrauterine Wachstumsretardierung auslösen von denen, die zu einem reduzierten postnatalen Wachstum führen. Neben der Möglichkeit einer durch eine plazentare Insuffizienz bedingten Wachstumsretardierung wurde in den IGF2BP1-defizienten Mäusen auch eine Reduktion der Translation der IGF2-mRNA im Stadium E12.5 beobachtet und als mögliche Erklärung der intrauterinen Wachstumsretardierung angeführt. Die IGF2-*leader-3*-mRNA gehört zu den erstbeschriebenen IGF2BP-Zieltraskripten, war aus diesem Grund namensgebend für die Proteinfamilie (*Erstbeschreibung* in [18]) und kodiert für einen wichtigen embryonalen Wachstumsfaktor (*Übersichtsartikel* in [113]). Die IGF2-mRNA und IGF2BP1 zeigen im Zeitraum des zweiten Expressionsmaximums (Stadium E12.5) eine ähnliche Verteilung im embryonalen Mausgeweben, sodass der Phänotyp der homozygot IGF2BP1-defizienten Mäuse auf eine fehlende Regulierung dieses Wachstumsfaktors durch IGF2BP1 zurückzuführen sein könnte [96]. Damit übereinstimmend zeigen IGF2-*knockout*-Mäuse einen ähnlichen Phänotyp mit sowohl intrauteriner Wachstumsretardierung, reduziertem Körpergewicht im adulten Stadium sowie eine kleindimensionierte Plazenta zu einem Zeitpunkt, der nach dem Stadium E12.5 liegt [96, 114–117] und damit zeitlich mit den Veränderungen aus den IGF2BP1-defizienten-Mäusen zusammenfällt. Auch weisen beide *knockout*-Mausstämme skelettalen Entwicklungsanomalien auf. IGF2 ist im sich entwickelnden Skelettsystem stark exprimiert [118] und fördert die Differenzierung und die Proliferation von Chondrozyten und Osteoblasten sowie die Synthese der Extrazellulärmatrix des Knorpel- und Knochengewebes durch diese Zelltypen [119] (*Übersichtsartikel* in [120]). Die sich durch Abwesenheit von IGF2BP1 einstellende Translationshemmung von IGF2 kann somit ebenfalls als mögliche Ursache der skelettalen Entwicklungsanomalien in Frage kommen.

Als eine mögliche Erklärung für das postnatale Wachstumsdefizit und als gleichzeitig auffälligste anatomische Anomalie der überlebenden IGF2BP1-*knockout*-Mäuse zeigten sich in Dünn- und Dickdarm nekrotische Areale mit abgeflachten und verschmälerten Villi sowie eine reduzierte Tiefe der Krypten [96]. Die postnatale Gedeihstörung wäre nun unabhängig von dem IGF2BP1-Defizit durch ein Malabsorptions- oder Malassimilationssyndrom hinreichend erklärt [96].

Die Autoren erklären die hohe Variation von überlebensfähigen bis hin zu letalen Phänotypen der homozygot IGF2BP1-defizienten-Mäuse mit der denkbaren Möglichkeit einer Kompensation der IGF2BP1-Funktion durch die anderen eine ähnliche Bindungsspezifität für RNA aufweisenden Paraloge IGF2BP2 und -3 [96].

Im Genom von *Drosophila melanogaster* kommt nur das zu IGF2BP1 orthologe Protein dIMP vor (*Übersichtsartikel* in [101]). Eine Funktionsverlust-Mutation ist in diesem Organismus nicht überlebensfähig [102] und verdeutlicht die Wichtigkeit dieses Faktors in der Ontogenese bei ausbleibender Kompensationsmöglichkeit durch paraloge Proteine.

1.5.3 IGF2BP1 in der embryonalen Neurogenese und Zellmigration

Eine Vielzahl von Zellen im Embryo und im adulten Organismus nutzt einen dynamischen Auf- und Abbau spezialisierter Membranprotrusionen um eine zielgerichtete Fortbewegung und Migration auszuführen (*Übersichtsartikel* in [121]). Diese im Allgemeinen als Pseudopodien bezeichneten Zellkompartimente sind reich an Aktinfilamenten und können auf Basis morphologischer Charakteristika unter anderem in Lamelli- und Filopodien eingeteilt werden (*Übersichtsartikel* in [122]).

In noch undifferenzierten Neuronen verläuft sowohl das Auswachsen des Neuriten in Form eines Wachstumskegels als auch das Wachstum des Dendriten nach einem gleichartigen Prinzip und bildet die Basis der Synaptogenese (*Übersichtsartikel* in [123]). Als treibende Kraft und als zugrundeliegender molekularer Mechanismus dieser komplexen Bewegung kann eine gegen die Membran gerichtete Polymerisation von Tubulin- und Aktinfillamenten angesehen werden (*Übersichtsartikel* in [122, 124]). Die gerichtete Bewegung dieser Kompartimente in den heranwachsenden Neuriten und Dendriten ist dabei abhängig von der lokalen β-Aktin-Konzentration [21, 55, 57]. Diese Differenzierungsprozesse von Nervenzellen sind in einer Reihe von Spezies mit einer IGF2BP1-abhängigen Regulation der β-Aktin-mRNA in Verbindung gebracht worden und sind passend zur wiederholt beobachteten Expression des IGF2BP1 im neuronalen Gewebe von Embryonen unterschiedlicher Organismen (Abschn. 1.5.1, S. 8). Der durch IGF2BP1 realisierte Transport der β-Aktin-mRNA in unreifen Neuronen mit Ermöglichung einer lokalisierten Translation ist eine Voraussetzung für die gerichtete Bewegung des Wachstumskegels heranwachsender Neuriten in *Xenopus laevis* [125, 126], *Mus musculus* [127] und *Rattus norvegicus* [128] sowie für das axonale Wachstum embryonaler Neuronen der Maus *per se* [111]. In den embryonalen hippocampalen Neuronen des Huhns (*Gallus gallus*) führte eine IGF2BP1-Defizienz ferner zu einer Beeinträchtigung der Motilität der Wachstumskegel der Neuriten [57]. Hinsichtlich des Dendriten konnte in IGF2BP1-depletierten embryonalen Hippocampus-Neuronen der Ratte eine verringerte Anzahl der dendritischen Filopodien und Synapsen [56] sowie ein niedrigeres Verzweigungsniveau der Dendriten [99] und damit eine Reduktion der Aufnahmefläche synaptischer Kontakte beobachtet werden.

Nicht nur in Bezug auf die Ausreifung der zentralen, sondern auch auf die der peripheren Neuronen kommt dem IGF2BP1 eine entscheidende Bedeutung zu. So ist das zu IGF2BP1 orthologe dIMP aus *Drosophila melanogaster* wesentlich für die Entwicklung der motorischen Endplatte von Motoneuronen und möglicherweise auch für eine fehlerfreie

neuromuskuläre Erregung [102] sowie für ein auf die Muskelfaser gerichtetes Wachstum der Axone [129, 130].

In der Embryonalentwicklung von *Xenopus laevis* ist das dem IGF2BP1 orthologe VG1RBP/Vera essentiell für die Migrationseigenschaften der Zellen von Neuralleisten und Neuralrohr. Ein Funktionsverlust des Proteins im Zweizellstadium führt hier zu einem ausbleibenden Schluss der Deckplatte des Neuralrohrs und zu einer gestörten Migration von Neuralleistenabkömmlingen wie den Melanozyten mit resultierender irregulärer Pigmentierung der Embryonen [106].

Neben den beschriebenen Funktionen in Neuronen ist der IGF2BP1-vermittelte Transport der β-Aktin-mRNA auch im Zusammenhang mit den Migrationseigenschaften der Fibroblasten des Hühnerembryos gebracht worden. Die durch IGF2BP1 ermöglichte lokalisierte β-Aktin-Translation innerhalb des die Zellbewegung anführenden Membranfortsatzes ist essentiell für die Etablierung einer Zellpolarität [100] und einer gerichteten Motilität [29] dieser Zellart.

Abgesehen von den Eigenschafen im Embryo ist die regelrechte Regeneration axonal geschädigter adulter muriner Neurone von einem ungestörten Transport von mRNAs durch IGF2BP1 abhängig [111].

Die Regulationsmechanismen der β-Aktin-mRNA durch IGF2BP1 sind demnach speziell und umfassen neben der bei den anderen Zieltranskripten vornehmlich wahrgenommenen reinen Translationskontrolle auch den Transport der mRNA und damit die spatiotemporale β-Aktin-Expression. Somit ist IGF2BP1 als ein wichtiger Faktor für den Ausreifungsprozess und für die synaptische Plastizität embryonaler Neurone, für die Regenerationsfähigkeit adulter axonal geschädigter Neurone als auch für die Migrationseigenschaften von Fibroblasten anzusehen.

1.5.4 Die Bedeutung von IGF2BP1 in der Aufrechterhaltung von Stammzelleigenschaften

In traumatisch erzeugten Gewebeschäden der Kolonschleimhaut von adulten Mäusen konnten MANIERI *et al.* (2012) [107] eine Expression von IGF2BP1 in den an der Geweberegeneration beteiligten mesenchymalen Stammzellen (MSCs) des Kolons zeigen. Die Autoren postulieren eine stabilisierende Funktion des Proteins auf die mRNA von PTGS2 (*prostaglandin-endoperoxide synthase 2*, auch *cyclooxigenase-2*), dessen volle Expression aus zwei funktionierenden Allelen in MSCs für eine regelhaft ablaufende Wundheilung unabdingbar ist [107]. In Anbetracht dieser Ergebnisse stellt sich die Frage nach einer grundsätzlichen Funktion von IGF2BP1 im Rahmen von Regenerationsprozessen im Sinne einer Wundheilung auch anderer adulter Gewebe, da PTGS2 bei allen akuten Inflammationsprozessen induziert wird und zu den wichtigen Enzymen bei der Synthese von Entzündungsmediatoren gezählt wird [131].

In neuronalen Stammzellen des dorsalen Telecephalons muriner Embryonen ist IGF2BP1 durch Stabilisierung der mRNA des Proteins HMGA2 für die Fähigkeit zur Selbsterneuerung und die Hemmung der Ausdifferenzierung und damit den Erhalt der Stammzelleigenschaften von Bedeutung. In IGF2BP1-depletierten murinen Embryonen kommt es durch

einen frühzeitigen Verlust dieser Stammzelleigenschaften zu einer bis in das adulte Stadium persistierenden Reduktion der Hirnmasse [73].

Eine ähnliche Funktion bezüglich der Aufrechterhaltung von Stammzelleigenschaften konnte in humanen pluripotenten Stammzellen (hPSCs) ermittelt werden. Hier konnte eine stabilisierende Eigenschaft des IGF2BP1 auf die mRNA des für die Zelladhäsion wichtigen Proteins ITGB5 (*integrin subunit beta 5*) sowie ein positiver Einfluss auf das antiapoptotische Protein BCL2 (*B-cell lymphoma 2, apoptosis regulator*) gezeigt werden [33]. Eine Depletion von IGF2BP1 in hPSCs führte schlüssiger Weise zu einer reduzierten Zelladhäsion und einer gesteigerten Apoptose [33].

Schließlich ist IGF2BP1 für die Erhaltung der Stammzellnische des Hodens von *Drosophila melanogaster* von Bedeutung. Im Rahmen des Alterungsprozesses kommt es in den Stammzellen der Keimbahn männlicher Fruchtfliegen zu einem Verlust des für das Selbsterneuerungspotenzial dieser Zellen wichtigen Faktors Upd (*unpaired*) bei gleichzeitigem Verlust von IGF2BP1 [82]. Es konnte demonstriert werden, dass IGF2BP1 nach Bindung an die Upd-mRNA eine Stabilisierung dieser durch Maskierung einer Bindungsstelle der endogenen siRNA (*small interfering RNA*) siRNA2 bewirkt und das Transkript damit vor Abbau schützt. In Anwesenheit der im Alterungsprozess hochregulierten let-7 Expression sinkt auch die IGF2BP1-Expression (Abschn. 1.4, S. 7) mit der Folge einer Reduktion der Upd-Expression [82].

1.6 IGF2BP1 im pathologischen Kontext

Eine Beziehung zwischen IGF2BP1 und pathologischen Prozessen wurde hauptsächlich in Verbindung mit malignen neoplastischen Prozessen untersucht. Die meisten dieser Untersuchungen zeigen eine das Überleben, die Migration und die Resistenzentwicklung der Tumorzellen fördernde Wirkung von IGF2BP1. Zur Erreichung dieser Effekte bedient sich das Protein in Tumorzellen offenbar an Mechanismen, die sich direkt aus den physiologischen Funktionen von IGF2BP1 im embryonalen Gewebe ableiten.

1.6.1 Pathologische Expression des IGF2BP1

Eine Re-/Expression der IGF2BPs konnte in zahlreichen Entitäten benigner und maligner humaner Tumore festgestellt werden. Der überwiegende Anteil dieser Untersuchungen zeigt eine erhöhte Expression der Paraloge IGF2BP1 und -3 (Manuskript 1 [7], Tab. 2 und 3, S. 53 und 54 und aktualisiert Tab. A1, S. 114) [14, 98]. Da IGF2BP1 und -3 abseits davon hauptsächlich in embryonalem Gewebe und nur in geringen Mengen in einzelnen adulten Zelltypen exprimiert sind, werden sie in der Literatur häufig als onkofetale Proteine betitelt [77, 93, 132, 133]. Im Gegensatz dazu ist IGF2BP2 in vielen adulten Geweben physiologischerweise exprimiert (Manuskript 1 [7], Abb. 3a, S. 52) [109]. Die bezüglich einer IGF2BP1-mRNA- und/oder Proteinexpresssion am ausführlichsten untersuchten Malignome sind die des Kolons [133–136], der Lunge [133, 137–139], des Ovars [43, 47, 48, 133, 140], der weiblichen Brustdrüse [72, 132, 133, 141, 142] sowie das Neuroblastom [143]. Weitere Untersuchungen berichten auch über eine Expression im pankreatischen [133, 144], hepatozellulären [79,

84, 88, 145, 146], oesophagealen [139], zervikalen, [90] und prostatischen Karzinom [133], im Basalzellkarzinom [147], Melanom [148], Meningeom [137] und in der B-Vorläufer-ALL (akute lymphatische Leukämie) [149] sowie in zahlreichen malignen Neoplasien des Nervensystems [85, 137] und des Hodens [109], als auch in zahlreichen malignen und benignen Weichteiltumore [52, 89, 93]. Mit einem alle Paraloge erkennenden Antikörper konnte außerdem eine IGF2BP-Expression in zahlreichen Neoplasien des myeloischen und des lymphatischen Systems [112] sowie in nicht näher bezeichneten Malignomen von Kolon, Lunge, Hirn, Prostata, Ovar, der weiblichen Brust und im Melanom [150] gezeigt werden. Des Weiteren ist eine IGF2BP1-Expression in vielen aus humanen Neoplasien zahlreicher Tumorentitäten gewonnenen Zelllinien nachweisbar (Manuskript 1 [7], Abb. 3b, S. 52) [47, 83]. Eine Überexpression des physiologischerweise bereits in vielen gesunden Geweben exprimmierten IGF2BP2 wurde bereits im Liposarkom [151], im HCC [152, 153], im kolorektalen Karzinom [154] und im oesophagealen Adenokarzinom [155] gesehen, allerdings ist eine möglicherweise tumorprogressive Rolle bisher nur wenig überzeugend dargestellt worden.

Neben den Studien, die einen direkten Nachweis von IGF2BP1 und/oder seiner mRNA analysierten, gab es auch eine Reihe von Untersuchungen an Blutseren von Patienten mit unterschiedlichen Tumorleiden mit einer Entdeckung von Autoantikörpern gegen IGF2BP1, -2 und -3 [156–165] (und *Übersichtsartikel* in [166–170]). Dies ist ein indirekter Hinweis auf die Expression der Proteine in neoplastischen Geweben, und die IGF2BPs sind in diesem Kontext als tumorassoziierte Antigene (TAAs) zu bezeichnen. In der Literatur rücken bezüglich dieses Phänomens insbesondere das HCC und seine präkanzerösen Läsionen in den Vordergrund mit Entdeckung signifikant erhöhter Autoantikörper gegen alle Paraloge [145, 146, 156–158, 166, 171–174] (Tab. A2, S. 122 und *Übersichtsartikel* in [170]). In Einzelfällen waren auch in Seren von Patienten mit Leberzirrhose oder einer chronischen Hepatitis Autoantikörper gegen zum Teil alle IGF2BPs nachweisbar [145, 146, 173, 174].

1.6.2 Genomische Alterationen des IGF2BP1-Gens

Abgesehen von den Veränderungen der IGF2BP1-Expression konnten einzelne Untersuchungen auch Alterationen des IGF2BP1-Gens in malignen Tumorerkrankungen nachweisen. Im Neuroblastom konnte eine Amplifikation des IGF2BP1-Gens in 84 % der untersuchten Fälle gezeigt werden und mit einer ungünstigen Überlebensprognose korreliert werden, wobei die Amplifikation insbesondere bei Neuroblastomen im Stadium 4 (nach *international neuroblastoma staging system*) zu finden war [143].

Zudem konnte IGF2BP1 in einem Einzelfallbericht eines 16-jährigen Patienten mit einer ALL der B-Zellreihe als IGH(*immunoglobulin heavy chain*)-Translokationspartner identifiziert werden [175] und könnte, ähnlich wie für Translokationen anderer RBPs in soliden und hämatologischen Neoplasien bereits gezeigt [176], möglicherweise als Tumorfördernde Funktionsgewinnmutation wirken. In einer davon unabhängigen Arbeit konnte eine IGF2BP1-Überexpression speziell in t(12;21)(p13;q22)/ETV6(*ETS variant 6*)-RUNX1(*Runt-related transcription factor 1*)-positiven Fällen der B-Vorläufer-ALL entdeckt werden [149]. In Verbindung mit der Tatsache, dass eine ETV6-RUNX1-Translokation alleine zur klinischen Manifestation einer B-Vorläufer-ALL nicht ausreichend ist [177], liegt

die Vermutung nahe, dass IGF2BP1 in diesen Fällen zur malignen Entartung beitragen könnte [175].

Zusätzlich gibt es zwei Berichte über eine Amplifikation des IGF2BP1-Gens in Mammakarzinomen [132, 141]. Das IGF2BP1-Gen ist auf dem Chromosom 17 in Nachbarschaft zu dem ERBB2(*erb-b2 receptor tyrosine kinase 2*, auch HER2/neu)-Gen lokalisiert und war in einer Untersuchung an 40 Mammakarzinomen in 14 Fällen gemeinsam oder auch unabhängig von ERBB2 amplifiziert [132].

1.6.3 IGF2BP1 als tumorinitiierender Faktor

In der Tat war im Zeitraum der Datenerhebung zur vorliegenden Arbeit nur ein einziges die Bedeutung von IGF2BP1 in einer Tumorerkrankung untersuchendes *in vivo* Experiment verfügbar. Von Tessier *et al.* (2004) [178] wurde eine transgene Mauslinie vorgestellt, welche unter Laktation ein durch den WAP(*whey acidic protein*)-Promotor kontrolliertes IGF2BP1 in den Brustdrüsenzellen exprimierte. Dosisabhängig entwickelten 95 % der stark-exprimierenden und 60 % der schwächer-exprimierenden Tiere ein Mammakarzinom binnen 60 Wochen. Dabei wiesen zahlreiche Tiere eine multifokale/ -zentrische Tumorgenese und einige der Tiere auch lymphonodale, Ovarial- oder Lungenmetastasen auf. Damit ist anzunehmen, dass ein alleiniges Auftreten einer hochregulierten Expression von IGF2BP1 im Brustdrüsengewebe der Maus für eine Tumorinitiation ausreichend ist.

1.6.4 IGF2BP1 als protumorigener Faktor

Bezug zu Manuskript 2, S. 66 [79]

1. Proliferation und Selbsterneuerung: In zahlreichen aus malignen Neoplasien isolierten Tumorzelllinien wurde eine Abhängigkeit der Proliferation von der IGF2BP1-Expression in Zellkulturexperimenten gezeigt [41–43, 47, 48, 52, 75, 80, 84–87, 89, 95, 136, 138, 142, 143, 147, 148, 179–183]. Die proliferationsfördernde Wirkung erreicht IGF2BP1 offenbar durch Erwirkung eines bereits häufig beschriebenen stabilisierenden Einflusses auf die mRNAs des Protoonkogens MYC [27, 39–44, 76, 79, 136, 184, 185] mit der Konsequenz, die Expression und damit den onkogenen Einfluss dieses Faktors in malignen Zellen zu unterstützen. Die Expressionsfördernde Wirkung von IGF2BP1 auf MYC wurde bereits in der Mammakarzinom-Zelllinie MCF-7 [41], der Kolonkarzinom-Zelllinie LIM-2405 [136], der Ovarialkarzinom-Zelllinie ES-2 [43, 48], der Melanom-Zelllinie 1241 mel [148], der Osteosarkom-Zelllinie U2OS [44], den Neuroblastom-Zelllinien BE(2)-C, IMR-32 und Kelly [143] sowie in MSCs der Nabelschnur [95] gezeigt.

Analog zu MYC hat IGF2BP1 auch eine stabilisierende Wirkung auf die mRNA von KRAS (*KRAS proto-oncogene, GTPase*), welches in malignen Neoplasien gehäuft aktivierende Mutationen zeigt und damit zu einer gesteigerten Proliferation der Tumorzellen führt (*Übersichtsartikel* in [186]). In der Kolonkarzinom-Zelllinie SW-480 führte eine siRNA-vermittelte IGF2BP1-Depletion zu einer Reduktion der KRAS-Expression, was scheinbar als Folge einer stabilisierenden, direkten Interaktion von IGF2BP1 mit dem kodierenden Abschnitt und dem 3‘-UTR der mRNA von KRAS angesehen werden kann [136].

Des Weiteren kann die Ursache des proliferationsfördernden Einflusses von IGF2BP1 in der Stabilisierung der GLI1(*GLI family zinc finger 1*)-mRNA begründet liegen, welche einen durch den Hedgehog(Hg)-Signalweg aktivierbaren Transkriptionsfaktor kodiert [95, 179]. Die Zielgene von GLI1 wirken stimulierend auf den Zellzyklus, womit ein aktivierter Hg-Signalweg in Tumorzellen in erster Linie als Proliferationsstimulus anzusehen ist (*Übersichtsartikel* in [187, 188]). In Kolonkarzinom-Zelllinien (genetisch modifizierte DLD-1 und LS-174T) konnte eine durch IGF2BP1-Depletion erzwungene Reduktion der Zellteilungsrate durch eine GLI1-Überexpression wiederhergestellt werden [179], womit GLI1 als ein Vermittler des von IGF2BP1 ausgehenden Proliferationsreizes gewertet werden kann.

In neueren Untersuchungen zeigte unsere Arbeitsgruppe außerdem, dass IGF2BP1 die Protein- [47] und mRNA-Expression [48] des RBPs LIN28B und des chromatinstrukturregulierenden Proteins HMGA2 [47] verstärkt. Beide Proteine sind sowohl in Stammzellen als auch in malignen Zellen zahlreicher Tumorentitäten exprimiert. Während HMGA2 in Stammzellen die Genexpression von proliferationsfördernden Faktoren begünstigt (*Übersichtsartikel* in [189]), verhindert LIN28B die Zellreifung durch Repression der heterochronie-miRNA let-7 und hütet so Stammzelleigenschaften wie beispielsweise die Fähigkeit zur Selbsterneuerung [190]. In der Ovarialkarzinom-Zelllinie ES-2 führte eine Depletion von HMGA2 zu einer Reduktion der Proliferationsfähigkeit, eine Depletion von LIN28B zu einer Reduktion der Potenziale zur Selbsterneuerung sowie zur *anoikis*-Resistenz und eine IGF2BP1-Depletion zu einer Kombination aller drei Resultate [47]. In derselben Zelllinie führte eine IGF2BP1-Überexpression durch Verhinderung eines let-7/miRISC-vermittelten Abbaus der mRNAs von HMGA2 und LIN28B zu einer erhöhten und eine Inaktivierung zu einer reduzierten Expression beider Faktoren, wodurch anzunehmen ist, dass IGF2BP1 in malignen Tumorzellen überlebensfördernde Eigenschaften von HMGA2 und LIN28B vermittelt [47].

Für den speziellen Fall der ALL mit ETV6/RUNX1-Translokation gibt es Hinweise für eine stabilisierende Wirkung von IGF2BP1 auf die ETV6/RUNX1-mRNA mit resultierender Expressionssteigerung des Fusionsproteins [191]. Eine IGF2BP1-Depletion in REH-Zellen führte zu einer Reduktion der Proliferations- und einer Steigerung der Apoptoserate [182].

2. Apoptose & anoikis Resistenz: Wie bereits häufig in Tumor-Zellkulturen gezeigt, scheint IGF2BP1 nicht nur aufgrund des vom ihm ausgehenden Proliferationsvorteils, sondern auch aufgrund einer Unterbindung der Apoptose vorteilhaft für das Wachstumsverhalten von Tumorzellen zu sein [42, 45, 52, 136, 147, 148, 182]. Ein Anhaltspunkt für eine Hemmung der Apoptose durch IGF2BP1 ergibt sich durch seine stabilisierende Wirkung auf die mRNA der Proteine βTrCP1 (Genprodukt von BTRC) [42] und cIAP1 (*cellular inhibitor of apoptosis protein 1*; Genprodukt von BIRC2) [52], die jeweils einen aktivierenden Einfluss auf den NF-κB(*nuclear factor kappa-light-chain-enhancer of activated B cells*)-Signalweg haben und der seinerseits im aktiven Status Überleben, Proliferation und Inhibition von Apoptose in Tumorzellen steuert (*Übersichtsartikel* in [192]). Als substraterkennende Untereinheit der im Abschn. 1.4, (S. 7) beschriebenen Ubiquitin-Ligase fördert βTrCP1 den Abbau von Inhibitoren des NF-κB, was in der Kolonkarzinom-Zelllinie

HCT 116 [42, 45] und der Melanom-Zelllinie 1241 mel [148] einen Schutz vor Apoptose herbeiführte. In einer weiteren Untersuchung konnte gezeigt werden, dass IGF2BP1 die IRES-abhängige Translation des Apoptoseinhibitors cIAP1 (Abschn. 1.3, S. 2) verstärkt [52]. Das Protein cIAP1 erreicht seine antiapoptotische Wirkung durch Inhibition von Caspasen (*Übersichtsartikel* in [193]). Die Rhabdomyosarkom-Zelllinie RH36 konnte durch eine IGF2BP1-Depletion und daraus resultierender, cIAP1-vermittelter Reduktion der Effektor-Caspasen-Aktivität auf ein durch TNFα-provoziertes Apoptosesignal sensibilisiert werden [52]. Darüber hinaus führte eine IGF2BP1-Depletion in der Kolonkarzinom-Zelllinie SW-480 zu einer gesteigerten Expression des proapoptotischen Faktors CYFIP2 (*cytoplasmic FMR1 interacting protein 2*) und zu einer daraus resultierenden gesteigerten Apoptoserate [136]. Ferner zeigte eine IGF2BP1-Depletion eine Reduktion der Fähigkeit zur *anoikis*-Resistenz in Zellkulturen unterschiedlichster Tumorentitäten [47, 48].

3. Chemoresistenz: Schließlich ist ein IGF2BP1-vermittelter Mechanismus zur Entwicklung einer Chemoresistenz von Tumor-Zelllinien demonstriert worden. IGF2BP1 hat eine stabilisierende Wirkung auf das Transkript des zellmembranständigen Substrattransporters MDR1 (*multidrug resistance protein 1*) [184], welcher in Tumorzellen häufig überexprimiert ist und als ABC(*ATP binding cassette*)-Transporter für das Ausschleusen von hydrophoben Substanzen, wie sie im Rahmen von Chemotherapien eingesetzt werden, verantwortlich ist [194]. In der Ovarialkarzinom-Zelllinie NCI/ADR-RES führte eine IGF2BP1-Überexpression zu einem erhöhten Expressionsniveau von MDR1 und unterdessen zu einer gesteigerten Chemoresistenz gegenüber Taxanen [140].

Zusammengenommen sprechen die bislang publizierten Studien für eine das Überleben, das Wachstum und die Teilung von Tumorzellen fördernde Wirkung von IGF2BP1. Dabei erlangt IGF2BP1 diese Möglichkeit über die Stabilisierung von Transkripten, deren Genprodukte wichtige Steuerelemente in der Regulation des Proliferations- und Apoptoseverhaltens sowie des Umganges mit toxischen Substanzen darstellen.

Zielstellung: Aufgrund der bereits gezeigten hochregulierten Expression von IGF2BP1 in zahlreichen humanen Neoplasien (Manuskript 1 [7], Tab. 2 und 3, S. 53 und 54 und aktualisiert Tab. A1, S. 114) und der Erkenntnisse über den Einfluss von IGF2BP1 auf die Proliferation und Apoptose zahlreicher sehr unterschiedlicher Tumorzelllinien (Abschn. 1.6.4, S. 15), stellt sich die Frage nach einer vom Zelltypen und der Tumorentität unter Umständen unabhängigen wachstumsfördernden Funktion des IGF2BP1. Unterstützt wird diese Annahme durch die genannten regulatorischen Funktionen von IGF2BP1 hinsichtlich tumorassoziierter Faktoren *in vitro* und *in cellulo* (Abschn. 1.6.4, S. 15), als auch durch die gezeigte tumorigene Eigenschaft von IGF2BP1 im Mausmodell *in vivo* [178] (Abschn. 1.6.3, S. 15). Trotz vorhandener Einzelfallberichte mit nachweisbarer IGF2BP1-Expression [145, 146] sowie der Entdeckung von IGF2BP1-Autoantikörpern in betroffenen Patienten [145, 146, 156–158, 166, 171–174] (Tab. A1, S. 114 und Abschn. 1.6.1, S. 13) waren bis zum Zeitpunkt unserer Datenerhebung hinsichtlich des HCCs weder systematische Untersuchung über eine mutmaßlich erhöhte IGF2BP1-Expression noch funktionelle Untersuchungen verfügbar.

In der vorliegenden Arbeit sollte der Einfluss von IGF2BP1 auf die Proliferation und Apoptose von HCC-Zelllinien zunächst *in vitro* analysiert werden. Bei Identifizierung eines

für die Tumorzellen günstigen Einflusses sollte ferner das Wachstumsverhalten von subkutanen HCC *xenograft*-Tumoren in der thymusaplatischen Maus charakterisiert werden. Dafür sollte weiterhin die für ein *xenograft*-Experiment geeignetste HCC-Zelllinie ermittelt und möglichst optimale Bedingungen für ein quantitatives *monitoring* der Versuchstiere geschaffen werden. Da für die Aufrechterhaltung einer hinreichenden IGF2BP1-Depletion über den notwendigen Gesamtzeitraum der langwierigen *in vivo*-Experimente eine transiente Depletion von IGF2BP1 unter Umständen unzureichend wäre, sollte die Etablierung eines lentiviralen Gentransfer-Systems zur genomischen Integration einer gegen IGF2BP1 gerichteten shRNA angestrebt werden. Zu Beginn der Arbeiten waren weder das lentivirale Gentransfer-System, noch die subkutane Xenotransplantation von humanen Tumorzellen in thymusaplastische Mäuse, noch das *monitoring* am Institut erprobt (Thematik publiziert in Manuskript 2, S. 66 [79]).

1.6.5 IGF2BP1 als migrationsfördernder Faktor

Bezug zu Manuskript 3, S. 79 [185]

Im Zusammenhang mit der Transportfunktion der β-Aktin-mRNA und der dadurch bedingten lokalisierten Translation mit Akkumulation von Aktinmonomeren in spezialisierte Membranprotrusionen von Neuronen, Neuralleistenabkömmlingen und Fibroblasten (Abschn. 1.5.3, S. 11) kommt dem IGF2BP1 eine Schlüsselfunktion in Migrationsprozessen von Zellen und der Motilität spezialisierter Zellfortsetze im Embryo zu. Die im Rahmen dieser physiologischen Zellmigration ablaufenden und von IGF2BP1 abhängigen Prozesse lassen sich grundsätzlich in malignen Zellen wiederfinden. Auch wenn zunächst nicht paralogspezifisch gezeigt, verhelfen insbesondere IGF2BP1 und -3 verschiedenen Tumorzelllinien zu einer für Migrationszwecke geeigneten Oberflächendifferenzierung. So ist anzunehmen, dass IGF2BP1 die Zelladhäsion und die Anzahl von Invadopodien in der Zervixkarzinom-Zelllinie Hela fördert [38] und einen essentiellen Faktor für die Formation von Lamellipodien in der Kolonkarzinom-Zelllinie SW-480 darstellt [150]. Außerdem zeigte sich nach einer Depletion aller IGF2BPs eine Reduktion der Motilität der Blasenkarzinom-Zelllinie TSU-Pr1 [195]. Des Weiteren ist speziell IGF2BP1 wichtig für die Migration der Zervixkarzinom-Zelllinien Hela und C33A [90], der Osteosarkom-Zelllinien U2OS [44] und MG63 [89], der Nierenzellkarzinom-Zelllinie 786-O [87], der HCC-Zelllinien QGY-7703 und SMMC-7721 [84] und der Glioblastom-Zelllinien U87 und U373 [85] *in vitro* sowie für die Migration und Migrationsgeschwindigkeit der Ovarialkarzinom-Zelllinie ES-2 *in vitro* [44, 47, 48] und die Metastasierung *in vivo* [48]. Diese Phänotypen werden dabei nicht durch IGF2B1 direkt verursacht, sondern müssen in letzter Instanz von anderen Faktoren etabliert werden [150, 195]. Mit seiner Fähigkeit die Expression von mRNAs zu regulieren, nimmt IGF2BP1 als posttranskriptioneller Modifikator hierbei eine Vermittlerrolle ein. So ist in der Zelllinie Hela ein stabilisierender Einfluss von IGF2BP1 auf die mRNA des Hyaluronsäurerezeptors CD44 gezeigt worden [38], welcher seinerseits als wichtiger Faktor für die Migrationsvorgänge in neoplastischen und nicht-neoplastischen Zellen anzusehen ist (*Übersichtsartikel* in [196]). Der Einfluss von IGF2BP1 auf das Zusammenspiel der für eine Migration im Gewebe notwendigen Anpassungen der Zytoarchitektur durch dynamische

Umbauvorgänge des Zytoskelets (ACTB) (*Übersichtsartikel* in [197]) einerseits und der Interaktionen zwischen Zelloberfläche und Extrazellulärmatrix (CD44) (*Übersichtsartikel* in [196]) andererseits konnten erstmals durch Stöhr *et al.* (2012) [44] umfassend aufgelöst werden und in einen Zusammenhang mit übergeordneten Regelkreisen des Aktinmetabolismus gebracht werden (*Übersichtsartikel* in [198]). Diese Studie zeigte, dass IGF2BP1 in der Osteosarkom-Zelllinie U2OS die mRNA des Signalproteins MAPK4 bindet und ihre Translation inhibiert und umgekehrt die mRNA des Tumorsuppressors PTEN bindet, stabilisiert und ihre Translation steigert. Diese Effekte resultierten in einer Steigerung der Migrationsgeschwindigkeit und der Konstituierung einer Richtungspersistenz der Tumorzellbewegung (*Übersichtsartikel* in [198]). Die Reduktion der MAPK4-Expression führte zu einer reduzierten MK5(auch MAPKAPK5, *mitogen-activated protein kinase-activated protein kinase 5*)-abhängigen Phosphoaktivierung von HSPB1 (*heat shock protein family B [small] member 1*) und damit zu einer Mobilisierung von in der Regel sonst durch HSPB1 sequestrierten Aktinmonomeren (G-Aktin). Analog dazu führte die Translationssteigerung von PTEN ebenfalls zu einer Inhibition der sonst durch AKT1 (*KT serine/threonine kinase 1*) vorgenommenen Phosphoaktivierung von HSPB1. Damit entsteht bezüglich beider Faktoren ein synergistischer Effekt, welcher über eine erhöhte G-Aktin-Konzentration zu einer Dynamisierung der Polymerisation von filamentärem (F-)Aktin führte. Des Weiteren scheint IGF2BP1 durch die Translationssteigerung von PTEN die Aktivität von RAC1 (*Rac family small GTPase 1*) zu inhibieren, wodurch U2OS-Zellen eine polarisierte Architektur mit definierter Vorder- und Hinterkante erhalten und dadurch eine Richtungspersistenz der Migration etabliert wird [44]. Passend dazu konnten in davon unabhängigen Studien auch in der Mammakarzinom-Zelllinie MDA-MB-231 [199] und MTLn3 [200] eine Richtungspersistenz der Zellbewegungen und in der Zelllinien MTLn3 eine Polarisierung des Zellkörpers [200, 201] durch eine IGF2BP1-Überexpression bewirkt werden.

Betrachtet man die Situation im Organismus, so erwartet die Metastasierung maligner Tumorerkrankungen von einzelnen Zellen die Fähigkeit zur Migration in Gewebe und Invasion in anatomische Strukturen wie beispielsweise Blutgefäße und Nervenscheiden [202]. Dieses Phänomen wird allerdings auch stark von epithelialen Tumoren beansprucht [203, 204], obwohl ihre Ursprungszellen für gewöhnlich durch zahlreiche Zell-Zell-Kontakte und teilweise auch Zell-Matrix-Kontakte stark untereinander und mit der Basalmembran verzahnt sind und für gewöhnlich ihre Lokalisation oberhalb der Basalmembran nicht verlassen [205]. Schlussfolgernd kann für die dennoch häufig stattfindende Metastasierung aus einer solchen Tumormasse angenommen werden, dass die Transformation einer epithelialen Zelle in eine maligne Tumorzelle Änderungen umfassen muss, die zu einem Verlust ihrer epithelialen Eigenschaften führen. Dieser Prozess wird mithilfe des nicht unumstrittenen [206] Modells der epitheliomesenchymalen Transition (EMT) erklärt (*Erstbeschreibung* in [207]), welches besagt, dass epitheloide Zellen zumindest für den Zeitraum der Metastasierung Eigeschafften mesenchymaler Zellen annehmen müssen [208] (EMT-Typ 3 [209]). Tatsächlich konnte in solchen aus Epithelien hervorgegangenen Zellen sowohl eine Nutzung bestimmter Zellsignalwege, ein Expressionsprofil einer Reihe von sogenannten EMT-Marker als auch eine für Migrationszwecke geeignete Zellarchitektur charakterisiert wer-

den. Dazu zählen eine aktivierte TGFβ(*transforming growth factor beta*)-Signalkaskade, die Expression von Transkriptionsfaktoren wie SNAI1, -2 (*snail family transcriptional repressor 1 & -2*), TWIST1, -2 (*twist family bHLH transcription factor 1 & -2*), ZEB1, -2 (*zinc finger E-box binding homeobox 1 & -2*) und LEF1 (*lymphoid enhancer binding factor 1*), die Repression von posttranskriptionellen Regulatoren wie die miRNA-Familie miR-200, die Expression von typischen Oberflächenproteinen wie CD44 sowie Veränderungen und Umstrukturierungen von zytoskelettalen Elementen und Komponenten von Zellkontakten wie β-Aktin, epitheliales (E-)Cadherin und β-Catenin [210].

Zielstellung: Aufgrund der Erkenntnisse über den Einfluss von IGF2BP1 auf die Migrationsfähigkeit von Tumorzelllinien *in vitro* [44, 47, 48, 195] im Zusammenhang mit seiner regulatorischen Funktion der mRNAs von Schlüsselfaktoren der EMT wie β-Aktin [22, 29] (und Abschn. 1.5.3, S. 11), β-Catenin [42, 71] (und Abschn. 1.4, S. 7) und CD44 [38], stellt sich die Frage nach einer Funktion von IGF2BP1 in Metastasierungsprozessen. Diese Annahme wird durch die Erkenntnisse über eine hochregulierte Expression von IGF2BP1 in malignen epithelialen Tumoren (Manuskript 1 [7], Tab. 2 und 3, S. 53 und 54 und aktualisiert Tab. A1, S. 114 und Abschn. 1.6.1, S. 13) sowie der Korrelation der IGF2BP1-Expression mit der Metastasierung von epithelialen Tumoren im Menschen [150] (Abschn. 1.6.1, S. 13) und im experimentellen Mausmodell [178, 180] (Abschn. 1.6.3, S. 15) unterstützt. Trotz dieser Faktenlage waren bis zum Zeitpunkt unserer Datenerhebung keine Untersuchungen bezüglich einer Funktion von IGF2BP1 im Zusammenhang mit den im Theorem der EMT konzeptuierten Vorgänge verfügbar.

In der vorliegenden Arbeit sollte der Einfluss von IGF2BP1 auf entsprechende morphologische Veränderungen in humanen Tumorzelllinien sowie möglicherweise damit verbundene regulatorische Effekte von IGF2BP1 auf EMT-Marker untersucht werden. Da hierfür unter Umständen transiente Funktionsgewinn- und -verlust-Experimente unzureichend wären, sollten diese durch eine stabile Überexpression beziehungsweise durch eine stabile Depletion von IGF2BP1 mithilfe des im Rahmen dieser Arbeit etablierten lentiviralen Gentransfersystems in einer Reihe von Zelllinien realisiert werden (Thematik publiziert in Manuskript 3, S. 79 [185]).

2 Diskussion

Die Karzinogenese ist als mehrstufiger Prozess zu verstehen, der mit einer Akkumulation genetischer Alterationen einer Ursprungszelle beginnt und in ein von dieser Zelle ausgehendes, expansives Wachstumsverhalten einer Tumormasse mit der Möglichkeit der Metastasierung übergeht. Für das Gelingen und vor allem das Überleben dieses Vorgangs müssen Tumorzellen jedoch eine Reihe von Fähigkeiten akquirieren, die normalerweise nicht zum Wesen somatischer Zellen gehören. So müssen sie den Status der Immortalität und ein autonomes Proliferationsverhalten sowie die Fähigkeit zur Selbsterneuerung und zur Immunevasion erlangen [211]. Ereignisse, die zu einer Manifestation dieses aggressiven Verhaltens führen, werden nach heutigem Verständnis unter anderem auf besonders ungünstige somatische Mutationen im Genom mit anschließender Dysfunktion betroffener Genprodukte zurückgeführt [212]. Eine Mutation des IGF2BP1-Gens im Sinne einer klassischen Funktionsverlust- oder -gewinn-Mutation eines Tumorsupressor- oder Onkogens mit tumortreibenden Eigenschaften schein nach Maßgabe der aktuellen Literatur nicht der im Vordergrund stehende molekulare Pathogenesemechanismus in der Entstehung von IGF2BP1-positiven Neoplasien wie dem HCC zu sein [79] (Abschn. 1.6.2, S. 14).

Außer den Veränderungen auf genomischer Ebene treten in malignen Zellen auch transkriptionelle Regulationsstörungen mit Veränderungen des Transkriptoms auf [213]. Die daraus resultierende veränderte Genexpression von auch nicht-mutierten Faktoren kann für Tumorzellen vorteilhafte Auswirkungen mit sich bringen [212]. Es ist nicht verwunderlich, dass während dieses Prozesses gerade die Expression von RBPs verändert ist [11] und für Tumorzellen vorteilhaft sein kann [12, 214, 215]. Die mit einer Re-/expression von IGF2BP1 unmittelbar verbundenen Alterationen der Genexpression seiner Zieltranskripte und die damit verbundene Modulation der Tumorzelleigenschaften scheinen vielmehr als krankheitsauslösendes Moment in Frage zu kommen.

2.1 Die Bedeutung von IGF2BP1 für das Wachstumspotential maligner Neoplasien

In Gutschner *et al.* (2014) [79] konnten wir erstmals durch eine lentiviral erzeugte shRNA-vermittelte Depletion von IGF2BP1 sowohl eine Hemmung der Proliferation als auch eine Steigerung der Apoptose von sechs HCC-Zelllinien *in cellulo* als auch eine Reduktion des Tumorwachstums von Hep G2-*xenograft*-Tumoren in der Maus *in vivo* zeigen. Da eine IGF2BP1-Depletion in den subkutanen *Xenografts* offenbar nur zu einer Wachstumshemmung sowie zu einer Persistenz von Mikrotumoren und nicht etwa zu einer Rückbildung des Tumors führte, scheint IGF2BP1 im HCC eher eine Rolle als Progressionsfaktor denn als Initiationsfaktor einzunehmen. Seine Überexpression ist damit vielmehr als sekundäres Ereignis in der Hepatokarzinogenese zu verstehen. Bezüglich IGF2BP1 stellte unser Mausmodell dabei neben dem transgenen Mausmodell aus Tessier *et al.* (2004) [178] das zweitbeschriebene Experiment *in vivo* und das erstbeschriebene Funktionsverlustexperiment *in vivo* dar. Nahezu zeitgleich mit der Veröf-

fentlichung unserer Daten stellten HAMILTON *et al.* (2013) [180] Tierversuche mit dem übereinstimmenden Ergebnis einer das Tumorwachstum fördernden Rolle von IGF2BP1 in Mausmodellen zum kolorektalen Karzinom vor. So zeigten mittels Kolonkarzinom-Zelllinien erzeugte subkutane *Xenografts* bei IGF2BP1-Überexpression ein vergrößertes Tumorvolumen (Zelllinien SW-480 und LoVo). Zusätzlich konnte in einem genetischen Mausmodell bei spezifischem IGF2BP1-Knockout in den Schleimhautepithelzellen einer Kolonkarzinom-entwickelnden APC$^{(\mathrm{Min}/+)}$(*adenomatous polyposis coli*)-Maus durch serielle Kreuzung mit einer IGF2BP1-LoxP-Maus und einer Villin-Cre-Maus eine Reduktion der sich entwickelnden Kolonkarzinome von 66 % beobachtet werden [180]. In jüngster Vergangenheit konnte unsere Arbeitsgruppe in MÜLLER *et al.* (2018) [48] unter Einsatz der im Rahmen der vorliegenden Arbeit etablierten Methodik des lentiviralen Gentransfers und der Fluoreszenzbildgebung nach Depletion von IGF2BP1 eine Reduktion des Tumorwachstums von ES-2-*Xenografts* in thymusaplastischen Mäusen beobachten und somit die Ergebnisse aus den Mausversuchen mit der HCC-Zelllinie Hep G2 in einem unabhängigen Zellsystem rekapitulieren. Dieser proliferationsfördernde [41–43, 47, 48, 52, 75, 80, 84–87, 89, 95, 136, 138, 142, 143, 147, 148, 179–183] und antiapoptotische [42, 45, 52, 85, 86, 89, 136, 147, 148, 182] Einfluss von IGF2BP1 konnte bereits in zahlreichen Tumor-Zelllinien unterschiedlicher Herkunft gezeigt werden (Abschn. 1.6.4, S. 15). Es gibt jedoch auch einzelne gegensätzliche Berichte, die vor dem Hintergrund eines möglichen *publication bias* eine besondere Betrachtung verdienen. So zeigte sich nach IGF2BP1-Depletion eine Steigerung der Proliferationsrate der Leukämie-Zelllinie K-562 [216] und nach IGF2BP1-Überexpression eine Reduktion der Proliferationsrate der Mammakarzinom-Zelllinie MTLn3 aus *Rattus norvegicus* [72]. Zunächst ist festzuhalten, dass nicht jeder Tumor und nicht jede Tumor-Zelllinie (Manuskript 1 [7], Abb. 3b, S. 52 und Abschn. 1.6.1, S. 13) im gleichen Maße IGF2BP1 exprimiert. Bezüglich der oben genannten Zelllinie MTLn3 ist zu sagen, dass IGF2BP1 in dieser [72] und in anderen Mammakarzinom-Zelllinien des Menschen [83] sowohl auf Protein- als auch auf mRNA-Ebene nur zu geringen Mengen exprimiert, bis nicht detektierbar zu sein scheint. Diese Tatsache weist darauf hin, dass Mammakarzinome zur Aufrechterhaltung ihres Wachstums durchaus Mechanismen verwenden können, die von IGF2BP1 unabhängig sind. Vor dem Hintergrund der hohen genetischen und epigenetischen Heterogenität von Tumorzellen unterschiedlicher Herkunft und der damit verbundenen Diversität der von Tumorzellen zur Etablierung ihrer proliferativen Leistung verwendeten Signalwege [217, 218] und anderen Mechanismen [211] ist es nicht verwunderlich, dass nicht alle malignen Zellen auf IGF2BP1 angewiesen sind. Auch im Falle einer IGF2BP1-Positivität wie bei der oben genannten Leukämie-Zelllinie K-562 muss das Proliferationsverhalten nicht unmittelbar mit IGF2BP1 verknüpft sein. Beispielsweise fördert IGF2BP1 die Proliferation einer Reihe von Pankreaskarzinom-Zelllinien nur in Anwesenheit der Histon-Deacetylase SIRT6 (*sirtuin 6*) [181], welche eine wichtige Funktion für die genomische Stabilität und die DNA(*deoxyribonucleic acid*)-Reparatur erfüllt [219]. Damit wird deutlich, dass durch eine Depletion oder Überexpression von IGF2BP1 in den meisten bisher untersuchten, aber nicht in allen Tumorzellen unweigerlich ähnliche Effekte erwartet werden können. Die Proliferationsfördernde Wirkung von IGF2BP1 muss sich

scheinbar erst bei günstiger Konstellation des im speziellen Fall gegenwärtigen Transkriptoms entfalten können. In unseren Untersuchungen konnten wir zeigen, dass IGF2BP1 in Hep G2-Zellen die mRNA des Protoonkogens MYC durch direkte Bindung stabilisiert und daraus eine Translationssteigerung von MYC resultiert. MYC ist für seine wachstums- und proliferationsfördernden Wirkungen in malignen Tumorzellen gut bekannt (*Übersichtsartikel* in [220, 221]), und eine Stabilisierung seiner mRNA durch IGF2BP1 [27, 39–44, 76, 136, 184, 185] mit anschließender expressionsfördernder Wirkung [41, 43, 44, 74, 95, 136, 143, 148] wurde bereits in zahlreichen Tumorzelllinien aus unterschiedlichsten Entitäten gezeigt. Jedoch konnte der stabilisierende Einfluss von IGF2BP1 auf die mRNA von MYC nicht in allen von uns getesteten HCC-Zelllinien (Daten nicht gezeigt) und auch nicht in allen in der Literatur zu findenden Beschreibungen nachgewiesen werden. So kam es nach IGF2BP1-Depletion in der ALL-Zelllinie REH [182] und in Hela-Zellen [38] nicht zur erwarteten Reduktion der MYC-Expression. Hierbei sind verschiedene Erklärungen denkbar. Zum einen kann angenommen werden, dass das Maß der IGF2BP1-Depletion in diesen Zelllinien für eine Reduktion der MYC-Expression unzureichend war, so wie es die Autoren im Falle der REH-Zellen selbst vermutet haben [182, 191]. Zum anderen wird die Expression und die Stabilität der MYC-mRNA und des Proteins durch sehr viele Faktoren und auf unterschiedlichen Ebenen reguliert [222, 223], sodass die MYC-Stabilität nicht in jeder Zelllinie von IGF2BP1 abhängig sein muss. Darüber hinaus führten gut charakterisierte MYC-Mutationen in unterschiedlichen Lymphom-Entitäten zu einer erhöhten Stabilität des Proteins [224]. Eine MYC-Mutation wurde in den oben genannten Zelllinien nicht ausgeschlossen. In allen diesen Fällen würde die Stabilität der MYC-mRNA nicht exklusiv von IGF2BP1 abhängen und die Veränderung der Proteinexpression nach Funktionsverlust- oder -gewinn-Experimenten unter Umständen nicht mehr messbar sein. Weiterhin ist auch naheliegend, dass bei Abwesenheit von IGF2BP1 das sehr ähnliche Paralogon IGF2BP3 (Abschn. 1.2, S. 2) die entstehende Instabilität der MYC-mRNA kompensiert. Wie bereits gezeigt wurde, kann auch IGF2BP3 die MYC-mRNA binden [20], und eine IGF2BP3-Depletion führte in der murinen pre-B-ALL-Zelllinie 70Z/3 zu einer reduzierten MYC-Expression [225].

Wir konnten in unseren Untersuchungen auch zeigen, dass IGF2BP1 in der HCC-Zelllinie Hep G2 neben der mRNA von MYC auch die des Proliferationsmarkers MKI67 (*marker of proliferation ki-67*) direkt bindet und stabilisiert. Das Genprodukt Ki-67 findet als Proliferationsmarker breite Verwendung in der immunhistochemischen Diagnostik von Tumorerkrankungen und korreliert in zahlreichen malignen Neoplasien (*Übersichtsartikel* in [226, 227]) sowie im HCC mit einem fortgeschrittenen Tumorstadium und einer unvorteilhaften Prognose (*Metaanalyse* in [228]). Die Minderung der Expression des Genprodukts Ki-67 durch *antisense*-Oligonukleotide führte in verschiedenen Tumor-Zelllinien sowie in subkutanen und orthotopen *xenograft*-Experimenten zu einer Proliferationshemmung *ex vivo* [229–234] und einer Reduktion der Tumormasse *in vivo* [231] und damit im Vergleich zur IGF2BP1-Depletion gleichlautenden Ergebnissen in Zellkultur und *xenograft*-Experimenten aus unseren Versuchen. Folglich ist anzunehmen, dass IGF2BP1 die Proliferation von HCC-Zelllinien durch Unterstützung des onkogenen Einflusses von MYC

und Ki-67 fördert.

Wie in unserem Übersichtsartikel Köhn *et al.* (2013) [69] (Abschn. 1.3, S. 2) herausgearbeitet, könnte sich aufgrund des scheinbar von IGF2BP1 abhängigen Funktionserhalts der yRNPs eine für Tumorzellen vorteilhafte Situation ergeben. Eine Überexpression der Y RNAs ist bereits in humanen Tumorzelllinien [62, 70] und in einer Vielzahl maligner humaner Tumoren beschrieben [70]. Da Y3 eine wichtige Funktion in der Initiation der Replikation von Hela-Zellen [235], der Gallenblasenkarzinom-Zelllinie EJ-30 [236, 237] und in der Fibroblasten-Zelllinie NIH 3T3 [235] *in vitro* zu haben scheint, führte ihre Depletion in Hela-Zellen [68] und in der Zelllinie XL177 aus *Xenopus laevis* [238] schlüssiger Weise zu einem Zellzyklusarrest und damit zu einer verminderten Proliferationsrate *ex vivo* [70] sowie zu einem letalen Phenotypen in *Xenopus laevis* [238] *in vivo* (*Übersichtsartikel* in [239]). Aufgrund der offenbar von IGF2BP1 abhängigen subzellulären Lokalisation von Y3 [24] ergibt sich mutmaßlich eine weitere Möglichkeit für IGF2BP1, proliferationssteigernde Wirkungen eines seiner Zieltranskripte zu fördern.

2.2 Die Bedeutung von IGF2BP1 für das Metastasierungspotenzial maligner Neoplasien

Es gibt nicht nur eine genomische, sondern auch eine das Transkriptom betreffende inter- und intratumorale Heterogenität maligner Neoplasien [213]. Was die Zieltranskripte von IGF2BP1 angeht, kann das Protein in unterschiedlichen Tumorzellen demnach verschiedene Situationen antreffen. Um ein vollständiges Bild aller von IGF2BP1 regulierten Transkripte zu erlangen, wurden nach möglichst minimaler Manipulation von HEK-293T-Zellen sowohl die Komposition von IGF2BP1-m-/RNPs [26] als auch die direkt gebundenen Transkripte [31] analysiert. Dabei stellte sich heraus, dass sich innerhalb der IGF2BP1-mRNPs etwa 3 % des HEK-293T-Transkriptoms befinden [26] und IGF2BP1 über 1000 verschiedene Transkripte anhand eines distinkten Sequenzmotivs binden kann [31]. Die Genprodukte dieser Transkripte konnten in Beziehung zu vielen unterschiedlichen zellulären Prozessen gebracht werden und sind nicht etwa einer bestimmten zellulären Funktion zuzuordnen gewesen. Allerdings ist zu beachten, dass es sich bei HEK-293T-Zellen um embryonale Zellen mit unklarer Gewebeherkunft und mit schweren genomischen Alterationen sowie stark dereguliertem Transkriptom und damit um ein sehr artefizielles Zellsystem handelt [240]. Ferner ist eine Bindung allein kein hinreichender Beweis für eine tatsächliche funktionelle Wertigkeit dieser Interaktion [241]. In einer späteren Studie zeigten Strukturanalysen von an IGF2BP1 gebundener β-Aktin-mRNA ein RNA-Sequenzmotiv auf, welches eine Vorhersage von nur etwa 100 IGF2BP1-Zieltranskripten *in silico* definierte [32]. Die Zieltranskripte konnten dann in einem weitaus physiologischeren Kontext an murinen embryonalen Hirnlysaten verifiziert werden, und ihre Genprodukte ließen sich gut in funktionelle Gruppen kategorisieren, die auf eine wichtige Rolle des Proteins in der Ontogenese hinwiesen [32]. Im Kontext von Tumorzelllinien zeichnete sich bei Anwendung von Funktionsverlust- und -gewinn-Experimenten ein teilweise besser kategorisierbares Muster der durch IGF2BP1 regulierten Transkripte ab. In U2OS-Zellen wurden nach IGF2BP1-

Depletion und Anwendung von zytotoxischem Stress etwa 70 durch IGF2BP1 regulierte Zieltranskripte identifiziert, die sich in ihrer Gesamtheit wiederum nicht eindeutig einer bestimmten zellulären Eigenschafft zuordnen ließen [44]. In Hela-Zellen führte dann eine IGF2BP1-Depletion aber zu einer Reduktion von nur fünf und zu einer Steigerung der Expression von nur 17 Transkripten. Unter den zuletzt genannten Transkripten sind 11 Transkripte für Funktionen im Zusammenhang mit Adhäsion, Motilität, Invasion und der Extrazellulärmatrix beschrieben [38]. Übereinstimmend scheint IGF2BP1 in MTLn3-Zellen [72, 242] und in ES-2-Zellen (Ovarialkarzinom) [48] mit Faktoren zu assoziieren, die eine Rolle für Motilität und Adhäsion oder für Migration spielen. Die Annahme, dass IGF2BP1 in unterschiedlichen Tumorentitäten und unter bestimmten exogenen Konditionen Tumorzellen auf unterschiedliche Art und Weise beeinflussen kann, ist also naheliegend. Es ist folglich nicht verwunderlich, dass die Vielzahl der durch IGF2BP1 regulierten Transkripte dem Protein neben seinem Einfluss auf das Proliferations- und Apoptoseverhalten von Tumorzellen auch eine Einflussnahme auf andere Tumorzelleigenschaften, wie das Migrationsverhalten, ermöglicht.

In Zirkel *et al.* (2013) [185] konnten wir zeigen, dass IGF2BP1 beinahe ausschließlich in Tumorzelllinien mit mesenchymalen Expressionsprofil koexprimiert war und in diesen Zellen für die Aufrechterhaltung der mesenchymalen Eigenschaften und ihr Migrationsverhalten mitverantwortlich war. Entsprechend führte eine IGF2BP1-Depletion in Tumorzelllinien mit mesenchymalen Expressionsprofil zu morphologischen Änderungen, die eher dem Phänotypen epithelialer Zellen entsprachen. Zunächst zeigte sich bei IGF2BP1-Depletion in humanen transformierten embryonalen HEK-293A-Zellen eine Zunahme von β-Catenin und E-Cadherin beinhaltenden Zell-Zell-Kontakten, eine Akzentuierung des subplasmalemmal lokalisierten F-Aktins sowie eine Abflachung der Zellkörper bei gleichbleibendem Zellvolumen. Dieser Phänotyp konnte in Grundzügen in Tumorzelllinien mit mesenchymalen Expressionsprofil, wie den Melanom-Zelllinien HT-144 und 1F6 sowie der Ovarialkarzinom-Zelllinie ES-2, rekapituliert werden. Ferner konnten wir zeigen, dass das Migrationspotenzial von HT-144-Zellen durch eine stabile lentiviral erzeugte, shRNA-vermittelte Depletion von IGF2BP1 beeinträchtigt wurde. Auf molekularbiologischer Ebene fanden wir eine mögliche Erklärung für diese zytomorphologischen Änderungen und für den Migrationsphänotypen in der Identifizierung eines expressionsfördernden Einfluss von IGF2BP1 auf die Transkriptionsfaktoren LEF1 und SNAI2 in allen oben genannten Zelllinien. Vor allem SNAI2, aber auch LEF1 sind für ihre Aktivität als Transkriptionsfaktoren im Rahmen der EMT gut bekannt [210]. Folglich konnten wir die durch eine IGF2BP1 verursachte Beeinträchtigung der Migration von HT-144-Zellen durch eine LEF1- oder SNAI2-Expression rekonstituieren. Wir konnten weiterhin spezifizieren, dass IGF2BP1 die LEF1-mRNA durch direkte Bindung stabilisiert und daraus eine Steigerung der LEF1-Expression in allen oben genannten Zelllinien resultierte. LEF1 bildet eine Endstrecke des Wnt-Signalwegs und steigerte durch Bindung an den Promotor die Expression der für eine Zellmigration wichtigen extrazellulären Matrix-Komponente Fibronectin (FN1) in ebenfalls allen oben genannten Zelllinien sowie in U2OS-Zellen. Beachtenswert ist in diesem Zusammenhang, dass MSCs des Colons physiologischerweise IGF2BP1 expri-

mieren und im Rahmen einer *reparatio* aus den benachbarten Regionen *per migrationem* in das betroffene Gewebe einwandern [107, 243] (Abschn. 1.5.4, S. 12). Damit kommt eine generelle Funktion von IGF2BP1 in Migrationsprozessen auch im adulten Organismus in Frage und kann als Phänomen einer physiologischen EMT (EMT-Typ 2 [209]) verstanden werden. Die Regulation von SNAI2 scheint in unseren Untersuchungen jedoch ohne direkte Interaktion von IGF2BP1 mit der SNAI2-mRNA abzulaufen. Allerdings konnten wir in IGF2BP1-negativen Zelllinien mit epithelialem Expressionsprofil wie MCF-7 und der spontan immortalisierten Nierenzelllinie MDCK im Umkehrschluss auch durch eine stabile Expression von IGF2BP1 keinen mesenchymalen Phänotypen und kein mesenchymales Expressionsprofil etablieren. Daraus kann geschlussfolgert werden, dass IGF2BP1 nicht das Potenzial hat, selbst eine EMT zu initiieren, sondern vielmehr die Fähigkeit besitzt bei bereits erfolgter EMT die Expression und damit den Einfluss von für diesen Prozess wichtigen Transkriptionsfaktoren auf posttranskriptioneller Ebene zu unterstützen. Unsere Ergebnisse stehen damit im Einklang mit den bereits dargestellten Funktionen von IGF2BP1 in Bezug auf Zellmigration im physiologischen (Abschn. 1.6.5, S. 18) und im pathologischen (Abschn. 1.5.3, S. 11) Kontext. Die „Umprogrammierung“ des Expressionsprofils von Zellen eines epithelialen Malignoms im Sinne einer EMT ist zunächst als ein initial notwendiger Prozess für Migrationsvorgänge anzusehen und gilt als weitreichend akzeptierte Modellvorstellung für Metastasierungsereignisse im Menschen [202] (Abschn. 1.6.5, S. 18). Da IGF2BP1 laut den hier dargestellten Ergebnissen mesenchymale Zelleigenschaften unterhält und ein migrationsfördernder Faktor zu sein scheint, stellt sich die Frage, ob IGF2BP1 eventuell auch ein wichtiger Faktor für Metastasierungsvorgänge ist.

Was das Migrationsverhalten, aber nicht die genannten morphologischen Änderungen angeht, finden sich in der Literatur bezüglich IGF2BP1 auch zu unseren Ergebnissen im Widerspruch stehende Berichte. Sämtliche dieser Studien wurden dabei von der Arbeitsgruppe Singer (*Albert Einstein College of Medicine*, New York) veröffentlicht. In murinen Mammakarzinom-Zelllinien scheint IGF2BP1 in Zellen, die ein geringes Metastasierungs- oder Invasionspotenzial (MTC) haben, höher exprimiert zu sein als in Zellen, die ein höheres Metastasierungspotenzial (MTLn3) aufweisen [72, 200, 201, 242]. Des Weiteren ist die durch EGF (*epidermal growth factor*) stimulierte Chemotaxis von normalerweise IGF2BP1-negativen MTLn3-Zellen bei IGF2BP1-Überexpression *in vitro* und *in vivo* reduziert [200, 201]. Dem stehen neben unseren Ergebnissen aus HT-144-Zellen (Melanom) Ergebnisse aus U2OS- (Osteosarkom), ES-2- (Ovarialkarzinom) [44, 47, 48] und TSU-Pr1-Zellen (Blasenkarzinom) [195] gegenüber, die einen positiven Einfluss von IGF2BP1 auf die Motilität von Tumorzellen zeigen. Weiterhin konnte gezeigt werden, dass die von durch ebenfalls mit MTLn3-Zellen erzeugten orthotopen *Xenografts* in der Maus ausgehenden Lungenmetastasen bei IGF2BP1-Überexpression seltener waren und dass die Anzahl der zirkulierenden Tumorzellen im Blut reduziert war [201, 244]. Diskrepant dazu ist jedoch, dass die in Tessier *et al.* (2004) [178] gezeigte transgene Expression von IGF2BP1 in der Brustdrüse von Mäusen zur Entwicklung metastasierender Mammakarzinome führte. Ferner wiesen Mäuse mit einem durch IGF2BP1-depletierten SW480-Zellen (Kolonkarzinom) erzeugten *Xenografts* aus den bereits oben genannten Untersuchungen

von HAMILTON *et al.* (2013) [180] eine reduzierte Anzahl von zirkulierenden Tumorzellen im Blut auf. Ergänzend konnte unsere Arbeitsgruppe in neuen Untersuchungen schließlich zeigen, dass eine IGF2BP1-Depletion in *xenograft*-Experimenten mit der Ovarialkarzinom-Zelllinie ES-2 zu einer Reduktion von Metastasen in thymusaplastischen Mäusen *in vivo* führte [48]. Als Ursache für den negativen Effekt von IGF2BP1 auf die Migration von Mammakarzinom-Zelllinien postulierten die Autoren um SINGER eine durch IGF2BP1 induzierte lokalisierte Translation der β-Aktin-mRNA, die zur Etablierung einer Zellpolarität führte und damit die Fähigkeit der Zellen zur flexiblen Bewegung reduzierte [201, 244]. So waren diese Zellen bei Änderungen eines Gradienten von Chemoatraktanzien nicht in der Lage, eine effektive Änderung der Migration in entgegengesetzte Richtung durchzuführen [200]. Zu beachten ist aber, dass sowohl durch F-Aktin aufgebaute zytoskelettale Stressfasern als auch eine durch ein Lamellipodium definierte Vorder- und eine gegenüberliegende Hinterkante eines polarisierten Zellkörpers Charakteristika migrierender neoplastischer [245] und nicht-neoplastischer Zellen [246] sind. Die Konstituierung einer Polarität ist somit nicht Hindernis, sondern Erfordernis für Migrationsvorgänge. Der positive Einfluss von IGF2BP1 zur Realisierung einer solchen Zellarchitektur wurde bereits in unterschiedlichen Zellsystemen demonstriert [44, 199, 201].

Nicht nur bezüglich muriner, sondern auch humaner Mammakarzinom-Zelllinien liegen gleichlautende Studienergebnisse vor. In der IGF2BP1-exprimierenden humanen Mammakarzinom-Zelllinie T47D führte eine IGF2BP1-Depletion zu einer erhöhten Migrationsfähigkeit der Zellen [72, 199] und zu einer Reduktion der Migration bei Rekonstitution der IGF2BP1-Expression [72]. Entsprechend führte eine IGF2BP1-Expression in der IGF2BP1-negativen humanen Mammakarzinom-Zelllinie MDA-MB-231 zu einer Reduktion der Migration [72]. Zudem konnte für beide Zelllinien gezeigt werden, dass IGF2BP1 die Halbwertszeit von Zell-Matrix-Kontakten erhöht [201]. Allerdings ist aus einer unabhängigen Arbeit mit unabhängiger Fragestellung ersichtlich, dass T47D-Zellen äußerst wenig bis gar kein und dass MDA-MB-231-Zellen durchaus IGF2BP1 exprimieren [83], womit die Grundvoraussetzungen dieser Experimente offenbar nicht optimal gegeben waren.

Es wird auffällig, dass in allen in der Literatur beschriebenen Experimenten, die einen negativen Einfluss von IGF2BP1 auf das Migrationspotenzial von Tumorzellen zeigen, Tumorzelllinien aus Mammakarzinomen verwendet wurden. Zudem wurde in vielen dieser Experimente mit Zelllinien gearbeitet, die aus Mammakarzinomen der Ratte isoliert wurden (MTC und MTLn3), und für die Funktionsgewinn-Experimente wurde häufig das orthologe und deshalb nicht mit IGF2BP1 identische ZBP1 aus *Gallus gallus* verwendet. Im Gegensatz dazu zeigten wir die phänotypischen Änderungen und die Induktion eines mesenchymalen Expressionsprofils durch IGF2BP1 in einer Reihe sehr unterschiedlicher Tumor-Zelllinien, wodurch ein weitestgehend vom Zellsystem unabhängiger Effekt von IGF2BP1 anzunehmen ist. Bezüglich des Mammakarzinoms scheinen sich die Ergebnisse aus den genannten Zellkultur- und Tierexperimenten mit der Situation in Tumorerkrankungen im Menschen nur teilweise zu decken. Auch wenn nicht in jedem Fall im gleichen Maße wie im jeweiligen Primärtumor, so war IGF2BP1 in allen getesteten Metastasen ebenfalls exprimiert [72]. Im Gegensatz dazu ist die IGF2BP1-Expression in Metastasen

des kolorektalen Karzinoms stärker als im *primarius* und korreliert positiv mit der Anzahl der lymphonodalen Metastasen [150]. Hinsichtlich der Migration scheint IGF2BP1 damit speziell im Kontext von Mammakarzinom-Zelllinien ein im Vergleich mit den anderen genannten Tumorzelllinien aus anderen Entitäten gegensätzliches Verhalten zu begünstigen.

Unsere Ergebnisse belegen die Notwendigkeit des Faktors IGF2BP1 für die Aufrechterhaltung eines für die Zellmigration notwendigen mesenchymalen Expressionsprofils, welches durch die Regulation von Transkriptionsfaktoren der EMT auf posttranskriptioneller Ebene erreicht wird, und rücken das Protein in den Verdacht, eine wichtige Rolle in Metastasierungsprozessen einzunehmen.

2.3 IGF2BP1 im klinischen Kontext

Aus den Erkenntnissen, dass IGF2BP1 in neoplastischen Prozessen des Menschen reexprimiert wird und in diesen Neoplasien sehr wahrscheinlich eine wachstums- und migrationsfördernde Wirkung ausübt, ergeben sich klinisch nutzbare Situationen:

1. Diagnostik: Aus dem Expressionsprofil von IGF2BP1 in den neoplastischen Erkrankungen des Menschen (Manuskript 1 [7], Tab. 2 und 3, S. 53 und 54 und aktualisiert Tab. A1, S. 114 und Abschn. 1.6.1, S. 13) kann geschlussfolgert werden, dass die histologische Analyse einer IGF2BP1-Expression im neoplastischen Gewebe weder einen Aussagewert bezüglich der Dignität des Tumors hat, noch kann eine Positivität allein bei bekannter Dignität zur Identifizierung einer speziellen Tumorentität herangezogen werden. Da die Ursprungszellen der IGF2BP1-positiven Malignome Derivate aller Keimblätter repräsentieren, ist bei bekanntem Expressionsstatus ferner auch kein Hinweis bezüglich einer Liniendifferenzierung der Tumorzellen möglich, die zu einer Diskrimination zwischen einem epithelialen, mesenchymalen, neuroendokrinen, lymphatischen oder hämatologischen Ursprung des Tumors führen könnte. Der diagnostische Wert einer IGF2BP1-Expressionsanalyse für die Bestimmung der Entität eines malignen Prozesses ist demzufolge eher gering. Die Expression von IGF2BP1 in benignen Neoplasien ist nur in einzelnen Arbeiten untersucht worden, sodass ein diagnostischer Wert einer IGF2BP1-Positivität für die Bestimmung der Dignität momentan nicht gut bewertet werden kann.

Da es insbesondere im Falle von hepatozellulären Karzinomen zu einer Autoantikörperbildung gegen IGF2BP1 kommt (Tab. A2, S. 122 und Abschn. 1.6.1, S. 13), stellt sich die Frage nach dem diagnostischen Stellenwert einer serologischen Untersuchung eben dieser Autoantikörper. Bei Patienten mit HCC war die Autoantikörperbildung in den überwiegenden Fällen erst bei klinisch manifestem Karzinom und damit erst nach der malignen Entartung messbar, jedoch gab es auch Fälle mit positiven Autoantikörpern bei Patienten mit präkanzeröser Läsion. Der Einzelfallcharakter der zuletzt genannten Fälle legt aber die Vermutung nahe, dass bei diesen Patienten bereits zum Analysezeitpunkt ein klinisch ansonsten unauffälliges HCC vorlag. Somit stehen die IGF2BPs einerseits im Verdacht, einen tumorinitiierenden Effekt in der Transformation von präkanzeröser Läsion in das hepatozellulären Karzinom zu besitzen, andererseits kann ihre Re-/Expression auch nur als Folge der Transformation angesehen werden, ohne mit dieser im kausalen Zusammenhang zu stehen, und die anschließende Immunantwort als reines Epiphänomen der Tumorerkran-

kung gewertet werden. In beiden Fällen sind die IGF2BP-Autoantikörper zumindest als möglicher Tumormarker im Rahmen eines unspezifischen Suchtests bei Malignomverdacht und als Verlaufs- und Rezidivparameter in Erwägung zu ziehen. Im Speziellen könnte bei bekannter präkanzeröser Kondition in der Leber ein Nachweis von Autoantikörpern gegen IGF2BP1 zur Früherkennung eines HCCs genutzt werden.

2. Prognostik: Auch wenn der diagnostische Wert einer IGF2BP1-Expression im neoplastischen Gewebe im Rahmen der histologischen Untersuchung eher gering ist, so ist dennoch hervorzuheben, dass eine IGF2BP1-Positivität im kolorektalen [135], hepatozellulären [84, 88], ovarialen [43, 48, 133, 140], oesophagealen [139] und im Lungenkarzinom [138, 139] sowie im Neuroblastom [143] mit einer ungünstigen Überlebensprognose für den Patienten assoziiert werden konnte. Außerdem besteht im hepatozellulären Karzinom (HCC) eine Korrelation mit einer multifokalen Tumorgenese [84] sowie eine negative Korrelation mit einem ereignisfreien Überleben im kolorektalen [135], hepatozellulären [84], und ovariellen [43, 48] Karzinom sowie im Neuroblastom [143]. Weiterhin bestehen Korrelationen zwischen einer erhöhten IGF2BP1-Expression und einem hohen TNM-Stadium beim Kolon- [135, 150] und Lungenkarzinom [138], einem höheren *grading* im Lungen- [138] und Ovarialkarzinom [43, 133] sowie einem höheren klinischen Stadium nach FIGO (*Fédération Internationale de Gynécologie et d'Obstétrique*) im Ovarialkarzinom [43, 133]. Zusätzlich konnten wir für das HCC eine signifikante, wenn auch schwache Korrelation einer IGF2BP1-Expression mit der Tumorgröße (T-Stadium) und dem Differenzierungsgrad (G-Stadium) des Tumors zeigen [79]. Somit wäre aufgrund des Zusammenhanges zwischen einer IGF2BP1-Positivität eines malignen Tumors mit negativen klinisch-pathologischen Merkmalen der Erkrankung sowie aufgrund eines Zusammenhanges mit einer ungünstigen Überlebensprognose die Bestimmung einer IGF2BP1-Expression in den genannten Tumorentitäten von prognostischem Wert.

3. Therapie: Eine zielgerichtete Therapie gegen IGF2BP1 ist aufgrund der Begünstigung des Wachstums einer breiten Masse verschiedener Tumorzellen *in vitro* und *in vivo* (Abschn. 1.6.3 und 1.6.4, jeweils S. 15), seiner Expression in einer hohen Anzahl von humanen Tumorgeweben unterschiedlichster Entitäten mit der damit teilweise einhergehenden Verschlechterung der Überlebensprognose (Abschn. 1.6.1, S. 13) und aufgrund seiner Auswirkungen auf die Migrationsfähigkeit (Absch. 1.6.5, S. 18) von Tumorzellen ein vielversprechender Therapieansatz. Für das Erreichen einer Reduktion der IGF2BP1-Expression in entsprechend positiven Neoplasien sind sowohl Methoden, mit denen ein direkter Angriff des Gens, der mRNA oder des Proteins, als auch indirekte Methoden, mit denen Faktoren beeinflusst werden, welche die Expression von IGF2BP1 regulieren (Abschn. 1.4, S. 7), in Erwägung zu ziehen. Ein modifizierender Eingriff in das Genom erfordert den eher schwierigen Einsatz gentherapeutischer Methoden. Allerding ergeben sich aus der in jüngster Vergangenheit entdeckten und sich bereits in präklinischen Entwicklungsstufen befindenden [247] Technologien zur Genom-Editierung neue und vielversprechende Methoden mit therapeutischem Potential [248–250]. Ein möglicher Ansatz bezüglich IGF2BP1 wäre die Realisierung einer Funktionsverlust-Mutation durch Modifikation der RNA-bindenden-Domänen. Da dadurch seine Fähigkeit m-/RNA zu binden aufgehoben wird [19, 20, 29,

36, 37], könnten auf diese Weise seine onkogenen Eigenschaften unterbunden werden.

Fernab der Gentherapie ist alternativ auch eine auf transkriptioneller Ebene und damit noch vor der Translation positionierte Hemmung von IGF2BP1 denkbar. So könnten gegen die mRNA von IGF2BP1 gerichtete *antisense*-Oligonukleotide (ASOs) oder siRNAs eine Therapieoption darstellen [251]. Auch wenn bereits häufig im klinischen Kontext für unterschiedlichste neoplastische und nicht-neoplastische genetische Erkrankungen erfolgreich erprobt [251, 252], sind Therapeutika, die als Wirkprinzip eine Modifikation von DNA oder eine direkte Einflussnahme auf den mRNA-Metabolismus anstreben, nicht Gegenstand einer routinemäßigen Anwendung in der Klinik. Ein Grund dafür ist sicherlich die noch nicht gut gelöste Frage nach der Formulierung und der Etablierung einer geeigneten Pharmakokinetik des eigentlichen Therapeutikums im Menschen [247, 250, 253]. Bezüglich der Leber im Speziellen ist dennoch hervorzuheben, dass sich dieses Organ als äußerst empfänglich gegenüber gentherapeutischen Verfahren [247, 254] und dem Einsatz von ASOs [253] erwiesen hat. Somit kann die Therapie von primären Leber-Malignomen mit den genannten Methoden durchaus realistisch sein.

Des Weiteren sind auch Therapeutika vorstellbar, die ihr Wirkprinzip auf Ebene des Proteins entfalten. Auch wenn intrazellulär lokalisierte Proteine wie IGF2BP1 als Ziele von Antikörper-Therapien zunächst weniger in Frage kommen [255], ist eine Internalisierung eines solchen Antikörpers in Tumorzellen mit dann Zugang des Antikörpers zu seinem Antigen nicht ausgeschlossen [256]. Aussichtsreich wird dieser Ansatz insbesondere aufgrund der immer größer werdenden Anzahl an Möglichkeiten, Modifikationen an Antikörpern vorzunehmen oder spezielle Trägersubstanzen zu nutzen, die den Antikörpern einen Zugang in das Zytoplasma verschaffen [257]. Auch wurden bereits erfolgreich Antikörper entwickelt, die kurze Peptide von sonst intrazellulär lokalisierten, tumorassoziierten Antigenen an der Zelloberfläche von Tumorzellen erkennen. Dieser Ansatz macht sich den Umstand zu Nutze, dass Tumorzellen ihre intrazellulären Proteine auf MHC(*major histocompatibility complex*)-Klasse-1-Molekülen an der Zelloberfläche präsentieren und therapeutisch eingesetzte Antikörper somit entsprechend positiv exprimierende Tumorzellen aufspüren können [258].

Die Abhängigkeit der IGF2BP1-Expression von regulierenden Faktoren (Abschn. 1.4, S. 7) macht das Protein außerdem auch indirekt durch eine pharmakologische Hemmung seiner Transkriptionsfaktoren angreifbar. In diesem Zusammenhang rückt insbesondere der onkogene Transkriptionsfaktor MYC in den Vordergrund, da seine Hemmung durch den Einsatz von BET(*bromo-and extraterminal domain family of proteins*)-Inhibitoren bereits gezeigt werden konnte [259]. Die Wirkung basiert auf einer Verhinderung der Rekrutierung des BET-Proteins BRD4 (*bromodomain containing 4*) an den MYC-Lokus und einer damit einhergehenden Hemmung der MYC-Transkription [260]. BET-Inhibitoren werden bereits in zahlreichen Phase-I-Studien in Verbindung mit verschiedenen Entitäten solider, hämatologischer und lymphatischer Malignome erprobt [261]. Aufgrund der Tatsache, dass die Quantität von MYC und IGF2BP1 in malignen Zellen als das Produkt einer sich verstärkenden Rückkopplungsschleife angesehen werden kann, wird durch die gezielte Hemmung einer der beiden Faktoren der jeweils andere Faktor ebenfalls unterdrückt. Bei der durch

den Einsatz von BET-Inhibitoren herbeigeführten Reduktion der MYC-Expression ist somit eine konsekutive Reduktion der IGF2BP1-Expression zu erwarten. Die potente antitumorale Wirkung von BET-Inhibitoren kann also zumindest teilweise als Ausdruck einer Hemmung der IGF2BP1-Transkription verstanden werden. Interessanterweise kann die für BET-Inhibitoren resistente Leukämie-Zelllinie K-562 durch eine IGF2BP1-Depletion sensibilisiert werden. Wie zu erwarten war, wurde dabei zum einen eine reduzierte Proliferationsrate und zum anderen eine reduzierte MYC-Expression gemessen [74].

Anhand dieses Beispiels wird deutlich, dass für die Entwicklung von effizienten Tumortherapien genaue Kenntnisse über die Funktion und die Regulation von tumorassoziierten Proteinen wie dem IGF2BP1 notwendig sind. Bezüglich IGF2BP1 ist festzustellen, dass die an seiner Expressionkontrolle beteiligten molekularen Elemente (Abschn. 1.4, S. 7) wohl bekannte Charakteristika embryonaler und adulter Stammzellen sind und von diesen zur Aufrechterhaltung ihrer Stammzellidentität benötigt werden [262–265]. Ausgerechnet ein aktivierter Wnt-Signalweg [266–271], eine niedrige let-7-Expression [81, 263, 272] und ein hypomethyliertes Genom [273, 274] können aber als typische tumortreibende Zustände maligner Zellen angesehen werden und führten bereits häufig zu vergleichenden Gegenüberstellungen von Stammzellen und malignen Tumorzellen [275–278]. Diese und andere Analogien prägten den Begriff der Tumorstammzelle (CSC) (*Erstbeschreibung* in [279]). Darunter sind Subpopulationen von Tumorzellen zu verstehen, die aufgrund ihrer Fähigkeiten zur Proliferation, zur Selbsterneuerung und zur Erzeugung differenzierter Schwesterzellen die Tumormasse unterhalten und ihr ein aggressives biologisches Verhalten verleihen (*Übersichtsartikel* in [280]). In diesem Bild spiegelt sich die von uns gezeigte proliferationsfördernde und antiapoptotische Wirkung von IGF2BP1 (Manuskript 2, S. 66 [79] und Manuskript 4, S. 99 [69]) genauso wider, wie seine Expression gleichsam im embryonalen wie im neoplastischen Gewebe (Manuskript 1, S. 46 [7]). Auch scheinen CSCs die Ursprungszellen der EMT und Metastasierung (*Übersichtsartikel* in [281]) und der Entwicklung einer Chemoresistenz [282, 283] zu sein. Diese Situationen steht ebenfalls im Einklang mit unseren Untersuchungen zur Rolle von IGF2BP1 im Rahmen der EMT (Manuskript 3, S. 79 [185]) und passen zu den Hinweisen einer Mitwirkung von IGF2BP1 bei der Entwicklung einer Chemoresistenz im Ovarialkarzinom [140, 184](Abschn. 1.6.4, S. 15). In Zusammenschau der bisher zugänglichen Daten kann somit die Vermutung ausgesprochen werden, dass IGF2BP1 in physiologischen Zellelementen als integraler Bestandteil eines posttranskriptionellen Netzwerks zur Aufrechterhaltung eines Stammzellcharakters aufzufassen ist (Abschn. 1.5.4, S. 12) und bei Re-/Expression in malignen Zellen seine physiologischen Funktionen vor dem Hintergrund eines dysregulierten Transkriptoms wiederaufnimmt, wodurch unter Umständen die Etablierung einer CSC begünstigt wird. Seine Expression ist somit als Malignitätskriterium einzustufen und mit onkogenen Eigenschaften neoplastischer Zellen assoziiert. Für ein besseres Verständnis der Regulation von Transkription und Translation des IGF2BP1 sind weiterführende Analysen notwendig und würden nicht nur ein besseres Verständnis der Expressionskontrolle im Embryo, sondern auch zu einem besseren Verständnis der Dysregulation der IGF2BP1-Expression in malignen Zellen beitragen und so möglicherweise neue Wege für eine gezielte Tumortherapie eröffnen.

Literaturverzeichnis

[1.] Gerstberger, S., Hafner, M. und Tuschl, T. (2014). A census of human RNA-binding proteins. *Nat Rev Genet 15*, 829–845.

[2.] Dreyfuss, G., Kim, V. N. und Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages they carry. *Nat Rev Mol Cell Biol 3*, 195–205.

[3.] Lunde, B. M., Moore, C. und Varani, G. (2007). RNA-binding proteins: modular design for efficient function. *Nat Rev Mol Cell Biol 8*, 479–490.

[4.] Tartaglia, G. G. (2016). The Grand Challenge of Characterizing Ribonucleoprotein Networks. *Front Mol Biosci 3*, 24.

[5.] Santoro, M. R., Bray, S. M. und Warren, S. T. (2012). Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu Rev Pathol 7*, 219–245.

[6.] Moore, A. E., Chenette, D. M., Larkin, L. C. und Schneider, R. J. (2014). Physiological networks and disease functions of RNA-binding protein AUF1. *Wiley Interdiscip Rev RNA 5*, 549–564.

[7.] Bell, J. L., Wächter, K., Mühleck, B., Pazaitis, N., Köhn, M., Lederer, M. und Hüttelmaier, S. (2013). Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell Mol Life Sci 70*, 2657–2675.

[8.] Verkerk, A. J., Pieretti, M., Sutcliffe, J. S., Fu, Y. H., Kuhl, D. P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M. F. und Zhang, F. P. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell 65*, 905–914.

[9.] De Boulle, K., Verkerk, A. J., Reyniers, E., Vits, L., Hendrickx, J., Van Roy, B., Van den Bos, F., de Graaff, E., Oostra, B. A. und Willems, P. J. (1993). A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet 3*, 31–35.

[10.] Myrick, L. K., Nakamoto-Kinoshita, M., Lindor, N. M., Kirmani, S., Cheng, X. und Warren, S. T. (2014). Fragile X syndrome due to a missense mutation. *Eur J Hum Genet 22*, 1185–1189.

[11.] Kechavarzi, B. und Janga, S. C. (2014). Dissecting the expression landscape of RNA-binding proteins in human cancers. *Genome Biol 15*, R14.

[12.] Pascale, A. und Govoni, S. (2012). The complex world of post-transcriptional mechanisms: is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. *Cell Mol Life Sci 69*, 501–517.

[13.] Gouble, A., Grazide, S., Meggetto, F., Mercier, P., Delsol, G. und Morello, D. (2002). A new player in oncogenesis: AUF1/hnRNPD overexpression leads to tumorigenesis in transgenic mice. *Cancer Res 62*, 1489–1495.

[14.] Lederer, M., Bley, N., Schleifer, C. und Hüttelmaier, S. (2014). The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer. *Semin Cancer Biol 29*, 3–12.

[15.] Köhn, M., Lederer, M., Wächter, K. und Hüttelmaier, S. (2010). Near-infrared (NIR) dye-labeled RNAs identify binding of ZBP1 to the noncoding Y3-RNA. *RNA 16*, 1420–1428.

[16.] Nielsen, J., Adolph, S. K., Rajpert-De Meyts, E., Lykke-Andersen, J., Koch, G., Christiansen, J. und Nielsen, F. C. (2003). Nuclear transit of human zipcode-binding protein IMP1. *Biochem J 376*, 383–391.

[17.] Yisraeli, J. K. (2005). VICKZ proteins: a multi-talented family of regulatory RNA-binding proteins. *Biol Cell 97*, 87–96.

[18.] Nielsen, J., Christiansen, J., Lykke-Andersen, J., Johnsen, A. H., Wewer, U. M. und Nielsen, F. C. (1999). A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol 19*, 1262–1270.

[19.] Chao, J. A., Patskovsky, Y., Patel, V., Levy, M., Almo, S. C. und Singer, R. H. (2010). ZBP1 recognition of beta-actin zipcode induces RNA looping. *Genes Dev 24*, 148–158.

[20.] Wächter, K., Köhn, M., Stöhr, N. und Hüttelmaier, S. (2013). Subcellular localization and RNP formation of IGF2BPs (IGF2 mRNA-binding proteins) is modulated by distinct RNA-binding domains. *Biol Chem 394*, 1077–1090.

[21.] Oleynikov, Y. und Singer, R. H. (2003). Real-time visualization of ZBP1 association with beta-actin mRNA during transcription and localization. *Curr Biol 13*, 199–207.

[22.] Hüttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., Bassell, G. J., Condeelis, J. und Singer, R. H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature 438*, 512–515.

[23.] Pan, F., Hüttelmaier, S., Singer, R. H. und Gu, W. (2007). ZBP2 facilitates binding of ZBP1 to beta-actin mRNA during transcription. *Mol Cell Biol 27*, 8340–8351.

[24.] Sim, S., Yao, J., Weinberg, D. E., Niessen, S., Yates, J. R. und Wolin, S. L. (2012). The zipcode-binding protein ZBP1 influences the subcellular location of the Ro 60-kDa autoantigen and the noncoding Y3 RNA. *RNA 18*, 100–110.

[25.] Nielsen, F. C., Nielsen, J., Kristensen, M. A., Koch, G. und Christiansen, J. (2002). Cytoplasmic trafficking of IGF-II mRNA-binding protein by conserved KH domains. *J Cell Sci 115*, 2087–2097.

[26.] Jønson, L., Vikesaa, J., Krogh, A., Nielsen, L. K., Hansen, T. v., Borup, R., Johnsen, A. H., Christiansen, J. und Nielsen, F. C. (2007). Molecular composition of IMP1 ribonucleoprotein granules. *Mol Cell Proteomics 6*, 798–811.

[27.] Weidensdorfer, D., Stöhr, N., Baude, A., Lederer, M., Köhn, M., Schierhorn, A., Buchmeier, S., Wahle, E. und Hüttelmaier, S. (2009). Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *RNA 15*, 104–115.

[28.] Kwon, S., Abramson, T., Munro, T. P., John, C. M., Köhrmann, M. und Schnapp, B. J. (2002). UUCAC- and vera-dependent localization of VegT RNA in Xenopus oocytes. *Curr Biol 12*, 558–564.

[29.] Farina, K. L., Huttelmaier, S., Musunuru, K., Darnell, R. und Singer, R. H. (2003). Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. *J Cell Biol 160*, 77–87.

[30.] Munro, T. P., Kwon, S., Schnapp, B. J. und St Johnston, D. (2006). A repeated IMP-binding motif controls oskar mRNA translation and anchoring independently of Drosophila melanogaster IMP. *J Cell Biol 172*, 577–588.

[31.] Hafner, M., Landthaler, M., Burger, L., Khorshid, M., Hausser, J., Berninger, P., Rothballer, A., Ascano, M., Jungkamp, A.-C., Munschauer, M., Ulrich, A., Wardle, G. S., Dewell, S., Zavolan, M. und Tuschl, T. (2010). Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell 141*, 129–141.

[32.] Patel, V. L., Mitra, S., Harris, R., Buxbaum, A. R., Lionnet, T., Brenowitz, M., Girvin, M., Levy, M., Almo, S. C., Singer, R. H. und Chao, J. A. (2012). Spatial arrangement of an RNA zipcode identifies mRNAs under post-transcriptional control. *Genes Dev 26*, 43–53.

[33.] Conway, A. E., Van Nostrand, E. L., Pratt, G. A., Aigner, S., Wilbert, M. L., Sundararaman, B., Freese, P., Lambert, N. J., Sathe, S., Liang, T. Y., Essex, A., Landais, S., Burge, C. B., Jones, D. L. und Yeo, G. W. (2016). Enhanced CLIP Uncovers IMP Protein-RNA Targets in Human Pluripotent Stem Cells Important for Cell Adhesion and Survival. *Cell reports 15*, 666–679.

[34.] Dominguez, D., Freese, P., Alexis, M. S., Su, A., Hochman, M., Palden, T., Bazile, C., Lambert, N. J., Van Nostrand, E. L., Pratt, G. A., Yeo, G. W., Graveley, B. R. und Burge, C. B. (2018). Sequence, Structure, and Context Preferences of Human RNA Binding Proteins. *Mol Cell 70*, 854–867.e9.

[35.] Runge, S., Nielsen, F. C., Nielsen, J., Lykke-Andersen, J., Wewer, U. M. und Christiansen, J. (2000). H19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein. *J Biol Chem 275*, 29562–29569.

[36.] Nielsen, J., Kristensen, M. A., Willemoës, M., Nielsen, F. C. und Christiansen, J. (2004). Sequential dimerization of human zipcode-binding protein IMP1 on RNA: a cooperative mechanism providing RNP stability. *Nucleic Acids Res 32*, 4368–4376.

[37.] Nicastro, G., Candel, A. M., Uhl, M., Oregioni, A., Hollingworth, D., Backofen, R., Martin, S. R. und Ramos, A. (2017). Mechanism of β-actin mRNA Recognition by ZBP1. *Cell Rep 18*, 1187–1199.

[38.] Vikesaa, J., Hansen, T. V. O., Jønson, L., Borup, R., Wewer, U. M., Christiansen, J. und Nielsen, F. C. (2006). RNA-binding IMPs promote cell adhesion and invadopodia formation. *EMBO J 25*, 1456–1468.

[39.] Bernstein, P. L., Herrick, D. J., Prokipcak, R. D. und Ross, J. (1992). Control of c-myc mRNA half-life in vitro by a protein capable of binding to a coding region stability determinant. *Genes Dev 6*, 642–654.

[40.] Lemm, I. und Ross, J. (2002). Regulation of c-myc mRNA decay by translational pausing in a coding region instability determinant. *Mol Cell Biol 22*, 3959–3969.

[41.] Ioannidis, P., Mahaira, L. G., Perez, S. A., Gritzapis, A. D., Sotiropoulou, P. A., Kavalakis, G. J., Antsaklis, A. I., Baxevanis, C. N. und Papamichail, M. (2005). CRD-BP/IMP1 expression characterizes cord blood CD34+ stem cells and affects c-myc and IGF-II expression in MCF-7 cancer cells. *J Biol Chem 280*, 20086–20093.

[42.] Noubissi, F. K., Elcheva, I., Bhatia, N., Shakoori, A., Ougolkov, A., Liu, J., Minamoto, T., Ross, J., Fuchs, S. Y. und Spiegelman, V. S. (2006). CRD-BP mediates stabilization of betaTrCP1 and c-myc mRNA in response to beta-catenin signalling. *Nature 441*, 898–901.

[43.] Köbel, M., Weidensdorfer, D., Reinke, C., Lederer, M., Schmitt, W. D., Zeng, K., Thomssen, C., Hauptmann, S. und Hüttelmaier, S. (2007). Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. *Oncogene 26*, 7584–7589.

[44.] Stöhr, N., Köhn, M., Lederer, M., Glass, M., Reinke, C., Singer, R. H. und Hüttelmaier, S. (2012). IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. *Genes Dev 26*, 176–189.

[45.] Elcheva, I., Goswami, S., Noubissi, F. K. und Spiegelman, V. S. (2009). CRD-BP protects the coding region of betaTrCP1 mRNA from miR-183-mediated degradation. *Mol Cell 35*, 240–246.

[46.] Goswami, S., Tarapore, R. S., Teslaa, J. J., Grinblat, Y., Setaluri, V. und Spiegelman, V. S. (2010). MicroRNA-340-mediated degradation of microphthalmia-associated transcription factor mRNA is inhibited by the coding region determinant-binding protein. *The Journal of biological chemistry 285*, 20532–20540.

[47.] Busch, B., Bley, N., Müller, S., Glaß, M., Misiak, D., Lederer, M., Vetter, M., Strauß, H.-G., Thomssen, C. und Hüttelmaier, S. (2016). The oncogenic triangle of HMGA2, LIN28B and IGF2BP1 antagonizes tumor-suppressive actions of the let-7 family. *Nucleic Acids Res 44*, 3845–3864.

[48.] Müller, S., Bley, N., Glaß, M., Busch, B., Rousseau, V., Misiak, D., Fuchs, T., Lederer, M. und Hüttelmaier, S. (2018). IGF2BP1 enhances an aggressive tumor cell phenotype by impairing miRNA-directed downregulation of oncogenic factors. *Nucleic Acids Res 46*, 6285–6303.

[49.] Protter, D. S. W. und Parker, R. (2016). Principles and Properties of Stress Granules. *Trends Cell Biol 26*, 668–679.

[50.] Stöhr, N., Lederer, M., Reinke, C., Meyer, S., Hatzfeld, M., Singer, R. H. und Hüttelmaier, S. (2006). ZBP1 regulates mRNA stability during cellular stress. *J Cell Biol 175*, 527–534.

[51.] Hämmerle, M., Gutschner, T., Uckelmann, H., Ozgur, S., Fiskin, E., Gross, M., Skawran, B., Geffers, R., Longerich, T., Breuhahn, K., Schirmacher, P., Stoecklin, G. und Diederichs, S. (2013). Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology 58*, 1703–1712.

[52.] Faye, M. D., Beug, S. T., Graber, T. E., Earl, N., Xiang, X., Wild, B., Langlois, S., Michaud, J., Cowan, K. N., Korneluk, R. G. und Holcik, M. (2015). IGF2BP1 controls cell death and drug resistance in rhabdomyosarcomas by regulating translation of cIAP1. *Oncogene 34*, 1532–1541.

[53.] Weinlich, S., Hüttelmaier, S., Schierhorn, A., Behrens, S.-E., Ostareck-Lederer, A. und Ostareck, D. H. (2009). IGF2BP1 enhances HCV IRES-mediated translation initiation via the 3'UTR. *RNA 15*, 1528–1542.

[54.] Dahm, R. und Kiebler, M. (2005). Cell biology: silenced RNA on the move. *Nature 438*, 432–435.

[55.] Kislauskis, E. H., Zhu, X. und Singer, R. H. (1994). Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J Cell Biol 127*, 441–451.

[56.] Eom, T., Antar, L. N., Singer, R. H. und Bassell, G. J. (2003). Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J Neurosci 23*, 10433–10444.

[57.] Zhang, H. L., Eom, T., Oleynikov, Y., Shenoy, S. M., Liebelt, D. A., Dictenberg, J. B., Singer, R. H. und Bassell, G. J. (2001). Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron 31*, 261–275.

[58.] Lerner, M. R., Boyle, J. A., Hardin, J. A. und Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science 211*, 400–402.

[59.] Hendrick, J. P., Wolin, S. L., Rinke, J., Lerner, M. R. und Steitz, J. A. (1981). Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. *Mol Cell Biol 1*, 1138–1149.

[60.] Francoeur, A. M. und Mathews, M. B. (1982). Interaction between VA RNA and the lupus antigen La: formation of a ribonucleoprotein particle in vitro. *Proc Natl Acad Sci U S A 79*, 6772–6776.

[61.] Wolin, S. L. und Steitz, J. A. (1984). The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. *Proc Natl Acad Sci U S A 81*, 1996–2000.

[62.] Pruijn, G. J., Wingens, P. A., Peters, S. L., Thijssen, J. P. und van Venrooij, W. J. (1993). Ro RNP associated Y RNAs are highly conserved among mammals. *Biochim Biophys Acta 1216*, 395–401.

[63.] Farris, A. D., O'Brien, C. A. und Harley, J. B. (1995). Y3 is the most conserved small RNA component of Ro ribonucleoprotein complexes in vertebrate species. *Gene 154*, 193–198.

[64.] Farris, A. D., Koelsch, G., Pruijn, G. J., van Venrooij, W. J. und Harley, J. B. (1999). Conserved features of Y RNAs revealed by automated phylogenetic secondary structure analysis. *Nucleic Acids Res 27*, 1070–1078.

[65.] Teunissen, S. W., Kruithof, M. J., Farris, A. D., Harley, J. B., Venrooij, W. J. und Pruijn, G. J. (2000). Conserved features of Y RNAs: a comparison of experimentally derived secondary structures. *Nucleic Acids Res 28*, 610–619.

[66.] Perreault, J., Perreault, J.-P. und Boire, G. (2007). Ro-associated Y RNAs in metazoans: evolution and diversification. *Mol Biol Evol 24*, 1678–1689.

[67.] Mosig, A., Guofeng, M., Stadler, B. M. R. und Stadler, P. F. (2007). Evolution of the vertebrate Y RNA cluster. *Theory Biosci 126*, 9–14.

[68.] Gardiner, T. J., Christov, C. P., Langley, A. R. und Krude, T. (2009). A conserved motif of vertebrate Y RNAs essential for chromosomal DNA replication. *RNA 15*, 1375–1385.

[69.] Köhn, M., Pazaitis, N. und Hüttelmaier, S. (2013). Why YRNAs? About Versatile RNAs and Their Functions. *Biomolecules 3*, 143–156.

[70.] Christov, C. P., Trivier, E. und Krude, T. (2008). Noncoding human Y RNAs are overexpressed in tumours and required for cell proliferation. *Br J Cancer 98*, 981–988.

[71.] Gu, W., Wells, A. L., Pan, F. und Singer, R. H. (2008). Feedback regulation between zipcode binding protein 1 and beta-catenin mRNAs in breast cancer cells. *Mol Cell Biol 28*, 4963–4974.

[72.] Gu, W., Pan, F. und Singer, R. H. (2009). Blocking beta-catenin binding to the ZBP1 promoter represses ZBP1 expression, leading to increased proliferation and migration of metastatic breast-cancer cells. *J Cell Sci 122*, 1895–1905.

[73.] Nishino, J., Kim, S., Zhu, Y., Zhu, H. und Morrison, S. J. (2013). A network of heterochronic genes including Imp1 regulates temporal changes in stem cell properties. *eLife 2*, Original DateCompleted: 20131106, e00924.

[74.] Rathert, P., Roth, M., Neumann, T., Muerdter, F., Roe, J.-S., Muhar, M., Deswal, S., Cerny-Reiterer, S., Peter, B., Jude, J., Hoffmann, T., Boryń, Ł. M., Axelsson, E., Schweifer, N., Tontsch-Grunt, U., Dow, L. E., Gianni, D., Pearson, M., Valent, P., Stark, A., Kraut, N., Vakoc, C. R. und Zuber, J. (2015). Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature 525*, 543–547.

[75.] Noubissi, F. K., Nikiforov, M. A., Colburn, N. und Spiegelman, V. S. (2010). Transcriptional Regulation of CRD-BP by c-myc: Implications for c-myc Functions. *Genes Cancer 1*, 1074–1082.

[76.] Prokipcak, R. D., Herrick, D. J. und Ross, J. (1994). Purification and properties of a protein that binds to the C-terminal coding region of human c-myc mRNA. *J Biol Chem 269*, 9261–9269.

[77.] Leeds, P., Kren, B. T., Boylan, J. M., Betz, N. A., Steer, C. J., Gruppuso, P. A. und Ross, J. (1997). Developmental regulation of CRD-BP, an RNA-binding protein that stabilizes c-myc mRNA in vitro. *Oncogene 14*, 1279–1286.

[78.] Doyle, G. A., Betz, N. A., Leeds, P. F., Fleisig, A. J., Prokipcak, R. D. und Ross, J. (1998). The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. *Nucleic Acids Res 26*, 5036–5044.

[79.] Gutschner, T., Hämmerle, M., Pazaitis, N., Bley, N., Fiskin, E., Uckelmann, H., Heim, A., Groß, M., Hofmann, N., Geffers, R., Skawran, B., Longerich, T., Breuhahn, K., Schirmacher, P., Mühleck, B., Hüttelmaier, S. und Diederichs, S. (2014). Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma. *Hepatology 59*, 1900–1911.

[80.] Boyerinas, B., Park, S.-M., Shomron, N., Hedegaard, M. M., Vinther, J., Andersen, J. S., Feig, C., Xu, J., Burge, C. B. und Peter, M. E. (2008). Identification of let-7-regulated oncofetal genes. *Cancer Res 68*, 2587–2591.

[81.] Boyerinas, B., Park, S.-M., Hau, A., Murmann, A. E. und Peter, M. E. (2010). The role of let-7 in cell differentiation and cancer. *Endocr Relat Cancer 17*, F19–F36.

[82.] Toledano, H., D'Alterio, C., Czech, B., Levine, E. und Jones, D. L. (2012). The let-7-Imp axis regulates ageing of the Drosophila testis stem-cell niche. *Nature 485*, 605–610.

[83.] Mayr, C. und Bartel, D. P. (2009). Widespread shortening of 3'UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell 138*, 673–684.

[84.] Zhou, X., Zhang, C. Z., Lu, S.-X., Chen, G. G., Li, L.-Z., Liu, L.-L., Yi, C., Fu, J., Hu, W., Wen, J.-M. und Yun, J.-P. (2015). miR-625 suppresses tumour migration and invasion by targeting IGF2BP1 in hepatocellular carcinoma. *Oncogene 34*, 965–977.

[85.] Wang, R.-j., Li, J.-w., Bao, B.-h., Wu, H.-c., Du, Z.-h., Su, J.-l., Zhang, M.-h. und Liang, H.-q. (2015). MicroRNA-873 (miRNA-873) inhibits glioblastoma tumorigenesis and metastasis by suppressing the expression of IGF2BP1. *J Biol Chem 290*, 8938–8948.

[86.] Rebucci, M., Sermeus, A., Leonard, E., Delaive, E., Dieu, M., Fransolet, M., Arnould, T. und Michiels, C. (2015). miRNA-196b inhibits cell proliferation and induces apoptosis in HepG2 cells by targeting IGF2BP1. *Mol Cancer 14*, 79.

[87.] Huang, X., Huang, M., Kong, L. und Li, Y. (2015). miR-372 suppresses tumour proliferation and invasion by targeting IGF2BP1 in renal cell carcinoma. *Cell Prolif 48*, 593–599.

[88.] Zhang, J., Cheng, J., Zeng, Z., Wang, Y., Li, X., Xie, Q., Jia, J., Yan, Y., Guo, Z., Gao, J., Yao, M., Chen, X. und Lu, F. (2015). Comprehensive profiling of novel microRNA-9 targets and a tumor suppressor role of microRNA-9 via targeting IGF2BP1 in hepatocellular carcinoma. *Oncotarget 6*, 42040–42052.

[89.] Qu, Y., Pan, S., Kang, M., Dong, R. und Zhao, J. (2016). MicroRNA-150 functions as a tumor suppressor in osteosarcoma by targeting IGF2BP1. *Tumour Biol 37*, 5275–5284.

[90.] Su, Y., Xiong, J., Hu, J., Wei, X., Zhang, X. und Rao, L. (2016). MicroRNA-140-5p targets insulin like growth factor 2 mRNA binding protein 1 (IGF2BP1) to suppress cervical cancer growth and metastasis. *Oncotarget 7*, 68397–68411.

[91.] Qin, X., Sun, L. und Wang, J. (2017). Restoration of microRNA-708 sensitizes ovarian cancer cells to cisplatin via IGF2BP1/Akt pathway. *Cell Biol Int 41*, 1110–1118.

[92.] Sorenson, E. C., Khanin, R., Bamboat, Z. M., Cavnar, M. J., Kim, T. S., Sadot, E., Zeng, S., Greer, J. B., Seifert, A. M., Cohen, N. A., Crawley, M. H., Green, B. L., Klimstra, D. S. und DeMatteo, R. P. (2017). Genome and transcriptome profiling of fibrolamellar hepatocellular carcinoma demonstrates p53 and IGF2BP1 dysregulation. *PLoS One 12*, e0176562.

[93.] Ioannidis, P., Trangas, T., Dimitriadis, E., Samiotaki, M., Kyriazoglou, I., Tsiapalis, C. M., Kittas, C., Agnantis, N., Nielsen, F. C., Nielsen, J., Christiansen, J. und Pandis, N. (2001). C-MYC and IGF-II mRNA-binding protein (CRD-BP/IMP-1) in benign and malignant mesenchymal tumors. *Int J Cancer 94*, 480–484.

[94.] Thomas, B. J., Rubio, E. D., Krumm, N., Broin, P. O., Bomsztyk, K., Welcsh, P., Greally, J. M., Golden, A. A. und Krumm, A. (2011). Allele-specific transcriptional elongation regulates monoallelic expression of the IGF2BP1 gene. *Epigenetics Chromatin 4*, 14.

[95.] Mahaira, L. G., Katsara, O., Pappou, E., Iliopoulou, E. G., Fortis, S., Antsaklis, A., Fotinopoulos, P., Baxevanis, C. N., Papamichail, M. und Perez, S. A. (2014). IGF2BP1 expression in human mesenchymal stem cells significantly affects their proliferation and is under the epigenetic control of TET1/2 demethylases. *Stem Cells Dev 23*, 2501–2512.

[96.] Hansen, T. V. O., Hammer, N. A., Nielsen, J., Madsen, M., Dalbaeck, C., Wewer, U. M., Christiansen, J. und Nielsen, F. C. (2004). Dwarfism and impaired gut development in insulin-like growth factor II mRNA-binding protein 1-deficient mice. *Mol Cell Biol 24*, 4448–4464.

[97.] Nielsen, F. C., Nielsen, J. und Christiansen, J. (2001). A family of IGF-II mRNA binding proteins (IMP) involved in RNA trafficking. *Scand J Clin Lab Invest Suppl 234*, 93–99.

[98.] Yaniv, K. und Yisraeli, J. K. (2002). The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. *Gene 287*, 49–54.

[99.] Perycz, M., Urbanska, A. S., Krawczyk, P. S., Parobczak, K. und Jaworski, J. (2011). Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons. *J Neurosci 31*, 5271–5285.

[100.] Ross, A. F., Oleynikov, Y., Kislauskis, E. H., Taneja, K. L. und Singer, R. H. (1997). Characterization of a beta-actin mRNA zipcode-binding protein. *Mol Cell Biol 17*, 2158–2165.

[101.] Nielsen, J., Cilius Nielsen, F., Kragh Jakobsen, R. und Christiansen, J. (2000). The biphasic expression of IMP/Vg1-RBP is conserved between vertebrates and Drosophila. *Mech Dev 96*, 129–132.

[102.] Boylan, K. L. M., Mische, S., Li, M., Marqués, G., Morin, X., Chia, W. und Hays, T. S. (2008). Motility screen identifies Drosophila IGF-II mRNA-binding protein–zipcode-binding protein acting in oogenesis and synaptogenesis. *PLos Genet 4*, e36.

[103.] Adolph, S. K., DeLotto, R., Nielsen, F. C. und Christiansen, J. (2009). Embryonic expression of Drosophila IMP in the developing CNS and PNS. *Gene Expr Patterns 9*, 138–143.

[104.] Müeller-Pillasch, F., Pohl, B., Wilda, M., Lacher, U., Beil, M., Wallrapp, C., Hameister, H., Knöchel, W., Adler, G. und Gress, T. M. (1999). Expression of the highly conserved RNA binding protein KOC in embryogenesis. *Mech Dev 88*, 95–99.

[105.] Zhang, Q., Yaniv, K., Oberman, F., Wolke, U., Git, A., Fromer, M., Taylor, W. L., Meyer, D., Standart, N., Raz, E. und Yisraeli, J. K. (1999). Vg1 RBP intracellular distribution and evolutionarily conserved expression at multiple stages during development. *Mech Dev 88*, 101–106.

[106.] Yaniv, K., Fainsod, A., Kalcheim, C. und Yisraeli, J. K. (2003). The RNA-binding protein Vg1 RBP is required for cell migration during early neural development. *Development 130*, 5649–5661.

[107.] Manieri, N. A., Drylewicz, M. R., Miyoshi, H. und Stappenbeck, T. S. (2012). Igf2bp1 is required for full induction of Ptgs2 mRNA in colonic mesenchymal stem cells in mice. *Gastroenterology 143*, 110–21.e10.

[108.] Katsara, O., Mahaira, L. G., Iliopoulou, E. G., Moustaki, A., Antsaklis, A., Loutradis, D., Stefanidis, K., Baxevanis, C. N., Papamichail, M. und Perez, S. A. (2011). Effects of donor age, gender, and in vitro cellular aging on the phenotypic, functional, and molecular characteristics of mouse bone marrow-derived mesenchymal stem cells. *Stem Cells Dev 20*, 1549–1561.

[109.] Hammer, N. A., Hansen, T. v. O., Byskov, A. G., Rajpert-De Meyts, E., Grøndahl, M. L., Bredkjaer, H. E., Wewer, U. M., Christiansen, J. und Nielsen, F. C. (2005). Expression of IGF-II mRNA-binding proteins (IMPs) in gonads and testicular cancer. *Reproduction 130*, 203–212.

[110.] Fabrizio, J. J., Hickey, C. A., Stabrawa, C., Meytes, V., Hutter, J. A., Talbert, C. und Regis, N. (2008). Imp (IGF-II mRNA-binding protein) is expressed during spermatogenesis in Drosophila melanogaster. *Fly 2*, 47–52.

[111.] Donnelly, C. J., Willis, D. E., Xu, M., Tep, C., Jiang, C., Yoo, S., Schanen, N. C., Kirn-Safran, C. B., van Minnen, J., English, A., Yoon, S. O., Bassell, G. J. und Twiss, J. L. (2011). Limited availability of ZBP1 restricts axonal mRNA localization and nerve regeneration capacity. *EMBO J 30*, 4665–4677.

[112.] Natkunam, Y., Vainer, G., Chen, J., Zhao, S., Marinelli, R. J., Hammer, A. S., Hamilton-Dutoit, S., Pikarsky, E., Amir, G., Levy, R., Yisraeli, J. K. und Lossos, I. S. (2007). Expression of the RNA-binding protein VICKZ in normal hematopoietic tissues and neoplasms. *Haematologica 92*, 176–183.

[113.] Chao, W. und D'Amore, P. A. (2008). IGF2: epigenetic regulation and role in development and disease. *Cytokine Growth Factor Rev 19*, 111–120.

[114.] DeChiara, T. M., Efstratiadis, A. und Robertson, E. J. (1990). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature 345*, 78–80.

[115.] DeChiara, T. M., Robertson, E. J. und Efstratiadis, A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell 64*, 849–859.

[116.] Constância, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R., Stewart, F., Kelsey, G., Fowden, A., Sibley, C. und Reik, W. (2002). Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature 417*, 945–948.

[117.] Kent, L. N., Ohboshi, S. und Soares, M. J. (2012). Akt1 and insulin-like growth factor 2 (Igf2) regulate placentation and fetal/postnatal development. *Int J Dev Biol 56*, 255–261.

[118.] Conover, C. A. und Khosla, S. (2003). Role of extracellular matrix in insulin-like growth factor (IGF) binding protein-2 regulation of IGF-II action in normal human osteoblasts. *Growth Horm IGF Res 13*, 328–335.

[119.] Fisher, M. C., Meyer, C., Garber, G. und Dealy, C. N. (2005). Role of IGFBP2, IGF-I and IGF-II in regulating long bone growth. *Bone 37*, 741–750.

[120.] Jones, J. I. und Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev 16*, 3–34.

[121.] Gardel, M. L., Schneider, I. C., Aratyn-Schaus, Y. und Waterman, C. M. (2010). Mechanical integration of actin and adhesion dynamics in cell migration. *Annu Rev Cell Dev Biol 26*, 315–333.

[122.] Mattila, P. K. und Lappalainen, P. (2008). Filopodia: molecular architecture and cellular functions. *Nat Rev Mol Cell Biol 9*, 446–454.

[123.] Schelski, M. und Bradke, F. (2017). Neuronal polarization: From spatiotemporal signaling to cytoskeletal dynamics. *Mol Cell Neurosci 84*, 11–28.

[124.] Ryan, G. L., Watanabe, N. und Vavylonis, D. (2012). A review of models of fluctuating protrusion and retraction patterns at the leading edge of motile cells. *Cytoskeleton 69*, 195–206.

[125.] Leung, K.-M., van Horck, F. P. G., Lin, A. C., Allison, R., Standart, N. und Holt, C. E. (2006). Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci 9*, 1247–1256.

[126.] Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J. und Zheng, J. Q. (2006). An essential role for beta-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nat Neurosci 9*, 1265–1273.

[127.] Welshhans, K. und Bassell, G. J. (2011). Netrin-1-induced local β-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J Neurosci 31*, 9800–9813.

[128.] Sasaki, Y., Welshhans, K., Wen, Z., Yao, J., Xu, M., Goshima, Y., Zheng, J. Q. und Bassell, G. J. (2010). Phosphorylation of zipcode binding protein 1 is required for brain-derived neurotrophic factor signaling of local beta-actin synthesis and growth cone turning. *J Neurosci 30*, 9349–9358.

[129.] Kraut, R., Menon, K. und Zinn, K. (2001). A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in Drosophila. *Curr Biol 11*, 417–430.

[130.] Norga, K. K., Gurganus, M. C., Dilda, C. L., Yamamoto, A., Lyman, R. F., Patel, P. H., Rubin, G. M., Hoskins, R. A., Mackay, T. F. und Bellen, H. J. (2003). Quantitative analysis of bristle number in Drosophila mutants identifies genes involved in neural development. *Curr Biol 13*, 1388–1396.

[131.] Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature 454*, 428–435.

[132.] Doyle, G. A., Bourdeau-Heller, J. M., Coulthard, S., Meisner, L. F. und Ross, J. (2000). Amplification in human breast cancer of a gene encoding a c-myc mRNA-binding protein. *Cancer Res 60*, 2756–2759.

[133.] Gu, L., Shigemasa, K. und Ohama, K. (2004). Increased expression of IGF II mRNA-binding protein 1 mRNA is associated with an advanced clinical stage and poor prognosis in patients with ovarian cancer. *Int J Oncol 24*, 671–678.

[134.] Ross, J., Lemm, I. und Berberet, B. (2001). Overexpression of an mRNA-binding protein in human colorectal cancer. *Oncogene 20*, 6544–6550.

[135.] Dimitriadis, E., Trangas, T., Milatos, S., Foukas, P. G., Gioulbasanis, I., Courtis, N., Nielsen, F. C., Pandis, N., Dafni, U., Bardi, G. und Ioannidis, P. (2007). Expression of oncofetal RNA-binding protein CRD-BP/IMP1 predicts clinical outcome in colon cancer. *Int J Cancer 121*, 486–494.

[136.] Mongroo, P. S., Noubissi, F. K., Cuatrecasas, M., Kalabis, J., King, C. E., Johnstone, C. N., Bowser, M. J., Castells, A., Spiegelman, V. S. und Rustgi, A. K. (2011). IMP-1 displays cross-talk with K-Ras and modulates colon cancer cell survival through the novel proapoptotic protein CYFIP2. *Cancer Res 71*, 2172–2182.

[137.] Ioannidis, P., Kottaridi, C., Dimitriadis, E., Courtis, N., Mahaira, L., Talieri, M., Giannopoulos, A., Iliadis, K., Papaioannou, D., Nasioulas, G. und Trangas, T. (2004). Expression of the RNA-binding protein CRD-BP in brain and non-small cell lung tumors. *Cancer Lett 209*, 245–250.

[138.] Kato, T., Hayama, S., Yamabuki, T., Ishikawa, N., Miyamoto, M., Ito, T., Tsuchiya, E., Kondo, S., Nakamura, Y. und Daigo, Y. (2007). Increased expression of insulin-like growth factor-II messenger RNA-binding protein 1 is associated with tumor progression in patients with lung cancer. *Clin Cancer Res 13*, 434–442.

[139.] Lin, E. W., Karakasheva, T. A., Lee, D.-J., Lee, J.-S., Long, Q., Bass, A. J., Wong, K. K. und Rustgi, A. K. (2017). Comparative transcriptomes of adenocarcinomas and squamous cell carcinomas reveal molecular similarities that span classical anatomic boundaries. *PLos Genet 13*, e1006938.

[140.] Boyerinas, B., Park, S.-M., Murmann, A. E., Gwin, K., Montag, A. G., Zillhardt, M., Hua, Y.-J., Lengyel, E. und Peter, M. E. (2012). Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. *Int J Cancer 130*, 1787–1797.

[141.] Ioannidis, P., Mahaira, L., Papadopoulou, A., Teixeira, M. R., Heim, S., Andersen, J. A., Evangelou, E., Dafni, U., Pandis, N. und Trangas, T. (2003). 8q24 Copy number gains and expression of the c-myc mRNA stabilizing protein CRD-BP in primary breast carcinomas. *Int J Cancer 104*, 54–59.

[142.] Fakhraldeen, S. A., Clark, R. J., Roopra, A., Chin, E. N., Huang, W., Castorino, J., Wisinski, K. B., Kim, T., Spiegelman, V. S. und Alexander, C. M. (2015). Two Isoforms of the RNA Binding Protein, Coding Region Determinant-binding Protein (CRD-BP/IGF2BP1), Are Expressed in Breast Epithelium and Support Clonogenic Growth of Breast Tumor Cells. *J Biol Chem 290*, 13386–13400.

[143.] Bell, J. L., Turlapati, R., Liu, T., Schulte, J. H. und Hüttelmaier, S. (2015). IGF2BP1 harbors prognostic significance by gene gain and diverse expression in neuroblastoma. *J Clin Oncol 33*, 1285–1293.

[144.] Müeller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Büchler, M., Beger, H. G., Vila, M. R., Adler, G. und Gress, T. M. (1997). Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. *Oncogene 14*, 2729–2733.

[145.] Himoto, T., Kuriyama, S., Zhang, J.-Y., Chan, E. K. L., Nishioka, M. und Tan, E. M. (2005). Significance of autoantibodies against insulin-like growth factor II mRNA-binding proteins in patients with hepatocellular carcinoma. *Int J Oncol 26*, 311–317.

[146.] Himoto, T., Kuriyama, S., Zhang, J.-Y., Chan, E. K. L., Kimura, Y., Masaki, T., Uchida, N., Nishioka, M. und Tan, E. M. (2005). Analyses of autoantibodies against tumor-associated antigens in patients with hepatocellular carcinoma. *Int J Oncol 27*, 1079–1085.

[147.] Noubissi, F. K., Kim, T., Kawahara, T. N., Aughenbaugh, W. D., Berg, E., Longley, B. J., Athar, M. und Spiegelman, V. S. (2014). Role of CRD-BP in the growth of human basal cell carcinoma cells. *J Invest Dermatol 134*, 1718–1724.

[148.] Elcheva, I., Tarapore, R. S., Bhatia, N. und Spiegelman, V. S. (2008). Overexpression of mRNA-binding protein CRD-BP in malignant melanomas. *Oncogene 27*, 5069–5074.

[149.] Stoskus, M., Gineikiene, E., Valceckiene, V., Valatkaite, B., Pileckyte, R. und Griskevicius, L. (2011). Identification of characteristic IGF2BP expression patterns in distinct B-ALL entities. *Blood Cells Mol Dis 46*, 321–326.

[150.] Vainer, G., Vainer-Mosse, E., Pikarsky, A., Shenoy, S. M., Oberman, F., Yeffet, A., Singer, R. H., Pikarsky, E. und Yisraeli, J. K. (2008). A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. *J Pathol 215*, 445–456.

[151.] Cleynen, I., Brants, J. R., Peeters, K., Deckers, R., Debiec-Rychter, M., Sciot, R., Van de Ven, W. J. M. und Petit, M. M. R. (2007). HMGA2 regulates transcription of the Imp2 gene via an intronic regulatory element in cooperation with nuclear factor-kappaB. *Mol Cancer Res 5*, 363–372.

[152.] Lu, M., Nakamura, R. M., Dent, E. D., Zhang, J. Y., Nielsen, F. C., Christiansen, J., Chan, E. K. und Tan, E. M. (2001). Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. *Am J Pathol 159*, 945–953.

[153.] Kessler, S. M., Pokorny, J., Zimmer, V., Laggai, S., Lammert, F., Bohle, R. M. und Kiemer, A. K. (2013). IGF2 mRNA binding protein p62/IMP2-2 in hepatocellular carcinoma: antiapoptotic action is independent of IGF2/PI3K signaling. *Am J Physiol Gastrointest Liver Physiol 304*, G328–G336.

[154.] Ye, S., Song, W., Xu, X., Zhao, X. und Yang, L. (2016). IGF2BP2 promotes colorectal cancer cell proliferation and survival through interfering with RAF-1 degradation by miR-195. *FEBS Lett 590*, 1641–1650.

[155.] Barghash, A., Golob-Schwarzl, N., Helms, V., Haybaeck, J. und Kessler, S. M. (2016). Elevated expression of the IGF2 mRNA binding protein 2 (IGF2BP2/IMP2) is linked to short survival and metastasis in esophageal adenocarcinoma. *Oncotarget 7*, 49743–49750.

[156.] Zhang, J. Y., Chan, E. K., Peng, X. X., Lu, M., Wang, X., Mueller, F. und Tan, E. M. (2001). Autoimmune responses to mRNA binding proteins p62 and Koc in diverse malignancies. *Clin Immunol 100*, 149–156.

[157.] Zhang, J. und Chan, E. K. L. (2002). Autoantibodies to IGF-II mRNA binding protein p62 and overexpression of p62 in human hepatocellular carcinoma. *Autoimmun Rev 1*, 146–153.

[158.] Soo Hoo, L., Zhang, J. Y. und Chan, E. K. L. (2002). Cloning and characterization of a novel 90 kDa 'companion' auto-antigen of p62 overexpressed in cancer. *Oncogene 21*, 5006–5015.

[159.] Shi, F.-D., Zhang, J.-Y., Liu, D., Rearden, A., Elliot, M., Nachtsheim, D., Daniels, T., Casiano, C. A., Heeb, M. J., Chan, E. K. L. und Tan, E. M. (2005). Preferential humoral immune response in prostate cancer to cellular proteins p90 and p62 in a panel of tumor-associated antigens. *Prostate 63*, 252–258.

[160.] Su, Y., Qian, H., Zhang, J., Wang, S., Shi, P. und Peng, X. (2005). The diversity expression of p62 in digestive system cancers. *Clin Immunol 116*, 118–123.

[161.] Liu, W., Wang, P., Li, Z., Xu, W., Dai, L., Wang, K. und Zhang, J. (2009). Evaluation of tumour-associated antigen (TAA) miniarray in immunodiagnosis of colon cancer. *Scand J Immunol 69*, 57–63.

[162.] Liu, X., Ye, H., Li, L., Li, W., Zhang, Y. und Zhang, J.-Y. (2014). Humoral autoimmune responses to insulin-like growth factor II mRNA-binding proteins IMP1 and p62/IMP2 in ovarian cancer. *J Immunol Res 2014*, 326593.

[163.] Zhou, S. L., Yue, W. B., Fan, Z. M., Du, F., Liu, B. C., Li, B., Han, X. N., Ku, J. W., Zhao, X. K., Zhang, P., Cui, J., Zhou, F. Y., Zhang, L. Q., Fan, X. P., Zhou, Y. F., Zhu, L. L., Liu, H. Y. und Wang, L. D. (2014). Autoantibody detection to tumor-associated antigens of P53, IMP1, P16, cyclin B1, P62, C-myc, Survivn, and Koc for the screening of high-risk subjects and early detection of esophageal squamous cell carcinoma. *Dis Esophagus 27*, 790–797.

[164.] Liu, W., Li, Y., Wang, B., Dai, L., Qian, W. und Zhang, J.-Y. (2015). Autoimmune Response to IGF2 mRNA-Binding Protein 2 (IMP2/p62) in Breast Cancer. *Scand J Immunol 81*, 502–507.

[165.] Zhou, S. L., Ku, J. W., Fan, Z. M., Yue, W. B., Du, F., Zhou, Y. F., Liu, Y. L., Li, Y., Tang, S., Hu, Y. L., Hu, X. P., Hou, Z. C., Liu, J., Liu, Y., Feng, X. S. und Wang, L. D. (2015). Detection of autoantibodies to a panel of tumor-associated antigens for the diagnosis values of gastric cardia adenocarcinoma. *Dis Esophagus 28*, 371–379.

[166.] Tan, E. M. und Zhang, J. (2008). Autoantibodies to tumor-associated antigens: reporters from the immune system. *Immunol Rev 222*, 328–340.

[167.] Tan, H. T., Low, J., Lim, S. G. und Chung, M. C. M. (2009). Serum autoantibodies as biomarkers for early cancer detection. *FEBS J 276*, 6880–6904.

[168.] Zhang, J.-Y. und Tan, E. M. (2010). Autoantibodies to tumor-associated antigens as diagnostic biomarkers in hepatocellular carcinoma and other solid tumors. *Expert Rev Mol Diagn 10*, 321–328.

[169.] Liu, W., Peng, B., Lu, Y., Xu, W., Qian, W. und Zhang, J.-Y. (2011). Autoantibodies to tumor-associated antigens as biomarkers in cancer immunodiagnosis. *Autoimmun Rev 10*, 331–335.

[170.] Hong, Y. und Huang, J. (2015). Autoantibodies against tumor-associated antigens for detection of hepatocellular carcinoma. *World J Hepatol 7*, 1581–1585.

[171.] Zhang, J. Y., Chan, E. K., Peng, X. X. und Tan, E. M. (1999). A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. *J Exp Med 189*, 1101–1110.

[172.] Zhang, J.-Y., Casiano, C. A., Peng, X.-X., Koziol, J. A., Chan, E. K. L. und Tan, E. M. (2003). Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. *Cancer Epidemiol Biomarkers Prev 12*, 136–143.

[173.] Zhang, J.-Y., Megliorino, R., Peng, X.-X., Tan, E. M., Chen, Y. und Chan, E. K. L. (2007). Antibody detection using tumor-associated antigen mini-array in immunodiagnosing human hepatocellular carcinoma. *J Hepatol 46*, 107–114.

[174.] Chen, Y., Zhou, Y., Qiu, S., Wang, K., Liu, S., Peng, X.-X., Li, J., Tan, E. M. und Zhang, J.-Y. (2010). Autoantibodies to tumor-associated antigens combined with abnormal alpha-fetoprotein enhance immunodiagnosis of hepatocellular carcinoma. *Cancer Lett 289*, 32–39.

[175.] Gu, G., Sederberg, M. C., Drachenberg, M. R. und South, S. T. (2014). IGF2BP1: a novel IGH translocation partner in B acute lymphoblastic leukemia. *Cancer Genet 207*, 332–334.

[176.] Lukong, K. E., Chang, K.-w., Khandjian, E. W. und Richard, S. (2008). RNA-binding proteins in human genetic disease. *Trends Genet 24*, 416–425.

[177.] Wiemels, J. L., Cazzaniga, G., Daniotti, M., Eden, O. B., Addison, G. M., Masera, G., Saha, V., Biondi, A. und Greaves, M. F. (1999). Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet 354*, 1499–1503.

[178.] Tessier, C. R., Doyle, G. A., Clark, B. A., Pitot, H. C. und Ross, J. (2004). Mammary tumor induction in transgenic mice expressing an RNA-binding protein. *Cancer Res 64*, 209–214.

[179.] Noubissi, F. K., Goswami, S., Sanek, N. A., Kawakami, K., Minamoto, T., Moser, A., Grinblat, Y. und Spiegelman, V. S. (2009). Wnt signaling stimulates transcriptional outcome of the Hedgehog pathway by stabilizing GLI1 mRNA. *Cancer Res 69*, 8572–8578.

[180.] Hamilton, K. E., Noubissi, F. K., Katti, P. S., Hahn, C. M., Davey, S. R., Lundsmith, E. T., Klein-Szanto, A. J., Rhim, A. D., Spiegelman, V. S. und Rustgi, A. K. (2013). IMP1 promotes tumor growth, dissemination and a tumor-initiating cell phenotype in colorectal cancer cell xenografts. *Carcinogenesis 34*, 2647–2654.

[181.] Kugel, S., Sebastián, C., Fitamant, J., Ross, K. N., Saha, S. K., Jain, E., Gladden, A., Arora, K. S., Kato, Y., Rivera, M. N., Ramaswamy, S., Sadreyev, R. I., Goren, A., Deshpande, V., Bardeesy, N. und Mostoslavsky, R. (2016). SIRT6 Suppresses Pancreatic Cancer through Control of Lin28b. *Cell 165*, 1401–1415.

[182.] Stoskus, M., Eidukaite, A. und Griskevicius, L. (2016). Defining the significance of IGF2BP1 overexpression in t(12;21)(p13;q22)-positive leukemia REH cells. *Leuk Res 47*, 16–21.

[183.] Zhou, J., Bi, C., Ching, Y. Q., Chooi, J.-Y., Lu, X., Quah, J. Y., Toh, S. H.-M., Chan, Z.-L., Tan, T. Z., Chong, P. S. und Chng, W.-J. (2017). Inhibition of LIN28B impairs leukemia cell growth and metabolism in acute myeloid leukemia. *J Hematol Oncol 10*, 138.

[184.] Sparanese, D. und Lee, C. H. (2007). CRD-BP shields c-myc and MDR-1 RNA from endonucleolytic attack by a mammalian endoribonuclease. *Nucleic Acids Res 35*, 1209–1221.

[185.] Zirkel, A., Lederer, M., Stöhr, N., Pazaitis, N. und Hüttelmaier, S. (2013). IGF2BP1 promotes mesenchymal cell properties and migration of tumor-derived cells by enhancing the expression of LEF1 and SNAI2 (SLUG). *Nucleic Acids Res 41*, 6618–6636.

[186.] Haigis, K. M. (2017). KRAS Alleles: The Devil Is in the Detail. *Trends Cancer 3*, 686–697.

[187.] Pasca di Magliano, M. und Hebrok, M. (2003). Hedgehog signalling in cancer formation and maintenance. *Nat Rev Cancer 3*, 903–911.

[188.] Hanna, A. und Shevde, L. A. (2016). Hedgehog signaling: modulation of cancer properies and tumor mircroenvironment. *Mol Cancer 15*, 24.

[189.] Pfannkuche, K., Summer, H., Li, O., Hescheler, J. und Dröge, P. (2009). The high mobility group protein HMGA2: a co-regulator of chromatin structure and pluripotency in stem cells? *Stem Cell Rev 5*, 224–230.

[190.] Shyh-Chang, N. und Daley, G. Q. (2013). Lin28: primal regulator of growth and metabolism in stem cells. *Cell Stem Cell 12*, 395–406.

[191.] Stoskus, M., Vaitkeviciene, G., Eidukaite, A. und Griskevicius, L. (2016). ETV6/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive acute lymphoblastic leukemia. *Blood Cells Mol Dis 57*, 30–34.

[192.] Hoesel, B. und Schmid, J. A. (2013). The complexity of NF-κB signaling in inflammation and cancer. *Mol Cancer 12*, 86.

[193.] Gyrd-Hansen, M. und Meier, P. (2010). IAPs: from caspase inhibitors to modulators of NF-kappaB, inflammation and cancer. *Nat Rev Cancer 10*, 561–574.

[194.] Holohan, C., Van Schaeybroeck, S., Longley, D. B. und Johnston, P. G. (2013). Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer 13*, 714–726.

[195.] Oberman, F., Rand, K., Maizels, Y., Rubinstein, A. M. und Yisraeli, J. K. (2007). VICKZ proteins mediate cell migration via their RNA binding activity. *RNA 13*, 1558–1569.

[196.] Senbanjo, L. T. und Chellaiah, M. A. (2017). CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. *Front Cell Dev Biol 5*, 18.

[197.] Yamaguchi, H. und Condeelis, J. (2007). Regulation of the actin cytoskeleton in cancer cell migration and invasion. *Biochim Biophys Acta 1773*, 642–652.

[198.] Stöhr, N. und Hüttelmaier, S. (2012). IGF2BP1: a post-transcriptional "driveröf tumor cell migration. *Cell Adh Migr 6*, 312–318.

[199.] Gu, W., Katz, Z., Wu, B., Park, H. Y., Li, D., Lin, S., Wells, A. L. und Singer, R. H. (2012). Regulation of local expression of cell adhesion and motility-related mRNAs in breast cancer cells by IMP1/ZBP1. *J Cell Sci 125*, 81–91.

[200.] Lapidus, K., Wyckoff, J., Mouneimne, G., Lorenz, M., Soon, L., Condeelis, J. S. und Singer, R. H. (2007). ZBP1 enhances cell polarity and reduces chemotaxis. *J Cell Sci 120*, 3173–3178.

[201.] Wang, W., Goswami, S., Lapidus, K., Wells, A. L., Wyckoff, J. B., Sahai, E., Singer, R. H., Segall, J. E. und Condeelis, J. S. (2004). Identification and testing of a gene expression signature of invasive carcinoma cells within primary mammary tumors. *Cancer Res 64*, 8585–8594.

[202.] Lambert, A. W., Pattabiraman, D. R. und Weinberg, R. A. (2017). Emerging Biological Principles of Metastasis. *Cell 168*, 670–691.

[203.] AbBRAMS, H. L., SPIRO, R. und GOLDSTEIN, N. (1950). Metastases in carcinoma; analysis of 1000 autopsied cases. *Cancer 3*, 74–85.

[204.] Budczies, J., von Winterfeld, M., Klauschen, F., Bockmayr, M., Lennerz, J. K., Denkert, C., Wolf, T., Warth, A., Dietel, M., Anagnostopoulos, I., Weichert, W., Wittschieber, D. und Stenzinger, A. (2015). The landscape of metastatic progression patterns across major human cancers. *Oncotarget 6*, 570–583.

[205.] Nisticò, P., Bissell, M. J. und Radisky, D. C. (2012). Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. *Cold Spring Harbor Perspect Biol 4*, DOI: 10.1101/cshperspect.a011908.

[206.] Tarin, D., Thompson, E. W. und Newgreen, D. F. (2005). The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res 65*, 5996–6000, discussion 6000–1.

[207.] Hay, E. D. (1995). An overview of epithelio-mesenchymal transformation. *Acta Anat 154*, 8–20.

[208.] Nieto, M. A., Huang, R. Y.-J., Jackson, R. A. und Thiery, J. P. (2016). EMT: 2016. *Cell 166*, 21–45.

[209.] Kalluri, R. und Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *J Clin Invest 119*, 1420–1428.

[210.] Lamouille, S., Xu, J. und Derynck, R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol 15*, 178–196.

[211.] Hanahan, D. und Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell 144*, 646–674.

[212.] Vogelstein, B., Papadopoulos, N., Velculescu, V. E., Zhou, S., Diaz, L. A. und Kinzler, K. W. (2013). Cancer genome landscapes. *Science 339*, 1546–1558.

[213.] Uhlen, M., Zhang, C., Lee, S., Sjöstedt, E., Fagerberg, L., Bidkhori, G., Benfeitas, R., Arif, M., Liu, Z., Edfors, F., Sanli, K., von Feilitzen, K., Oksvold, P., Lundberg, E., Hober, S., Nilsson, P., Mattsson, J., Schwenk, J. M., Brunnström, H., Glimelius, B., Sjöblom, T., Edqvist, P.-H., Djureinovic, D., Micke, P., Lindskog, C., Mardinoglu, A. und Ponten, F. (2017). A pathology atlas of the human cancer transcriptome. *Science 357*, DOI: 10.1126/science.aan2507.

[214.] Kim, M. Y., Hur, J. und Jeong, S. (2009). Emerging roles of RNA and RNA-binding protein network in cancer cells. *BMB Rep 42*, 125–130.

[215.] Wurth, L. und Gebauer, F. (2015). RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim Biophys Acta 1849*, 881–886.

[216.] Liao, B., Patel, M., Hu, Y., Charles, S., Herrick, D. J. und Brewer, G. (2004). Targeted knockdown of the RNA-binding protein CRD-BP promotes cell proliferation via an insulin-like growth factor II-dependent pathway in human K562 leukemia cells. *J Biol Chem 279*, 48716–48724.

[217.] Vogelstein, B. und Kinzler, K. W. (2004). Cancer genes and the pathways they control. *Nat Med 10*, 789–799.

[218.] Giancotti, F. G. (2014). Deregulation of cell signaling in cancer. *FEBS Lett 588*, 2558–2570.

[219.] Kugel, S. und Mostoslavsky, R. (2014). Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci 39*, 72–81.

[220.] Meyer, N. und Penn, L. Z. (2008). Reflecting on 25 years with MYC. *Nat Rev Cancer 8*, 976–990.

[221.] Dang, C. V. (2012). MYC on the path to cancer. *Cell 149*, 22–35.

[222.] Vervoorts, J., Lüscher-Firzlaff, J. und Lüscher, B. (2006). The ins and outs of MYC regulation by posttranslational mechanisms. *J Biol Chem 281*, 34725–34729.

[223.] Farrell, A. S. und Sears, R. C. (2014). MYC degradation. *Cold Spring Harb Perspect Med 4*, DOI: 10.1101/cshperspect.a014365.

[224.] Salghetti, S. E., Kim, S. Y. und Tansey, W. P. (1999). Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc. *EMBO J 18*, 717–726.

[225.] Palanichamy, J. K., Tran, T. M., Howard, J. M., Contreras, J. R., Fernando, T. R., Sterne-Weiler, T., Katzman, S., Toloue, M., Yan, W., Basso, G., Pigazzi, M., Sanford, J. R. und Rao, D. S. (2016). RNA-binding protein IGF2BP3 targeting of oncogenic transcripts promotes hematopoietic progenitor proliferation. *J Clin Invest 126*, 1495–1511.

[226.] Brown, D. C. und Gatter, K. C. (2002). Ki67 protein: the immaculate deception? *Histopathology 40*, 2–11.

[227.] Li, L. T., Jiang, G., Chen, Q. und Zheng, J. N. (2015). Ki67 is a promising molecular target in the diagnosis of cancer (review). *Mol Med Rep 11*, 1566–1572.

[228.] Luo, Y., Ren, F., Liu, Y., Shi, Z., Tan, Z., Xiong, H., Dang, Y. und Chen, G. (2015). Clinicopathological and prognostic significance of high Ki-67 labeling index in hepatocellular carcinoma patients: a meta-analysis. *Int J Clin Exp Med 8*, 10235–10247.

[229.] Schlüter, C., Duchrow, M., Wohlenberg, C., Becker, M. H., Key, G., Flad, H. D. und Gerdes, J. (1993). The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. *J Cell Biol 123*, 513–522.

[230.] Kausch, I., Lingnau, A., Endl, E., Sellmann, K., Deinert, I., Ratliff, T. L., Jocham, D., Sczakiel, G., Gerdes, J. und Böhle, A. (2003). Antisense treatment against Ki-67 mRNA inhibits proliferation and tumor growth in vitro and in vivo. *Int J Cancer 105*, 710–716.

[231.] Kausch, I., Jiang, H., Brocks, C., Bruderek, K., Krüger, S., Sczakiel, G., Jocham, D. und Böhle, A. (2004). Ki-67-directed antisense therapy in an orthotopic renal cell carcinoma model. *Eur Urol 46*, 118–24, discussion 124–5.

[232.] Zheng, J.-N., Sun, Y.-F., Pei, D.-S., Liu, J.-J., Sun, X.-Q., Chen, J.-C., Cai, W.-Q., Li, W. und Cao, J.-Y. (2005). Anti-Ki-67 peptide nucleic acid affects the proliferation and apoptosis of human renal carcinoma cells in vitro. *Life Sci 76*, 1873–1881.

[233.] Zheng, J.-N., Ma, T.-X., Cao, J.-Y., Sun, X.-Q., Chen, J.-C., Li, W., Wen, R.-M., Sun, Y.-F. und Pei, D.-S. (2006). Knockdown of Ki-67 by small interfering RNA leads to inhibition of proliferation and induction of apoptosis in human renal carcinoma cells. *Life Sci 78*, 724–729.

[234.] Rahmanzadeh, R., Rai, P., Celli, J. P., Rizvi, I., Baron-Lühr, B., Gerdes, J. und Hasan, T. (2010). Ki-67 as a molecular target for therapy in an in vitro three-dimensional model for ovarian cancer. *Cancer Res 70*, 9234–9242.

[235.] Christov, C. P., Gardiner, T. J., Szüts, D. und Krude, T. (2006). Functional requirement of noncoding Y RNAs for human chromosomal DNA replication. *Mol Cell Biol 26*, 6993–7004.

[236.] Langley, A. R., Chambers, H., Christov, C. P. und Krude, T. (2010). Ribonucleoprotein particles containing non-coding Y RNAs, Ro60, La and nucleolin are not required for Y RNA function in DNA replication. *PLoS One 5*, e13673.

[237.] Zhang, A. T., Langley, A. R., Christov, C. P., Kheir, E., Shafee, T., Gardiner, T. J. und Krude, T. (2011). Dynamic interaction of Y RNAs with chromatin and initiation proteins during human DNA replication. *J Cell Sci 124*, 2058–2069.

[238.] Collart, C., Christov, C. P., Smith, J. C. und Krude, T. (2011). The midblastula transition defines the onset of Y RNA-dependent DNA replication in Xenopus laevis. *Mol Cell Biol 31*, 3857–3870.

[239.] Kowalski, M. P. und Krude, T. (2015). Functional roles of non-coding Y RNAs. *Int J Biochem Cell Biol 66*, 20–29.

[240.] Stepanenko, A. A. und Dmitrenko, V. V. (2015). HEK293 in cell biology and cancer research: phenotype, karyotype, tumorigenicity, and stress-induced genome-phenotype evolution. *Gene 569*, 182–190.

[241.] Marchese, D., de Groot, N. S., Lorenzo Gotor, N., Livi, C. M. und Tartaglia, G. G. (2016). Advances in the characterization of RNA-binding proteins. *Wiley Interdiscip Rev RNA 7*, 793–810.

[242.] Wang, W., Wyckoff, J. B., Frohlich, V. C., Oleynikov, Y., Hüttelmaier, S., Zavadil, J., Cermak, L., Bottinger, E. P., Singer, R. H., White, J. G., Segall, J. E. und Condeelis, J. S. (2002). Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res 62*, 6278–6288.

[243.] Manieri, N. A., Mack, M. R., Himmelrich, M. D., Worthley, D. L., Hanson, E. M., Eckmann, L., Wang, T. C. und Stappenbeck, T. S. (2015). Mucosally transplanted mesenchymal stem cells stimulate intestinal healing by promoting angiogenesis. *J Clin Invest 125*, 3606–3618.

[244.] Condeelis, J. und Singer, R. H. (2005). How and why does beta-actin mRNA target? *Biol Cell 97*, 97–110.

[245.] Rodriguez-Boulan, E. und Macara, I. G. (2014). Organization and execution of the epithelial polarity programme. *Nat Rev Mol Cell Biol 15*, 225–242.

[246.] Li, R. und Gundersen, G. G. (2008). Beyond polymer polarity: how the cytoskeleton builds a polarized cell. *Nat Rev Mol Cell Biol 9*, 860–873.

[247.] Nelson, C. E. und Gersbach, C. A. (2016). Engineering Delivery Vehicles for Genome Editing. *Annu Rev Chem Biomol Eng 7*, 637–662.

[248.] Sánchez-Rivera, F. J. und Jacks, T. (2015). Applications of the CRISPR-Cas9 system in cancer biology. *Nat Rev Cancer 15*, 387–395.

[249.] Fellmann, C., Gowen, B. G., Lin, P.-C., Doudna, J. A. und Corn, J. E. (2017). Cornerstones of CRISPR-Cas in drug discovery and therapy. *Nat Rev Drug Discov 16*, 89–100.

[250.] Chira, S., Gulei, D., Hajitou, A., Zimta, A.-A., Cordelier, P. und Berindan-Neagoe, I. (2017). CRISPR/Cas9: Transcending the Reality of Genome Editing. *Mol Ther Nucleic Acids 7*, 211–222.

[251.] Kole, R., Krainer, A. R. und Altman, S. (2012). RNA therapeutics: beyond RNA interference and antisense oligonucleotides. *Nat Rev Drug Discov 11*, 125–140.

[252.] Kumar, S. R., Markusic, D. M., Biswas, M., High, K. A. und Herzog, R. W. (2016). Clinical development of gene therapy: results and lessons from recent successes. *Mol Ther Methods Clin Dev 3*, 16034.

[253.] Watts, J. K. und Corey, D. R. (2012). Silencing disease genes in the laboratory and the clinic. *J Pathol 226*, 365–379.

[254.] Naldini, L. (2015). Gene therapy returns to centre stage. *Nature 526*, 351–360.

[255.] Weiner, L. M., Murray, J. C. und Shuptrine, C. W. (2012). Antibody-based immunotherapy of cancer. *Cell 148*, 1081–1084.

[256.] Guo, K., Li, J., Tang, J. P., Tan, C. P. B., Hong, C. W., Al-Aidaroos, A. Q. O., Varghese, L., Huang, C. und Zeng, Q. (2011). Targeting intracellular oncoproteins with antibody therapy or vaccination. *Sci Transl Med 3*, 99ra85.

[257.] Trenevska, I., Li, D. und Banham, A. H. (2017). Therapeutic Antibodies against Intracellular Tumor Antigens. *Front Immunol 8*, 1001.

[258.] Dubrovsky, L., Dao, T., Gejman, R. S., Brea, E. J., Chang, A. Y., Oh, C. Y., Casey, E., Pankov, D. und Scheinberg, D. A. (2016). T cell receptor mimic antibodies for cancer therapy. *Oncoimmunology 5*, e1049803.

[259.] Doroshow, D. B., Eder, J. P. und LoRusso, P. M. (2017). BET inhibitors: a novel epigenetic approach. *Ann Oncol 28*, 1776–1787.

[260.] Shi, J. und Vakoc, C. R. (2014). The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell 54*, 728–736.

[261.] Xu, Y. und Vakoc, C. R. (2017). Targeting Cancer Cells with BET Bromodomain Inhibitors. *Cold Spring Harb Perspect Med 7*, DOI: 10.1101/cshperspect.a026674.

[262.] Gordon, M. D. und Nusse, R. (2006). Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *J Biol Chem 281*, 22429–22433.

[263.] Roush, S. und Slack, F. J. (2008). The let-7 family of microRNAs. *Trends Cell Biol 18*, 505–516.

[264.] Messerschmidt, D. M., Knowles, B. B. und Solter, D. (2014). DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. *Genes Dev 28*, 812–828.

[265.] Clevers, H., Loh, K. M. und Nusse, R. (2014). Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science 346*, 1248012.

[266.] Peifer, M. und Polakis, P. (2000). Wnt signaling in oncogenesis and embryogenesis–a look outside the nucleus. *Science 287*, 1606–1609.

[267.] Polakis, P. (2012). Wnt signaling in cancer. *Cold Spring Harbor Perspect Biol 4*, DOI: 10.1101/cshperspect.a008052.

[268.] Clevers, H. und Nusse, R. (2012). Wnt/β-catenin signaling and disease. *Cell 149*, 1192–1205.

[269.] Holland, J. D., Klaus, A., Garratt, A. N. und Birchmeier, W. (2013). Wnt signaling in stem and cancer stem cells. *Curr Opin Cell Biol 25*, 254–264.

[270.] Ring, A., Kim, Y.-M. und Kahn, M. (2014). Wnt/catenin signaling in adult stem cell physiology and disease. *Stem Cell Rev 10*, 512–525.

[271.] Zhan, T., Rindtorff, N. und Boutros, M. (2017). Wnt signaling in cancer. *Oncogene 36*, 1461–1473.

[272.] Büssing, I., Slack, F. J. und Grosshans, H. (2008). let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med 14*, 400–409.

[273.] Ehrlich, M. (2009). DNA hypomethylation in cancer cells. *Epigenomics 1*, 239–259.

[274.] Park, Y. J., Claus, R., Weichenhan, D. und Plass, C. (2011). Genome-wide epigenetic modifications in cancer. *Prog Drug Res 67*, 25–49.

[275.] Pardal, R., Clarke, M. F. und Morrison, S. J. (2003). Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer 3*, 895–902.

[276.] Ben-Porath, I., Thomson, M. W., Carey, V. J., Ge, R., Bell, G. W., Regev, A. und Weinberg, R. A. (2008). An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet 40*, 499–507.

[277.] Ma, Y., Zhang, P., Wang, F., Yang, J., Yang, Z. und Qin, H. (2010). The relationship between early embryo development and tumourigenesis. *J Cell Mol Med 14*, 2697–2701.

[278.] Kim, J. und Orkin, S. H. (2011). Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine. *Genome Med 3*, 75.

[279.] Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M. A. und Dick, J. E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature 367*, 645–648.

[280.] Bomken, S., Fiser, K., Heidenreich, O. und Vormoor, J. (2010). Understanding the cancer stem cell. *Br J Cancer 103*, 439–445.

[281.] Peitzsch, C., Tyutyunnykova, A., Pantel, K. und Dubrovska, A. (2017). Cancer stem cells: The root of tumor recurrence and metastases. *Semin Cancer Biol 44*, 10–24.

[282.] Zhao, J. (2016). Cancer stem cells and chemoresistance: The smartest survives the raid. *Pharmacol Ther 160*, 145–158.

[283.] Bandhavkar, S. (2016). Cancer stem cells: a metastasizing menace! *Cancer Med 5*, 649–655.

Thesen

1. Ribonukleinsäure(RNA)-bindende Proteine (RBPs) sind zentrale Regulatoren des RNA-Metabolismus.

2. Das *Insulin-like growth factor 2 mRNA-binding protein 1* (IGF2BP1) gehört zu einer Familie stark konservierter RBPs und zeigt einen onkofetalen Expressionscharakter.

3. IGF2BP1 entfaltet seine Wirkungen im physiologischen sowie im pathologischen Kontext durch Beeinflussung der Stabilität, Lokalisation und Translation zahlreicher mRNAs, deren Genprodukte zu einem großen Teil in Zusammenhang mit malignen Tumorerkrankungen des Menschen gebracht werden können.

4. IGF2BP1 ist das am höchsten exprimierte RBP im humanen hepatozellulären Karzinom (HCC).

5. Eine transiente IGF2BP1-Depletion führt zu einer Reduktion der Proliferation und zu einer Steigerung der Apoptose in humanen HCC-Zelllinien *in vitro*.

6. Eine stabile shRNA(*short hairpin-RNA*)-vermittelte IGF2BP1-Depletion in der humanen HCC-Zelllinie Hep G2 führt zu einer mittels nichtinvasiver Fluoreszenzbildgebung messbaren Reduktion des Wachstums von subkutanen *xenograft*-Tumoren in thymusaplastischen Mäusen *in vivo*.

7. IGF2BP1 stabilisiert die Boten-RNAs (mRNAs) des Protoonkogens MYC (*myc proto-oncogene protein*) und des Proliferationsmarkers Ki-67 (*marker of proliferation ki-67*) in humanen HCC-Zelllinien *in vitro*.

8. IGF2BP1 ist essenziell für die Aufrechterhaltung eines mesenchymalen Expressionsprofils und der damit verbundenen Zellmorphologie in verschiedenen humanen Tumorzelllinien *in vitro*.

9. IGF2BP1 stabilisiert die mRNA des Transkriptionsfaktors LEF1 (*lymphoid enhancer binding factor 1*) in verschiedenen humanen Tumorzelllinien *in vitro*.

10. Die überlebens- und migrationsfördernde Wirkung von IGF2BP1 in zahlreichen unterschiedlichen humanen Tumorzelllinien *in vitro* und *in vivo* legt nahe, dass IGF2BP1 einen von der Tumorentität unabhängigen protumorigenen Faktor darstellt und als vielversprechendes Ziel zukünftiger Tumortherapien anzusehen ist.

Publikationsteil

Manuskript 1

Bell JL, Wächter K, Mühleck B, Pazaitis N,
Köhn M, Lederer M, Hüttelmaier S.

Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression?

Cellular and Molecular Life Sciences
Aug 2013, 70:2657-75.

Cell. Mol. Life Sci. (2013) 70:2657–2675
DOI 10.1007/s00018-012-1186-z

Cellular and Molecular Life Sciences

REVIEW

Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression?

Jessica L. Bell · Kristin Wächter · Britta Mühleck · Nikolaos Pazaitis · Marcel Köhn · Marcell Lederer · Stefan Hüttelmaier

Received: 1 August 2012 / Revised: 28 September 2012 / Accepted: 1 October 2012 / Published online: 16 October 2012

Abstract The insulin-like growth factor-2 mRNA-binding proteins 1, 2, and 3 (IGF2BP1, IGF2BP2, IGF2BP3) belong to a conserved family of RNA-binding, oncofetal proteins. Several studies have shown that these proteins act in various important aspects of cell function, such as cell polarization, migration, morphology, metabolism, proliferation and differentiation. In this review, we discuss the IGF2BP family's role in cancer biology and how this correlates with their proposed functions during embryogenesis. IGF2BPs are mainly expressed in the embryo, in contrast with comparatively lower or negotiable levels in adult tissues. IGF2BP1 and IGF2BP3 have been found to be re-expressed in several aggressive cancer types. Control of IGF2BPs' expression is not well understood; however, let-7 microRNAs, β-catenin (CTNNB1) and MYC have been proposed to be involved in their regulation. In contrast to many other RNA-binding proteins, IGF2BPs are almost exclusively observed in the cytoplasm where they associate with target mRNAs in cytoplasmic ribonucleoprotein complexes (mRNPs). During development, IGF2BPs are required for proper nerve cell migration and morphological development, presumably involving the control of cytoskeletal remodeling and dynamics, respectively. Likewise, IGF2BPs modulate cell polarization, adhesion and migration in tumor-derived cells. Moreover, they are highly associated with cancer metastasis and the expression of oncogenic factors (KRAS, MYC and MDR1). However, a pro-metastatic role of IGF2BPs remains controversial due to the lack of 'classical' in vivo studies. Nonetheless, IGF2BPs could provide valuable targets in cancer treatment with many of their in vivo roles to be fully elucidated.

Keywords Cancer · IGF2BP · IMP · CRD-BP · VICKZ · KOC · MYC · Migration · Proliferation

Abbreviations

Acc. no.	Accession number
CRD	Coding region stability determinant
CRD-BP	Crd binding protein (IGF2BP gene alias)
dIMP	*Drosophila* IGF2BP
IGF2BP	Insulin-like growth factor 2 mRNA-binding protein
IMP	IGF2 mRNA binding protein (gene alias)
KH	hnRNP-K homology domain
PAR-CLIP	Photoactivatable ribonucleoside-enhanced crosslinking and immuno-precipitation
RBP	RNA-binding protein
RIP	RNA immunoprecipitation
RNP	Ribonucleoprotein (granule)
RRM	RNA-recognition motif
T2D	Type 2 diabetes
VICKZ	*V*g1RBP/Vera *I*GF2BP *C*RD-BP *K*OC *Z*BP1 (gene family alias)

Introduction

The insulin-like growth factor-2 mRNA-binding proteins 1, 2, and 3 (gene symbols: IGF2BP1, IGF2BP2, IGF2BP3) belong to a highly conserved protein family, which as their

Electronic supplementary material The online version of this article (doi:10.1007/s00018-012-1186-z) contains supplementary material, which is available to authorized users.

J. L. Bell · K. Wächter · B. Mühleck · N. Pazaitis · M. Köhn · M. Lederer · S. Hüttelmaier (✉)
Section for Molecular Cell Biology,
Institute of Molecular Medicine, Martin-Luther-University Halle, 06120 Halle, Germany
e-mail: stefan.huettelmaier@medizin.uni-halle.de

name suggests can bind RNA and influence their transcript target's fate. Nomenclature of the IGF2BP protein family remains confusing due to the many synonyms used throughout recent literature including: IMP, CRD-BP, VICKZ, ZBP, Vg1RBP/Vera or KOC. These synonyms may reflect the evolution of the various fields of IGF2BP family research which suggest that these RNA-binding proteins (RBPs) modulate important aspects of cell function during development and in cancer. In this review, we discuss the rapidly growing research into the IGF2BP family's involvement in cancer biology and the mechanisms by which high expression of these RBPs could cause an aggressive malignancy phenotype. We also discuss the molecular mechanisms by which these proteins facilitate their various functions, their role in cell migration and the need for better research tools to facilitate the next generation of IGF2BP research.

In mammals, the canonical structures of the three IGF2BP proteins are strikingly similar in order and spacing of domains (Fig. 1a), leading to proteins of calculated molecular weights ranging from 58 to 66 kDa. There is over 56 % amino acid sequence identity between the three proteins with greater degree of similarity seen within the protein domains. These similarities suggest that the proteins share biochemical functions. Notably, IGF2BP1 and 3 show a higher identity of 73 % with each other (Fig. 1b). All three proteins carry two RNA-recognition motifs (RRMs) in their *N*-terminal part and four hnRNP-K homology (KH) domains in the *C*-terminal region. Notably, only one IGF2BP ortholog has been reported in *Xenopus*, termed Vg1RBP/Vera. This shows the highest homology to mammalian IGF2BP3. In *Drosophila*, a protein lacking the *N*-terminal RRM domain but comprising four KH-domains has been suggested as *Drosophila* IGF2BP (*dIMP*).

Regardless of organism or cell type, all members of the IGF2BP protein family have been shown to bind RNA, whereas an association with DNA has only been reported once for the *Xenopus* variant of protein [1]. In vitro studies revealed that RNA-binding is mainly facilitated via the KH-domains [2], although the RRM-domains could potentially contribute to the stabilization of IGF2BP-RNA complexes with target-dependent in vitro half-life greater than 2 h [3]. Recent structural analyses of human IGF2BP1 KH-domains 3 and 4 suggest the formation of an anti-parallel pseudo-dimer conformation in which KH3 and KH4 each contact the targeted RNA [4]. Although final proof of this hypothesis requires protein–RNA co-crystals, these findings suggest that IGF2BPs force associated transcripts into a specific conformation. In light of the surprisingly long half-life of IGF2BP-RNA complexes in vitro, this provides evidence for an essential role of IGF2BPs in promoting the formation of 'stable' protein–RNA complexes.

The ribonucleoprotein (RNP) granule connection

IGF2BPs are predominately cytoplasmic, usually with a granular appearance. A nuclear role of IGF2BPs remains controversial, although there is evidence that IGF2BPs may already associate with their target mRNAs at their site of transcription [5–7]. In agreement, IGF2BPs were observed in the nucleus of spermatogenic cells and were suggested to comprise nuclear export signals [8]. In the cytoplasm, IGF2BPs form distinct ribonucleoprotein (RNP) granules which are enriched in the peri-nuclear region but are also observed in neurites of developing neurons supporting a role of IGF2BPs in promoting mRNA localization [2, 9]. Like most RNA-binding proteins (RBPs), IGF2BPs associate with various other RBPs in an RNA-dependent manner [10, 11]. However, in contrast to other proteins involved in the control of cytoplasmic mRNA fate, IGF2BPs apparently associate predominantly with 'virgin' mRNAs. This notion is supported by the observed association with components of the exon junction complex (EJC) as well as CBP80 whereas IGF2BPs do not copurify with eIF4E protein [10, 11]. Hence, IGF2BPs apparently 'cage' their target mRNAs in cytoplasmic protein–RNA complexes, termed mRNPs. This prevents the premature decay of specific target transcripts, for instance, CD44, MYC, PTEN or BTRC, presumably by limiting the release of protein-associated transcripts [12–16]. IGF2BP-directed recruitment of targeted mRNAs to cytoplasmic mRNPs is also consistent with their role in controlling mRNA translation and transport. The formation of stable protein–RNA association, as suggested based on in vitro studies [3], provides a bona fide mechanism to prevent promiscuous translation of transported mRNAs. The stable 'caging' of transported mRNAs allows for their 'long-distance' transport as well as transient storage. Consistently, IGF2BPs have been shown to direct the localization and spatially restrict translation of the β-actin (ACTB) mRNA to exploratory growth cones of developing neuronal cells [6, 9]. Moreover, IGF2BP1 was shown to stabilize its target transcripts during cellular stress when global mRNA translation is severely reduced and mRNAs together with RBPs are recruited to transiently forming stress granules [17].

However, the efficient 'caging' of transcripts in cytoplasmic mRNPs requires signaling events allowing the controlled release of silenced mRNAs to induce protein synthesis or mRNA decay, respectively. In the case of IGF2BPs, this regulation is likely to involve phosphorylation of the proteins. Src-directed tyrosine phosphorylation in the linker region connecting KH-domains 2 and 3 of IGF2BP1 was proposed to induce the disassembly of cytoplasmic mRNPs and activate the translation of the ACTB mRNA [6]. Phosphorylation of Vg1RBP/Vera by

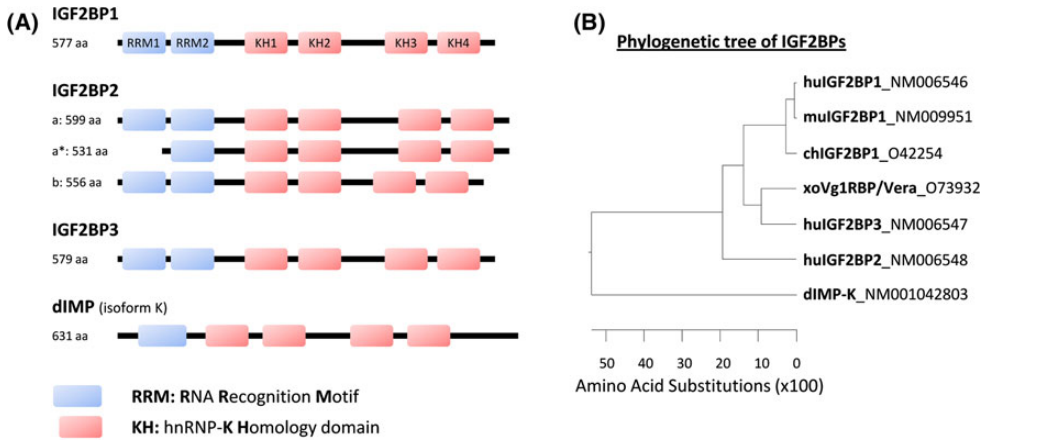


Fig. 1 The IGF2BP protein family. **a** Domain structure of humanIGF2BPs and additionally, the IMP ortholog (*dIMP*, isoform K) of *Drosophila melanogaster*. RNA-binding domains comprising RNA recognition motifs (*RRM*s, *blue*) and hnRNP-K homology domains (*KH*, *red*). The following proteins are shown: *IGF2BP1* (Acc. no.: NM006546), the longest IGF2BP1 protein isoform; *IGF2BP2-a* (Acc. no.: NM006548), the longest IGF2BP2 protein isoform; *IGF2BP2-a** (no Acc. no. available), truncated IGF2BP2-a resulting from leaky scanning during translation initiation [58]; *IGF2BP2-b* (Acc. no.: NM001007225.1), spliced IGF2BP-a lacking exon 10; *IGF2BP3* (Acc. no.: NM006547), the only reported variant of this paralogue; *dIMP* (Acc. no.: NM001042803), variant K of the *Drosophila melanogaster* ortholog of IGF2BPs. **b** Phylogentic tree indicating amino acid substitutions of distinct IGF2BP paralogues from different species (*hu* human, *mu* murine, *ch* chicken, *xo Xenopus*, *d Drosophila*). The accession number for each ortholog is indicated

MAPKs was suggested to modulate the release of Vg1 mRNA from mRNPs localized to the vegetal cortex during meiotic maturation [18]. Although not linked to mRNA localization, it was recently shown that phosphorylation of IGF2BP2 in the *N*-terminal linker region connecting RRM2 and KH1 by mTORC1 promotes the association with the leader3 5′-UTR of IGF2 resulting in elevated IGF2 protein synthesis [19]. Hence, the post-translational modifications of IGF2BPs emerge as an essential trigger modulating their role in controlling the cytoplasmic fate of specific transcripts. The underlying mechanism of these regulations would fit well with the idea that some target mRNAs of IGF2BPs are ‘caged’ in relatively stable cytoplasmic mRNPs (Fig. 2). However, why do we observe translational silencing of some target mRNAs whereas the association of IGF2BPs with other transcripts prevents their premature decay? Essentially, one could envision two mechanisms that are likely to cooperate in directing cytoplasmic mRNA fate. On the one hand, the protein composition of regulatory mRNPs could determine mRNA fate. Although this assumption remains largely speculative, transcript-specific mRNP compositions have been proposed [11]. Alternatively, final mRNA fate could be determined exclusively by cis-determinants of the regulated transcripts. In this scenario, the exclusive role of IGF2BPs would be to ensure the spatiotemporal execution of ‘final transcript fate’ by controlling the release of regulated transcripts from cytoplasmic mRNPs. Although not formally proven, this model is in agreement with various observations. For instance, IGF2BP1 was proposed to shield the BTRC (beta-transducin repeat containing E3 ubiquitin protein ligase) mRNA from microRNA-mediated degradation in the cytoplasm [20]. Likewise, IGF2BP1 was proposed to protect the MYC and MDR1 mRNAs from endonucleolytic attack [12, 21]. Moreover, it was proposed that the potential association of IGF2BPs with their target mRNAs already at the site of transcription provides an efficient mechanism to direct cytoplasmic mRNA fate by directing the assembly of mRNPs before cytoplasmic entry [5–7]. Consistently, IGF2BP1 was observed in ‘virgin’ mRNPs [10, 11]. Taken together, this suggests that IGF2BPs start controlling transcript fate right after transcription and modulate the rate at which associated transcripts encounter the translational apparatus, the decay machinery or microRNA attack by recruiting regulated transcripts in cytoplasmic mRNPs (Fig. 2). Although there is substantially more work required to clarify the molecular mechanisms by which IGF2BPs modulate mRNA fate, their role certainly involves cytoplasmic mRNPs and requires extensive control by cytoplasmic signaling.

The ‘RNA-binding puzzle’ of IGF2BPs

Despite various studies indicating a specific role of IGF2BPs in controlling the localization, translation or turnover of specific mRNA targets (Table 1), a comprehensive identification of targeted transcripts is still lacking. PAR-CLIP and RIP studies have suggested more than

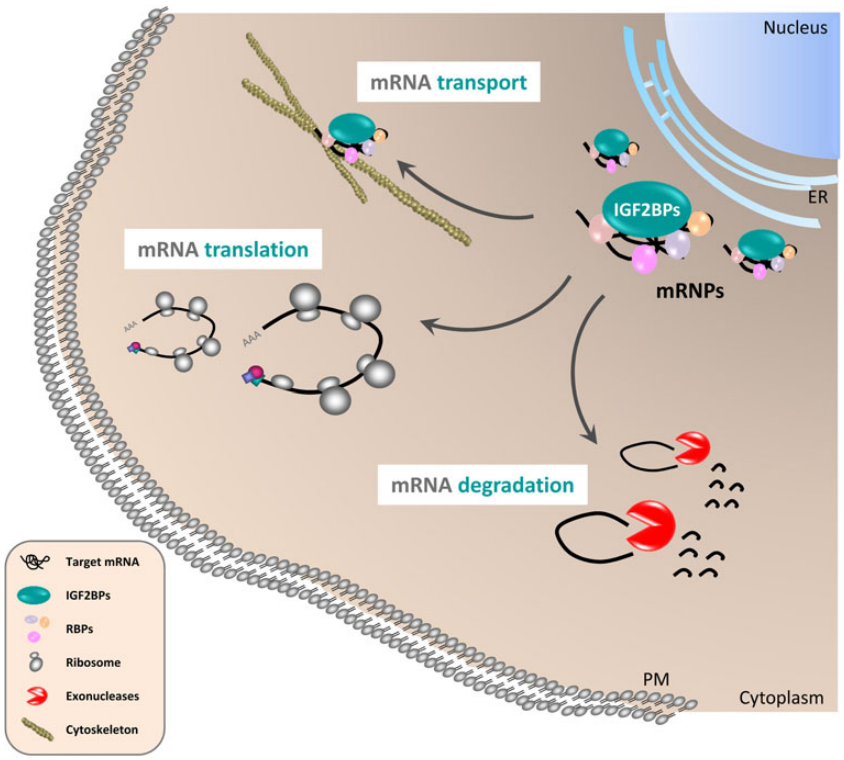


Fig. 2 Regulation of cytoplasmic mRNA fate by IGF2BPs. IGF2BPs associate with specific target mRNAs and other RNA-binding proteins (RBPs) in cytoplasmic mRNPs. The release of associated mRNAs from these mRNPs results in either their decay (*mRNA degradation*) of or protein synthesis (*mRNA translation*). The formation of 'stable' mRNPs is presumed to allow the directed transport of specific mRNAs along the microtubule and/or actin cytoskeleton (*mRNA transport*). To prevent promiscuous translation of sorted mRNAs, localized transcripts are likely to be translationally silenced during transport

1,000 target mRNAs for IGF2BP1 [10, 22]. However, it should be noted that these studies were based on the stable expression of Flag-tagged proteins in HEK293 cells, in which the stable expression of IGF2BP1 results in aberrant sedimentation in polysomal gradient centrifugation when compared with endogenous protein (Fig. S1). Recent studies focusing on structural constrains defined by the KH-domains 3 and 4 suggested just over 100 mRNAs to be regulated by IGF2BPs [23]. However, these studies do not take into account that KH-domains 1 and 2 are likely to be involved in RNA-binding; also, as the studies were based on IGF2BP1, the repertoire for the entire IGF2BP family could be significantly larger. A role of KH-1/2 in RNA-binding is supported for instance by the finding that in vitro KH3/4 do not associate with RNA below concentrations of 100 nM, unlike the full length protein [3]. Moreover, we observed that the KH1/2 domain modulates binding of IGF2BP1 to cis-determinants in the ACTB 3′UTR and, more strikingly, the MYC-CRD (coding region stability determinant) RNA in vitro (Fig. S2). This could indicate that KH1/2 are important for the stabilization of IGF2BP-RNA complexes.

Taken together, the currently available studies suggest a significant structural complexity of IGF2BP-RNA association. Structural studies of KH3/4, although still lacking protein–RNA co-crystal information, suggest that each KH-domain of IGF2BPs, presumably including KH-domains 1 and 2, forms direct contacts with associated transcripts [4]. Assuming that PAR-CLIP identifies specific binding consensus motifs, a putative binding motif for the KH-domains of IGF2BPs could be CAUH (H = A, U, or C) [22]. Thus, only the defined spacing of specific association motifs on substrate RNAs would determine the formation of specific IGF2BP–RNA complexes in vivo. Another layer of complexity to be considered is that IGF2BPs form homo- and potentially hetero-dimers on their target mRNAs and that this was proposed to promote the formation of stable protein–RNA complexes [3, 24]. In agreement, the stability of IGF2BP–RNA complexes was found to increase with the length of probed RNA baits in vitro whereas K_D-values were decreased [3]. Hence, it appears as if the identification of physiological relevant target mRNAs of IGF2BPs cannot be based solely on studying protein–RNA association, but presumably

Table 1 Target mRNAs of IGF2BPs

Target	Cis-element on RNA	IGF2BP	Regulation of target mRNA	References
ACTB	3′-UTR	1	Inhibition of mRNA translation	[6, 14, 43, 44]
ACTB	3′-UTR	1	mRNA transport	[2, 9, 42, 84]
BTRC	CDS	1	Inhibition of miR-dependent mRNA decay	[16, 20]
CD44	3′-UTR	1, 3	Inhibition of mRNA decay	[15]
CTNNB1	3′-UTR	1	Inhibition of mRNA decay	[50]
GLI1	Nd	1	Inhibition of mRNA decay	[98]
Gurken	5′-UTR	dIMP	mRNA transport/translation	[34]
IGF2	5′-UTR	1	Inhibition of mRNA translation	[31]
IGF2	5′-UTR	2, 3	Enhancement of mRNA translation	[19, 70–72]
MAPK4	3′-UTR	1	Inhibition of mRNA translation	[14]
MDR1	CDS	1	Inhibition of CRD-dependent mRNA decay	[21]
MYC	CDS	1	Inhibition of CRD-dependent mRNA decay	[11–13, 65, 66]
Oskar	3′-UTR	dIMP	mRNA transport/translation	[33]
PPP1R9B	3′-UTR	1	mRNA transport	[23]
PTEN	CDS	1	Inhibition of CRD-dependent mRNA decay	[14]
Vg1	3′UTR	Vg1RBP/Vera	mRNA transport/translation	[99–101]
HCV	5′-/3′-UTR	1	Enhancement of translation	[102]

Target	Cis-RNA	IGF2BPs	Proposed regulation of target RNA	References
CDH1	–	1	mRNA localization	[103]
H19	ncRNA (+)	1, (3)	mRNA localization, IGF2 expression	[3, 104]
LAMB2	–	2	Control of mRNA translation	[89]
LIMS2	–	2	Inhibition of mRNA decay	[90]
KRAS	CDS, 3′-UTR	1	Inhibition of mRNA decay	[57]
MAPT	–	1	mRNA localization	[105, 106]
PABPC1	5′-UTR	1	mRNA translation	[107]
PTGS2	–	1	mRNA increase (undefined)	[91]
TRIM54	–	2	Inhibition of mRNA decay	[90]
Y3	ncRNA(+)	1, 2, 3	RO60 protein localization	[97, 108]

requires functional screening approaches and correlation with cellular functions of the IGF2BP protein family.

The role of IGF2BPs during development

An important characteristic of the IGF2BP family is its high expression during the period between zygote and embryo stages [25]. There is a sharp peak in expression seen around embryonic day 12.5 before a decline in expression towards birth in mice [25, 26]. At E12.5, IGF2BPs are expressed at very high levels in the brain, limb buds, and muscle, and in the epithelia of many organs in mice. During *Xenopus* development, Vg1RBP/Vera is also expressed in the neural tube and neural crest cells [27]. Compared to their high expression in the embryo, IGF2BP1 and IGF2BP3 were reported to be expressed at negligible levels in adult organs, with the exception of reproductive tissues [26]. In contrast, IGF2BP2 was suggested to be expressed in various adult tissues (reviewed in [28–30]). Aiming to re-evaluate these observations, we analyzed the expression of IGF2BPs in various adult mouse tissues by semi-quantitative RT-PCR (Fig. 3a). These studies confirmed that IGF2BP1 expression is essentially abolished in the adult organism, although modest expression was observed in the brain, lung and spleen of 16-week-old male mice. Largely age-independent although modest expression of IGF2BP3 was observed in the lung, spleen, kidney, and gut of male mice. Surprisingly, expression in the brain and muscle was only observed in 16-week-old mice, whereas modest expression was observed in the heart and pancreas of 80-week-old mice. Consistent with previous reports, largely age-independent expression of IGF2BP2 was observed in all analyzed tissues, except pancreas. In the latter, IGF2BP2 expression appeared to be upregulated in 80-week-old mice. All family members were expressed in E17 mouse

embryonic fibroblasts (MEFs). Hence, the expression pattern observed for IGF2BP1 and IGF2BP3 can indeed be characterized as 'oncofetal', since they are largely absent from adult tissues but de novo synthesized or severely upregulated in various tumors and tumor-derived cells (Fig. 3b; reviewed in [28, 29]). In contrast, IGF2BP2 seems to be the only family member involved in directing mRNA fate in non-transformed adult tissues, supporting a role for this protein in metabolic control (reviewed in [30]).

The only family member for which knockout mice have been reported is IGF2BP1. Mice deficient for this family member have severely reduced viability, dwarfism and impaired gut development [25]. The smaller sized organs and 40 % smaller sized animals were suspected to be caused via hypoplasia. PCNA, a marker of proliferating cells, was reduced and a marker of apoptosis (TUNEL staining) was not significantly increased compared to wild-type mice. This indicates a pivotal role of IGF2BP1 in promoting cell growth and differentiation during development, presumably involving the regulation of IGF2 mRNA translation [31].

In *Drosophila,* loss of function *dIMP* mutations are zygotic lethal and the overexpression of dIMP disrupts dorsal/ventral polarity [32]. Consistently, *dIMP* could possibly direct the fate of localized mRNAs during early development, including gurken and oskar [33, 34]. As observed in vertebrates, *dIMP* shows a biphasic expression during embryogenesis and is expressed in reproductive tissues [35–37]. Moreover, *dIMP* plays a role in determining cell fate in testis stem cells and modulates neuronal differentiation [32, 38]. Hence, in all organisms analyzed so far, IGF2BPs were identified as essential modulators of cell growth and differentiation during development. IGF2BP1 and IGF2BP3 can be considered 'oncofetal' proteins with a biphasic expression during development and significant upregulation in various malignancies (see Tables 2, 3). Consistent with a suggested role in metabolic control, the only family member widely expressed in adult mouse tissues is IGF2BP2.

The role of IGF2BPs in the nervous system

The spatiotemporal control of mRNA localization is considered a key determinant of neuronal development, cytoskeletal remodeling, and finally synaptic function (reviewed in [39, 40]). IGF2BPs were identified as key players in these processes due to their role in directing subcellular mRNA sorting and spatial control of key mRNA translation. A few transcripts have been suggested to be regulated in a spatiotemporal manner by IGF2BPs in neurons (Table 1). However, the role of IGF2BP1 in controlling the fate of the ACTB mRNA is the most investigated (reviewed in [41]). The current view suggests

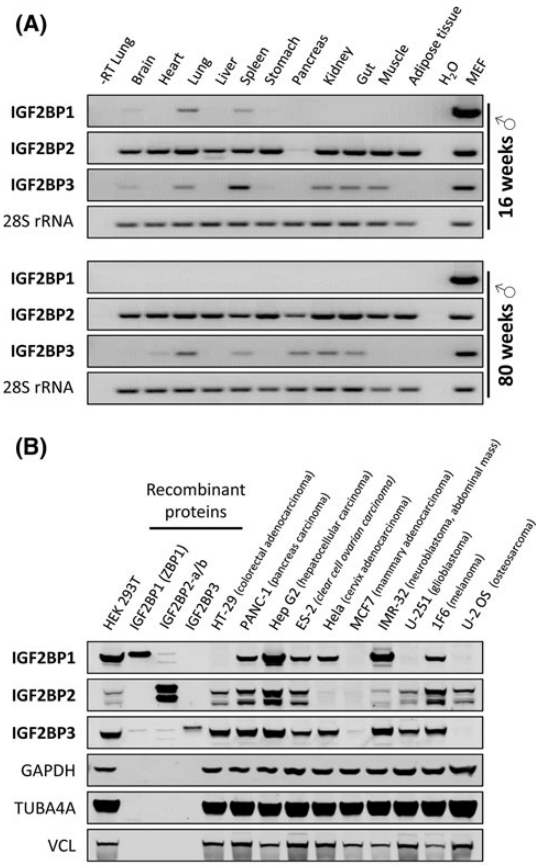


Fig. 3 IGF2BP expression in adult mice and tumor-derived cells. **a** Semi-quantitative RT-PCR analysis of IGF2BP expression (40 PCR cycles) in adult mouse tissues. Total RNA was analyzed from tissues isolated from either a 16- or 80-week-old male mouse. 28S RNA served as a loading control (20 PCR cycles). Total RNA isolated from E17 mouse embryonic fibroblasts (*MEF*) was used as positive control. Total lung RNA without reverse transcription (−RT) and water served as negative controls. **b** IGF2BP protein expression in indicated tumor-derived cells was analyzed by western blotting using mouse monoclonal antibodies directed against each of the three paralogues. Recombinant IGF2BP proteins (20 ng; including IGF2BP2-a and IGF2BP2-b) served as controls. Note, the IGF2BP3-directed antibody shows a significant cross-reactivity with IGF2BP1 (see also supplemental Fig. S4), presumably reflecting the high sequence similarity of both proteins. The cross-reactivity of both anti-IGF2BP1 (6A9) and anti-IGF2BP3 (6G8) with IGF2BP2 is low and presumably negligible for most studies (see also supplemental Fig. S4). Notably, one or two IGF2BP paralogues are expressed at very low levels in some tumor-derived cells, whereas all three paralogues are expressed in other cancer-derived cells. Additional controls for paralogue specificity of used monoclonal antibodies are shown in Fig. S4

that IGF2BP1 promotes the assembly of relatively stable cytoplasmic mRNPs comprising the ACTB mRNA. This allows the directed transport of the translationally silenced transcript into developing axons and dendrites [9, 42].

Table 2 IGF2BP1 expression in human cancers

Cancer	Method	Incidence	References
Breast	RT-PCR	59 % (69/118)	[109]
Ovarian carcinomas	IHC	69 % (73/106)	[13]
Ovarian	IHC	Not done (associated with MDR1)	[56]
Testis	IHC	90 % (30/33)	[26]
Brain tumors (various)	RT-PCR	55 % (28/51)	[110]
Melanoma	IHC	34 % (13/38)	[111]
Non-small cell lung	RT-PCR	27 % (4/11)	[110]
Pancreatic	Northern	33 % (5/15)	[112]
Colon, lung, ovarian	IHC	>60 %	[61]
Colon	IHC, RT-qPCR	50 % (36/78), 59 % (46/78)	[52]
Colorectal	RT-PCR	81 % (17/21)	[51]
Mesenchymal	RT-PCR	65 % (28/43)	[113]
Hodgkin lymphoma	IHC	94 % (101/108)	[78]
B cell lymphomas (various)	IHC	69 % (458/661)	[78]

Spatially restricted translation of localized ACTB mRNAs is presumably activated by Src-mediated phosphorylation of IGF2BP1 [6]. This spatiotemporal fine tuning of ACTB protein synthesis was suggested to promote growth cone guidance during development [43–45]. Recent studies indicate that IGF2BP1 also promotes the outgrowth and branching of neurites in hippocampal neurons, presumably by controlling Src-dependent spatiotemporal activation of ACTB protein synthesis [46]. Notably, these studies revealed that IGF2BP1 is not required for the maintenance of matured dendrites, correlating well with the observation that IGF2BP1 is not expressed in the adult mouse brain, although final proof of this assumption requires further in depth analyses (Fig. 3a). Notably, IGF2BP1 was recently implicated in nerve regeneration capacity of adult sensory neurons, suggesting that the protein could also play a role in the matured neuronal system, at least during regeneration [47]. Studies in *Drosophila* and *Xenopus* support essential roles of IGF2BPs in the nervous system. In *Drosophila, dIMP* was revealed to promote synaptic terminal growth and modulate protein synthesis at neuromuscular junctions [32]. In *Xenopus*, the ortholog Vg1RBP/Vera was shown to be required for migration of cells forming the neural tube of the embryo and, subsequently, migration of neural crest cells [27]. Taken together, these findings identify IGF2BPs as key regulators of neuronal development that modulate neurite outgrowth and neuronal cell migration, presumably by the spatiotemporal fine tuning of protein synthesis, as demonstrated for ACTB.

Control of IGF2BP expression

Surprisingly little is known about how the expression of IGF2BPs is regulated at the transcriptional level. In HEK293 cells, IGF2BP1 transcription was proposed to be induced by β-catenin (CTNNB1) in a TCF-dependent manner [16]. This observation remains puzzling, since the authors propose that, without CTNNB1/TCF4 overexpression, IGF2BP1 mRNA is not present or barely observed in HEK293 cells. In contrast, various studies indicate that IGF2BP1 is highly abundant in HEK293 cells (e.g., [6, 10, 48]). Despite this controversy, the CTNNB1-induced activation of IGF2BP1 expression was proposed to promote IGF2BP1-dependent stabilization of the BTRC and MYC mRNAs leading to elevated expression of both proteins [16]. While IGF2BP1 stabilizes the MYC mRNA presumably by protecting the transcript from endonucleolytic attack, the protein was proposed to prevent miR-182 directed degradation of the BTRC transcript [12, 13, 20]. These observations suggest that IGF2BP1 transcription is modulated by negative as well as positive feed-back regulation. Negative feed-back regulation should be facilitated by BTRC-dependent degradation of CTNNB1, whereas MYC was proposed to enhance the transcription of IGF2BP1, suggesting a positive feed-back loop [49]. Controversially, CTNNB1 was proposed to enhance the expression of IGF2BP1 expression by positive feed-back regulation in mammary carcinoma-derived tumor cells [50]. Taken together, the presented studies support the view of an oncogenic role of IGF2BP1 by providing evidence for CTNNB1/TCF4 as well as MYC-dependent transcriptional activation. This is consistent with the severe upregulation of IGF2BP1 in various malignancies (Table 2) and correlates well with IGF2BP1 de novo synthesis observed in colorectal carcinomas [51, 52]. However, substantially more work is required to decipher the cross-talk and feed-back regulations which are likely to orchestrate IGF2BP1 transcription in a cell- and malignancy-dependent manner.

Little information is available on the transcriptional control of other IGF2BPs. Transcriptional regulation of IGF2BP3 has never been studied to our knowledge. Two studies indicate that IGF2BP2, but not the two other family members, is regulated by the 'architectural' transcription factor HMGA2 and NFκB (NFKB1). The first report on the control of IGF2BP2 expression convincingly demonstrates that transcription of this paralogue is essentially abolished in HMGA2 (-/-) mice [53]. Consistently, HMGA2 was later proposed to promote the transcription of IGF2BP2 by

Table 3 IGF2BP3 expression in human cancers

Cancer	Incidence (%)	References
Gastrointestinal/pancreatic		
Pancreatic adenocarcinoma	63–97	[114–119]
Esophageal adenocarcinoma	66–94	[120, 121]
Gastric adenocarcinoma	60	[122]
Colorectal adenocarcinoma	65–74	[123, 124]
Hepatobiliary		
Hepatocellular carcinoma	53–68	[79, 125]
Bile duct carcinoma	58	[126]
Gynecologic		
Endometrial clear cell carcinoma	39	[74]
Endometrioid carcinoma	7–46	[74, 127]
Serous endometrial carcinoma	94–100	[59, 74, 127]
Cervical adenocarcinoma in situ	21–93	[75, 128]
Ovarian carcinoma	47	[76, 129]
Lung/pleura		
Non–small cell lung cancer	55	[130]
Squamous cell carcinoma lung	75–90	[130, 131]
Adenocarcinoma of the lung	70–90	[131, 132]
Bronchioloalveolar carcinoma	25–40	[130, 132]
Malignant mesothelioma	36–91	[133, 134]
Lymphoid		
Hodgkin lymphoma	100	[77]
Burkitt lymphoma	83	[77]
Follicular lymphoma	80	[77]
Diffuse large B cell lymphoma	85	[77]
Cutaneous		
Melanoma	40–50	[80, 135]
Merkel cell carcinoma	90	[136, 137]
Thyroid		
Papillary carcinoma, conventional	11–87	[138, 139]
Papillary carcinoma, follicular variant	38–67	[138, 139]
Follicular carcinoma	63–69	[138, 139]
Hürthle cell carcinoma	21	[138]
Poorly differentiated carcinoma	59	[140]
Nervous system		
Meningioma	6.5	[141]
Pituitary adenoma	31	[142]
Pituitary carcinoma	36	[142]
Neuroblastoma	58	[81]
Genitourinary		
Renal cell carcinoma, overall	11–21	[143, 144]
Renal cell carcinoma, clear cell	14–30	[143, 144]
Renal cell carcinoma, chromophobe	15–35	[143–145]
Renal cell carcinoma, papillary	9–65	[143–145]
Noninvasive papillary urothelial carcinoma	1–53	[146]
Urothelial carcinoma in situ	36–48	[146, 147]
Invasive urothelial carcinoma	34–59	[146, 147]
Breast		
Mammary carcinoma	33–41	[148–150]
Other		
Extrapulmonary small cell carcinoma	94	[151]
Mesothelioma	73	[152]
Osteosarcoma	17–96	[153, 154]

associating with an AT-rich region in the first intron of the IGF2BP2 gene [54]. Remarkably, the same region is targeted by NFKB1 that apparently synergizes with HMGA2 in enhancing the transcription of IGF2BP2. Hence, in contrast to IGF2BP1 where transcriptional control is proposed to be orchestrated via a bona fide promoter region located upstream of the start codon, IGF2BP2 expression is suggested to involve enhancer elements located in the first IGF2BP2 intron.

The post-transcriptional control of mRNA fate is a main regulatory crank in the control of gene expression. In this respect, a study by the Bartel laboratory provided a new perspective that emphasizes the 3′-end of IGF2BP transcripts, in particular IGF2BP1, in modulating the expression of this gene family [48]. Consistent with various in silico-predicted poly-adenylation sites in the approximately 7-kb-long 3′-UTR of the transcript (Fig. S3), at least three IGF2BP1 transcripts were observed in various tumor-derived cells and HEK293 cells. This supports the notion that IGF2BP1 expression is modulated by alternative polyadenylation (APA). Although the mechanism by which APA of IGF2BP1 is controlled remains largely elusive, it is commonly accepted that 3′-UTR shortening provides a potent escape strategy preventing the targeting of repressive microRNAs. This appears to be preferentially observed for transcripts encoding oncogenic factors which are targeted by tumor-suppressive microRNAs like the let-7 family, as demonstrated for IGF2BP1 [55]. Notably, APA-sites are only suggested for IGF2BP1 based on currently available sequence information (Fig. S3). Whether this indicates that 3′-UTR shortening provides an escape strategy only for IGF2BP1 remains to be elucidated.

The observed post-transcriptional control of IGF2BP1 expression by microRNAs was suggested to modulate tumor cell fate. Downregulation of let-7 expression, frequently observed in aggressive tumor cells, was correlated with increased drug-resistance and an upregulation of IGF2BP1 [56]. The latter was proposed to enhance the expression of the multi-drug-resistance factor 1 (MDR1) by preventing MDR1 mRNA degradation via endonucleases,

as previously proposed for MYC [21]. Hence, the microRNA-dependent upregulation of IGF2BP1 enhanced drug resistance by promoting the expression of MDR1. This supports other studies which indicate that regulatory post-transcriptional networks modulate tumor cell properties. For IGF2BP1, it was demonstrated that the protein promotes the expression of various bona fide let-7 targets including KRAS, Lin-28B and MYC [57]. Notably, the role of IGF2BP1 in the let-7-dependent post-transcriptional control of gene expression is apparently conserved through evolution. In *Drosophila*, let-7-controlled expression of *dIMP* was recently proposed to modulate the expression of the self-renewal factor Upd in the testis stem cell niche [38].

Although regulation of the other IGF2BP family members by microRNAs has not so far been demonstrated, the expression of at least IGF2BP2 seems to be also regulated at the post-transcriptional level. Recent studies indicate that leaky scanning during translation initiation results in the expression of a shorter protein isoform [58]. We have confirmed the expression of this isoform in osteosarcoma-derived U2OS cells and demonstrated that at least three protein isoforms of IGF2BP2 are expressed in several tumor-derived and transformed cells (Fig. 3b; Fig. S4). These include the longest protein isoform (IGF2BP2-a; Acc. no.: NM006548.4; calculated MW: 66 kDa), an alternatively spliced variant lacking exon 10 (IGF2BP2-b; Acc. no.: NM001007225.1; calculated MW: 61.8 kDa) and presumably the shortest isoform resulting from leaky scanning of IGF2BP2-a with a calculated molecular weight of 58.6 kDa (IGF2BP2-a*). As for IGF2BP2, an alternative splice variant lacking exons 6 and 7 was proposed for IGF2BP1 (Acc. no.: NM 001160423.1). However, although we were able to generate a cDNA encoding the shorter IGF2BP1 isoform by RT-PCR cloning from HEK293 cells, we have not been able to conclusively demonstrate expression of the shorter protein variant at the endogenous level (data not shown).

Taken together, it remains poorly understood how the transcription of IGF2BPs is regulated and how it might be modulated by epigenetic mechanisms. In contrast, there is substantial evidence for a significant role of post-transcriptional mechanisms directing the control of at least IGF2BP1 expression. The 'let-7-axis' appears to emerge as a highly conserved regulatory mechanism that antagonizes the expression of IGF2BP1. This supports the view that IGF2BP1 enhances tumor cell aggressiveness, since the let-7 microRNA family is considered to facilitate a tumor-suppressive role in most malignancies. Nonetheless, substantial efforts are required to promote our understanding of how the expression of IGF2BPs is modulated by the interplay of transcriptional and post-transcriptional networks. This will provide essential insights into how IGF2BP function is controlled during development and becomes deregulated in diseases.

Expression of IGF2BPs in cancer

Expression of IGF2BP family members has been implicated in various cancers; however, the vast majority of reports consider exclusively IGF2BP1 and IGF2BP3 (Tables 2, 3). For the latter, the most cited malignancies are those of the colon, liver, kidney, pancreas, and female reproductive tissues. There is sparse and less convincing evidence thus far for an oncogenic role for IGF2BP2, but studies have correlated the expression of this paralogue with liposarcoma, liver cancer, and endometrial adenocarcinomas [54, 59, 60]. This is consistent with the observation that IGF2BP1 and to a lesser extent also IGF2BP3 are mainly or even exclusively expressed during embryogenesis but become de novo synthesized in various malignancies. In contrast, IGF2BP2, which has barely been associated with a role in cancer, is the only paralogue observed to be expressed in all non-transformed mouse tissues so far analyzed (Fig. 3a).

The reported expression of IGF2BP1 and IGF2BP3 in primary malignancies does not allow concluding a specific expression pattern discriminating both paralogues. However, it should be noted that IGF2BP1 expression has been studied largely on the mRNA level by RT-PCR, whereas IGF2BP3 expression was analyzed mainly by immunohistochemistry. The latter is problematic with IGF2BPs due to the high sequence identity and homology. This imposes the difficulty to raise paralogue-specific antibodies which are useful for immunohistochemistry. Thus, isoform-specific expression analyses should be evaluated with caution and we expect that at least some of the reported observations have to be reconsidered.

IGF2BP1—oncogene(ic) or not?

For the majority of studies, there is a severe gap between pure functional in vitro studies and more descriptive clinical oncology/epidemiology studies. For example, even though there is a large body of in vitro evidence for IGF2BP1 in promoting cell movement, the significance of IGF2BP1 in the process of cancer metastasis has not been directly confirmed through in vivo studies. Likewise, we still have little information on a putative co-regulation of IGF2BP1 and target mRNA expression in primary tumor samples, although the expression of IGF2BP1 has, for instance, been correlated with lymph node metastasis of colorectal carcinomas [61].

Only one study provides strong in vivo evidence for a pro-oncogenic role of IGF2BP1 by applying classical methods. In a transgenic mouse model, the expression of

IGF2BP1 was induced in mammary epithelial cells of adult female mice via the whey acidic promoter (WAP) upon lactation [62]. The incidence of mammary tumors within 60 weeks was 95 % when IGF2BP1 was highly expressed, and still reached 60 % with lower relative expression of the paralogue. Tumors were generally multifocal and several tumor-bearing mice had metastases. The quantification of IGF2BP1 target RNAs demonstrated that levels of ACTB and MYC transcripts were unaffected by IGF2BP1 overexpression, whereas IGF2 and H19 were significantly and consistently induced at the RNA level in mammary tissue of transgenic mice after lactation. These findings are surprising for two reasons. In vitro, IGF2BP1 was shown to enhance the expression of MYC by preventing MYC mRNA degradation, whereas this was not observed in vivo, at least in the WAP-dependent mouse model [12, 13, 62]. Moreover, in vitro evidence indicates a role of IGF2BPs in modulating the translation of the IGF2 mRNA, mainly by associating with one of four known 5′-UTRs of IGF2, whereas total IGF2 mRNA levels were upregulated in vivo [31, 62]. In vitro studies revealed that IGF2BP1 also binds to the 3′-UTR of IGF2 mRNA which is identical in all IGF2 transcript variants. This could indicate that IGF2BP1 simply prevents IGF2 mRNA degradation in vivo [3]. Alternatively, one could envision a role of IGF2BP1 in modulating the activation and/or imprinting of the IGF2-H19 tandem locus (reviewed in [63]). Of note, the H19 RNA was reported to encode at least one microRNA, proposed to negatively affect cell proliferation, which would be consistent with the role of H19 as a tumor suppressor (reviewed in [64]). How this correlates with the observed induction of primary lesions as well as metastases in WAP-driven IGF2BP1 mouse models remains to be addressed [62].

IGF2BPs could be exploited in cancer through their influence on classical oncogenes, in particular MYC and KRAS [57]. Unlike various other targets to which IGF2BP1 binds via the 3′-UTR, IGF2BP1 was proposed to bind to the CRD in the MYC open reading frame [65]. There is a bulk of evidence accumulated indicating that IGF2BP1 sustains MYC expression in tumor cells derived from various cancers in vitro (e.g.: mammary carcinomas [66]; ovarian carcinomas [13]; colorectal carcinomas [57]). This regulatory role was mainly correlated with the role of IGF2BP1 in preventing cleavage of the MYC mRNA by endonucleases upon the stalling of ribosomes in a rare codon stretch at the 5′-end of the CRD [11, 12, 21, 67]. However, in light of the reported repression of MYC as well as KRAS expression by the let-7 microRNA family, which targets in the 3′-UTR of both transcripts, one could envision that IGF2BP1 also prevents the targeting of this miR-family. In the case of MYC, this could either be facilitated by blocking let-7 targeting to the MYC-3′-UTR or by recruiting the mRNA into cytoplasmic mRNPs upon association with the MYC-CRD. Alternatively, or in addition, the protein could prevent the targeting of miRs to the MYC-CRD, as previously proposed for the IGF2BP1-directed stabilization of the BTRC mRNA [20]. Evidence for an IGF2BP1-dependent enhancement of KRAS expression is presented by only one study, but the molecular mechanism of this regulation remains elusive [57]. However, the fact that both MYC and KRAS are targeted by microRNAs of the let-7 family, like IGF2BP1 itself, suggests that IGF2BP1 could prevent targeting of KRAS by this microRNA family.

Taken together, there is strong evidence for an ‘oncogenic’ role of at least IGF2BP1. However, there are obvious discrepancies between in vitro and the only available in vivo study. Hence, substantial efforts using in vivo models are required to elucidate the role of IGF2BPs in cancer.

What is the role of IGF2BP3 in cancer?

In contrast to IGF2BP1, which has been extensively studied in vitro, the role of IGF2BP3 remains barely investigated. However, of the three family members, IGF2BP3 has been associated the most with distinct cancer types. Accordingly, it was suggested as an important biomarker in systemic malignancies (reviewed in [68, 69]).

Functional studies addressing a regulatory role of IGF2BP3 revealed essentially two validated target mRNAs and some putative candidates. Evidence indicating IGF2BP3 to promote the mRNA translation of leader3 IGF2 mRNAs was presented by two laboratories [70, 71]. These studies suggest that the protein, like IGF2BP2 [19], enhances the translation of IGF2 mRNAs carrying a highly structured 5′-UTR, the so-called leader3. The latter presents one out of four distinct 5′-UTRs encoded by the human IGF2 locus. In agreement, it was demonstrated that IGF2BP3 promotes cell growth, proliferation, and resistance to ionic irradiation in an IGF2-dependent manner [72]. In contrast, IGF2BP1 was proposed to repress the translation of the IGF2 mRNA, either via the leader3 5′-UTR or potentially via the 3′-UTR of the IGF2 mRNA [3, 31]. Although the IGF2BP paralogue-specific regulation of IGF2 expression might well be regulated in a cell type- or cancer progression-dependent manner, these and various other findings indicate IGF2 as a key target transcript of the IGF2BP protein family. Interestingly, however, IGF2BP3 was also correlated with increased in vitro invasiveness and metastasis in *Xenograft* studies [15, 71, 73]. The only validated target mRNA which provides a conclusive hint how IGF2BP3 could facilitate a pro-invasive role is CD44. Together with IGF2BP1, IGF2BP3 was shown to enhance the formation of invadopodia by preventing the

degradation of the CD44 mRNA upon associating with the 3′-UTR of the CD44 mRNA [15].

In light of the poorly understood role of IGF2BP3 in modulating tumor cell functions, it is surprising to observe that there was an 'explosion' of descriptive studies published from 2007 onwards, which suggest IGF2BP3 expression to correlate with tumor aggressiveness in a broad variety of malignancies (Table 3). Among the various cancers for which an upregulation or de novo synthesis for IGF2BP3 was reported, lung, gastrointestinal, and ovarian cancers are the most frequently reported. Overall, in gastrointestinal cancers, there is the suggestion that IGF2BP3 expression, almost exclusively analyzed on the basis of immunostaining, correlates with an overall poor prognosis, tumor aggressiveness, and metastasis (for references, please refer to Table 3). In cancers of female tissues, positive staining was reported in 94 % of all serous endometrial carcinomas and 89 % of all serous endometrial intraepithelial carcinoma [74]. Notably, no expression was observed in endometrial intraepithelial neoplasia, whereas significant expression was observed in 93 % of cervical adenocarcinomas [75]. Notably, there is contradictive evidence for ovarian cancer suggesting IGF2BP3 expression to correlate with an improved survival [76]. One descriptive study by King et al. [77] displayed striking images of high IGF2BP3 protein expression by IHC in normal germinal centers of lymph nodes and negative staining in the periphery of the lymph nodes. Of note, a similar pattern was observed for IGF2BP1 in another lymphoma study [78]. The research of King and colleagues could support a role of IGF2BP3 in the proliferation and differentiation of B cells and possibly hints towards a broader role for IGF2BP3 in unrestricted proliferation and cell survival. Aside from these data, they also demonstrate a possible association of IGF2BP3 expression in specific subsets of lymphoma, such as 100 % of Hodgkin lymphoma. Although displaying less convincing IGF2BP3 staining in liver cancer, IGF2BP3 expression was correlated with cell proliferation by co-expression of ki67 [79]. This paralogue has also been associated with two cell types of neural crest origin; neuroblastoma and melanoma. IGF2BP3 has been found to be significantly highly expressed in metastatic melanomas, compared with thin melanomas. Thus, this paralogue may be useful diagnostically as a marker to differentiate melanoma from benign nevi cell types characterized by little or no IGF2BP3 expression [80]. Of relevance here is that Vg1RBP/Vera, the *Xenopus* ortholog of IGF2BPs, was revealed to promote the migration of neural crest cells during development [27]. This could indicate a significant role of IGF2BPs in the etiology of neuroblastoma and melanoma. In agreement, IGF2BP3 was proposed a marker of high clinical significance in neuroblastoma, with IGF2BP3-positive patients having decreased overall survival [81]. Interestingly, retinoic acid treatment of neuroblastoma cells revealed downregulation of IGF2BP3, and evidence within our laboratory shows this is also the case for IGF2BP1 (Bell et al., unpublished). Retinoid treatment causes the vast majority of neuroblastoma-derived cells to differentiate, decreases proliferation, and is therefore used in treating minimal residual disease neuroblastoma patients, but notably is also beneficial in many other cancers and proliferative disorders [82]. This could further implicate that high expression of IGF2BPs is associated with a de-differentiated highly proliferative cell state and speculatively nuclear receptor signaling pathways.

Taken together, evidence for an 'oncogenic' role of IGF2BP3 provided by in vitro studies is sparse and the paralogue specificity of used antibodies remains to be validated. Nonetheless, the bulk of correlative studies associating the upregulation of IGF2BP3 with various malignancies provide strong evidence for a pivotal role of IGF2BP3 in cancer.

IGF2BPs as pro-survival factors

Obviously, the ability of IGF2BPs to increase the expression of MYC, IGF2 and potentially other pro-survival proteins like KRAS tends towards IGF2BPs themselves having pro-survival traits. This is a major characteristic of both oncogenes and embryonic growth factors and thus supports the oncofetal expression of IGF2BP1 and IGF2BP3. Recent studies have suggested both these paralogues to promote cell survival in response to Taxanes treatment or ionizing radiation, respectively [56, 72]. Both articles discuss common treatment regimens in cancer therapy imposing cell cycle arrest and/or apoptosis. Thus, the pro-survival role of IGF2BP1 and IGF2BP3 in response to these therapeutic treatments in vitro suggests that IGF2BPs also serve a role in mediating chemo-/radioresistance of tumor cells. In support of this view, IGF2BP1 was shown to enhance the expression of MDR1 [21]. Notably, IGF2BP3 knockdown in K562 cells (chronic myeloid leukemia) does not induce apoptosis by itself, an observation we can also confirm for IGF2BP1 in tumor cells derived from gastrointestinal cancers (unpublished). However, IGF2BP3 knockdown enhances γ-irradiation-induced apoptosis by around 30 % in K562 cells [72]. This enhancement of apoptosis was largely abolished by supplementing recombinant IGF2, suggesting that IGF2BP3 may exert its protective effects essentially by promoting the expression of IGF2. In melanoma cells, knockdown of IGF2BP1 was also shown to be protective against chemotherapy-induced apoptosis [83]. Unfortunately, the role of p53 and involvement of the mitochondria in the observed apoptosis signaling was not investigated in the

above studies, and remains an important area of enquiry. This is emphasized by reported observations in colon carcinoma-derived cells in which IGF2BP1 knockdown was proposed to induce apoptosis, as suggested on the basis of increased Caspase3/8 abundance as well as cleaved PARP and LaminA/C proteins [57].

The role of IGF2BPs in cell migration

The identified target transcripts, in particular ACTB and CD44 (see Table 1), of IGF2BPs suggest a role of this protein family in controlling cytoskeletal organization, cell adhesion, and consequently cell migration. The most striking observation indicating a significant role of IGF2BPs in regulating cell motility was in *Xenopus* where the IGF2BP ortholog Vg1RBP/Vera promoted the directed migration of neuronal crest cells during development [27]. However, via which target mRNAs Vg1RBP/Vera modulates the migration of neural crest cells remains largely elusive .

The chicken ortholog of the human IGF2BP1, termed ZBP1 (Zipcode binding protein), was identified as a key regulator directing the localization of ACTB mRNA to the leading edge of fibroblasts as well as exploratory growth cones in primary neurons [2, 9, 84]. Although it remains unknown whether enhancement of neuronal crest cell migration by Vg1RBP/Vera also involves the localization of ACTB mRNA, these findings together indicated a pivotal role of IGF2BPs in modulating both cytoskeletal polarization and actin-driven cell migration. In support of this, IGF2BP1 was identified to control the spatially restricted translation of the ACTB mRNA in neuronal cells [6]. This suggested that the protein is an essential regulator of local ACTB monomer concentrations and thus F-actin polymerization, the driving force of cell protrusion. In developing mammalian neurons, the spatial control of ACTB protein levels by IGF2BPs or their orthologs is essentially involved in modulating neurite outgrowth and growth cone guidance [6, 43, 44]. Although actin remodeling and protrusion of growth cones is regulated by somewhat different mechanisms than observed in the migration of mesenchymal cells, IGF2BPs were also shown to enhance the migration of the latter. In tumor-derived cells, IGF2BPs were demonstrated to enhance the formation of lamellipodia, enforce intrinsic polarization, and thus promote directed cell migration [14, 61, 85, 86]. Although all these findings support the notion that IGF2BPs, in particular IGF2BP1, promote directed cell migration, it was unknown if this role was solely due to the spatiotemporal control of ACTB mRNA translation or involved the regulation of additional target mRNAs. However, recent studies by the Singer laboratory provide striking evidence that the localization of endogenous ACTB mRNA to the leading edge of fibroblasts lags behind the rapid change in migration directionality observed during random migration [87]. These findings suggest that the enhancement of ACTB mRNA localization sustains the directed migration in response to chemotactic cues rather than initiating cell protrusion. This obviously supports findings in neurons where IGF2BPs were suggested to support the guidance of growth cones during development [43, 44]. Despite this strong evidence indicating an essential role of IGF2BPs in the modulation of chemotactic movement, IGF2BP1 apparently also serves a role in controlling the random migration of tumor-derived cells. Our recent studies indicate that IGF2BP1 promotes the velocity of tumor cell migration and migration-supportive adhesion by limiting MAPK4 mRNA translation and consequently MK5-directed phosphorylation of HSP27 [14]. The latter is frequently upregulated in various cancers and is essentially involved in modulating cellular G-/F-actin ratios by an enhanced sequestering of ACTB monomers upon MK5-directed phosphorylation at two key serine residues [14]. Thus, by antagonizing MK5-directed phosphorylation of HSP27 and concomitantly limiting ACTB mRNA translation, IGF2BP1 serves as a 'post-transcriptional fine tuner' of ACTB monomer levels (reviewed in [88]). However, IGF2BP1 not only controls the speed of migration but also modulates intrinsic cell polarization, presumably via at least two target transcripts. The reported control of ACTB mRNA localization directs actin monomers to the site of active protrusion and thus determines a dynamic cytoskeletal polarization. Although this is presumably largely dispensable for randomly walking cells, it could have a severe impact on sustained motion during development or in chemotactic gradients [85, 87]. On the other hand, IGF2BP1 surprisingly enhances the expression of the tumor-suppressor PTEN and thereby shifts the cellular PIP3/PIP2 equilibrium [14]. This enhancement of PTEN expression enforces intrinsic cell polarization in a RAC1-dependent manner in vitro. Hence, in tumor-derived cells still expressing functional PTEN, IGF2BP1 can enhance both the speed and the directedness of cell movement. In glioblastoma-derived tumor-cells lacking PTEN, IGF2BP1 was found to exclusively promote the speed but not the directedness of random migration [14]. Despite conclusive evidence supporting IGF2BPs as key regulators of cell migration, their potential role in tumor cell invasion and metastasis remains poorly understood. However, it should be noted that the de novo synthesis of IGF2BP3 and to a lesser extent IGF2BP1 have been reported to correlate with enhanced metastasis and poor prognosis in various cancers. Moreover, the de novo synthesis of transgenic IGF2BP1 in mammary tissues of lactating mice induced both the formation of primary lesions as well as metastasis [62]. Consistently, IGF2BP1

and IGF2BP3 were shown to enhance the in vitro formation of invadopodia by promoting the expression of CD44 [15]. In agreement with this, we have observed that the forced expression of IGF2BP1 promoted the invasiveness of tumor cells in vitro, whereas the opposite was observed upon its knockdown (unpublished). Moreover, significant expression of IGF2BPs was observed in metastasizing colorectal carcinomas (CRC) with high expression of IGF2BPs at the invasive front [61]. Notably, IGF2BP expression apparently prevails during metastasis, since high levels of IGF2BPs were also observed in CRC-derived lymph node metastasis [61]. Although these studies fail to reveal which paralogues of the IGF2BP protein family potentially modulate the invasiveness of CRC, they support the view that IGF2BPs enhance the metastatic potential of tumor cells. In contrast, in vitro studies suggest that IGF2BP1 could interfere with metastasis by enhancing intrinsic cell polarization to a level which abolishes chemotactic responsiveness [85]. Surprisingly, IGF2BP1 depletion in mammary carcinoma-derived T47D cells was reported to enhance cell migration whereas the opposite was observed upon the overexpression of ZBP1, the chicken ortholog of IGF2BP1 [50]. These findings are puzzling, since we observe that IGF2BP1 promoted the migration of tumor-derived cells in vitro and enhanced cell polarization in a PTEN-dependent manner [14]. These observations are consistent with reports indicating IGF2BP1 to enhance cell polarization, as well as studies demonstrating that IGF2BPs promote cell migration and the formation of lamellipodia [61, 85, 86]. One simple explanation is that what is described to be IGF2BP1 in T47D is a specific IGF2BP1 mutant/isoform or another IGF2BP paralogue, since IGF2BP1 expression is barely observed in a panel of breast cancer-derived cells including T47D [48]. However, this does not explain why the overexpression of ZBP1 slows down T47D cell migration.

Despite controversial observations regarding a potential involvement of IGF2BPs in metastasis, IGF2BP1 and IGF2BP3 emerge as potent modulators of cell migration during development and in cancer. This role is likely to involve the spatiotemporal fine tuning of actin dynamics, the driving force of cell motility. Moreover, there is substantial evidence suggesting IGF2BPs modulate cell adhesion, the formation of invadopodia, and intrinsic cell polarization. Notably, IGF2BP2 could add to IGF2BP-directed control of cell migration, presumably by modulating cell adhesion. Recent reports suggest that IGF2BP2 controls the expression of proteins modulating cell matrix contact formation, LIMS2 and TRIM54, as well as the extracellular matrix protein LAMB2 [89, 90]. Hence, substantial in vitro and in particular in vivo studies are required to decipher how IGF2BPs modulate cell adhesion, migration, and most importantly metastasis. However, in view of the somewhat controversial observations reported, it appears likely that their role in metastasis is essentially determined by the cancer or cell type analyzed.

On a slightly different note, a recent publication has uncovered an unexpected role for IGF2BP1 in a mouse model of colon wound healing. IGF2BP1 was found to promote the expression of prostaglandin-endoperoxide synthase 2 (Ptgs2), presumably by preventing Ptgs2 mRNA degradation in colonic mesenchymal stem cells [91]. This was suggested to enable or enhance efficient wound closure, supporting a pivotal role of IGF2BPs in cell migration. Moreover, there is a hypothesis within the oncology field that has speculated that cancers are ‘wounds that never heal’ [92]. Recent papers on the subject have reported the importance of PTEN [93], IGFs, and MYC in these processes, significantly transcripts also regulated by IGF2BPs, and also that the majority of effected transcripts are shared in both wound healing and cancer. Further research is certainly required to elucidate further if IGF2BPs serve roles in the process of wound healing, and whether this role is exploited in tumors for growth and metastasis and may lead to the mechanisms of IGF2BP1/3 re-expression in adult tissues.

Current limitations and concluding remarks

Descriptive studies of IGF2BPs demonstrate well-correlated expression throughout development and in reproductive tissues (which have high proliferation requirements). To date, there are few mechanistic comparative studies involving paralogues and isoforms within the IGF2BP family. This poses a significant limitation in deciphering the role of individual IGF2BPs in cancer. In contrast to IGF2BP1 and IGF2BP3, for which de novo synthesis in various malignancies has been reported, IGF2BP2 has been implicated as a candidate gene involved in type 2 diabetes (T2D) (reviewed in [30]). However, it has to be noted that, except for a role in IGF2 mRNA translation proposed to be regulated by mTORC1-directed phosphorylation of IGF2BP2, there is currently no functional evidence for a role of this paralogue in glucose homeostasis, insulin signaling, or diabetes [19]. The only evidence for a putative role of IGF2BP2 in T2D is provided by various studies correlating SNPs in the second intron of the IGF2BP2 locus with T2D. Notably, some studies correlated IGF2BP2-SNPs with reduced pancreatic β-cell function rather than with reduced insulin sensitivity (reviewed in [30]). This could indicate a role of IGF2BP2 and potentially its paralogues in pancreatic development and/or function. Supporting this assumption, loss-of-function studies in *Xenopus* revealed that Vg1RBP/Vera is involved in determining pancreatic cell fate during

development [94]. Notably, we observed that IGF2BP2 and potentially the expression of IGF2BP3 were upregulated in old male mice (Fig. 3a). Hence, current evidence favors a role of IGF2BP2 in metabolic control and not in malignancy. This could point to a lack of research, rather than a lack of function, as there is little evidence towards it *not* being involved in malignancy either. It could be speculated that the family members act in balance to drive embryonic growth, with IGF2BP2 functioning as a cell survival and maintenance factor, unable to drive growth on its own, but nonetheless integral to aid growth in non-limited nutrient supply conditions in the embryo. This remains to be proven, but demonstrates the need for family members to be studied (where possible) within the same contexts. Multiple knockout/knock-in conditional mice studies are essential to determine which of the family members are required for carcinogenesis. It is relevant to note here that IGF2BP1 and IGF2BP3 were found to occupy the same mRNPs in one context, a finding supported by the observation that IGF2BPs could form homo- as well as hetero-dimers upon RNA-binding [3, 24, 95]. Although these findings provide strong evidence for cooperative regulation of mRNA fate by distinct paralogues, many cancer studies suggest that IGF2BPs could also act in an independent manner. As already eluded to, crossing of multiple IGF2BP paralogue knockouts would be advantageous in understanding the interactions and signaling effects, but first, formal characterization of conditional and tissue specific knock-out mice are required for each paralogue. The current models need to be improved. Transgenic mice (especially IGF2BP1 and 3) that replicate the re-expression observed in cancer pathology would be extremely useful for mechanistic studies, but also for anti-IGF2BP drug development and testing, in vivo. Notably, the only study addressing this aspect in mammary carcinomas revealed interesting differences of IGF2BP1 functions in vitro versus in vivo. For instance, IGF2BP1 expression in the mammary tissue of female mice led to an upregulation of IGF2 and H19 but not MYC mRNA levels [62].

Somewhat concerning is the specificity of currently available antibodies. Evidence within our laboratory has shown that development of paralogue specific antibodies is difficult (Fig. 3b; Fig. S4). Although we have achieved a significant paralogue specificity which allows for a largely unbiased analysis of IGF2BP expression in most cancer-derived cells, we currently cannot exclude slight paralogue cross-reactivity of monoclonal antibodies at high protein concentrations. Notably, we had no success with polyclonal peptide-directed antibodies, although other laboratories reported high paralogue specificity of their polyclonal antibodies [31, 58, 96]. This putative bias imposed by used antibodies is largely ignored, since many studies show specificity of used siRNA-mediated knockdown by western blotting, but, unfortunately, not all papers give evidence towards the specificity of their tools. The similarity in paralogue kDa size and amino acid sequence similarity makes differentiation of paralogues difficult by western blot, therefore confidence in antibodies and siRNAs is critical. With much of the research into IGF2BPs in the cancer context using immunohistochemistry, here, too, it is essential to generate and use paralogue-specific antibodies.

Even though IGF2BP1 and 3 have been demonstrated as putative targets for drug design for use as chemotherapy since the 1990s, there are no small molecules currently available for specific inhibition of IGF2BP function. Development of such compounds/molecules would have great therapeutic potential and also have a use towards mechanistic studies. Recent work on the IGF2BP1 protein structure has paved the way towards possible drug design, possibly through fragment-based screening or virtual ligand screening to inhibit binding of substrates such as the MYC or IGF2 mRNAs [4, 23]. However, structural analyses of all four KH-domains in complex with target RNAs are required for the development of specific compounds. The possibility of paralogue-specific transcript binding inhibition and/or specific-transcript binding inhibition is an exciting next stage for IGF2BP research.

Consistently, various studies indicate the IGF2BP family as powerful growth factors, critical in vertebrate development. Current evidence points to the more closely related IGF2BP1 and IGF2BP3 being pro-oncogenic and pro-migratory when re-expression is forced or induced in adult tissues, and to IGF2BP2 having a role in metabolic homeostasis and response to nutrients. More specific information is required as to the specific isoform and paralogue expression of significance in cancer etiology and patient outcome. Current literature highlights the close relationship between IGF2BP-dependent mechanisms in cell migration in both embryos and neoplasia. Future studies will hopefully bridge the gap in knowledge between in vitro mechanistic studies on cell migration and in vivo metastasis. Studies into IGF2BPs have shed light over the potential diversity and wide-reaching effects of individual RNA-binding proteins within cell homeostasis and cancer progression. More importantly, however, there is growing evidence indicating RNA-binding proteins, in particular IGF2BPs, as clinically significant markers and attractive targets for future anti-cancer/anti-metastatic drug design.

Acknowledgments The authors apologize for omitted references. This work was supported by DFG-funding (HU1547/2-2; SFB610-TP/C5; GRK1591) and BMBF-funding (Pronet-T^3 initiative) to S.H.

References

1. Griffin D, Penberthy WT, Lum H, Stein RW, Taylor WL (2003) Isolation of the B3 transcription factor of the *Xenopus* TFIIIA gene. Gene 313:179–188
2. Farina KL, Huttelmaier S, Musunuru K, Darnell R, Singer RH (2003) Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. J Cell Biol 160(1):77–87
3. Nielsen J, Kristensen MA, Willemoes M, Nielsen FC, Christiansen J (2004) Sequential dimerization of human zipcode-binding protein IMP1 on RNA: a cooperative mechanism providing RNP stability. Nucleic Acids Res 32(14):4368–4376
4. Chao JA, Patskovsky Y, Patel V, Levy M, Almo SC, Singer RH (2010) ZBP1 recognition of beta-actin zipcode induces RNA looping. Genes Dev 24(2):148–158
5. Oleynikov Y, Singer RH (2003) Real-time visualization of ZBP1 association with beta-actin mRNA during transcription and localization. Curr Biol 13(3):199–207
6. Huttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, Meng X, Bassell GJ, Condeelis J, Singer RH (2005) Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 438(7067):512–515
7. Pan F, Huttelmaier S, Singer RH, Gu W (2007) ZBP2 facilitates binding of ZBP1 to beta-actin mRNA during transcription. Mol Cell Biol 27(23):8340–8351
8. Nielsen J, Adolph SK, De-Meyts ER, Andersen JL, Koch G, Christiansen J, Nielsen FC (2003) Nuclear transit of human zipcode-binding protein IMP1. Biochem J 376:383–391
9. Zhang HL, Eom T, Oleynikov Y, Shenoy SM, Liebelt DA, Dictenberg JB, Singer RH, Bassell GJ (2001) Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. Neuron 31(2):261–275
10. Jonson L, Vikesaa J, Krogh A, Nielsen LK, Hansen T, Borup R, Johnsen AH, Christiansen J, Nielsen FC (2007) Molecular composition of IMP1 ribonucleoprotein granules. Mol Cell Proteomics 6(5):798–811
11. Weidensdorfer D, Stohr N, Baude A, Lederer M, Kohn M, Schierhorn A, Buchmeier S, Wahle E, Huttelmaier S (2009) Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. RNA 15(1):104–115
12. Lemm I, Ross J (2002) Regulation of c-myc mRNA decay by translational pausing in a coding region instability determinant. Mol Cell Biol 22(12):3959–3969
13. Kobel M, Weidensdorfer D, Reinke C, Lederer M, Schmitt WD, Zeng K, Thomssen C, Hauptmann S, Huttelmaier S (2007) Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. Oncogene 26(54):7584–7589
14. Stohr N, Kohn M, Lederer M, Glass M, Reinke C, Singer RH, Huttelmaier S (2012) IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. Genes Dev 26(2):176–189
15. Vikesaa J, Hansen TV, Jonson L, Borup R, Wewer UM, Christiansen J, Nielsen FC (2006) RNA-binding IMPs promote cell adhesion and invadopodia formation. EMBO J 25(7):1456–1468
16. Noubissi FK, Elcheva I, Bhatia N, Shakoori A, Ougolkov A, Liu J, Minamoto T, Ross J, Fuchs SY, Spiegelman VS (2006) CRD-BP mediates stabilization of betaTrCP1 and c-myc mRNA in response to beta-catenin signalling. Nature 441(7095):898–901
17. Stohr N, Lederer M, Reinke C, Meyer S, Hatzfeld M, Singer RH, Huttelmaier S (2006) ZBP1 regulates mRNA stability during cellular stress. J Cell Biol 175(4):527–534
18. Git A, Allison R, Perdiguero E, Nebreda AR, Houliston E, Standart N (2009) Vg1RBP phosphorylation by Erk2 MAP kinase correlates with the cortical release of Vg1 mRNA during meiotic maturation of Xenopus oocytes. RNA 15(6):1121–1133
19. Dai N, Rapley J, Angel M, Yanik MF, Blower MD, Avruch J (2011) mTOR phosphorylates IMP2 to promote IGF2 mRNA translation by internal ribosomal entry. Genes Dev 25(11):1159–1172
20. Elcheva I, Goswami S, Noubissi FK, Spiegelman VS (2009) CRD-BP protects the coding region of betaTrCP1 mRNA from miR-183-mediated degradation. Mol Cell 35(2):240–246
21. Sparanese D, Lee CH (2007) CRD-BP shields c-myc and MDR-1 RNA from endonucleolytic attack by a mammalian endoribonuclease. Nucleic Acids Res 35(4):1209–1221
22. Hafner M, Landthaler M, Burger L, Khorshid M, Hausser J, Berninger P, Rothballer A, Ascano M Jr, Jungkamp AC, Munschauer M, Ulrich A, Wardle GS, Dewell S, Zavolan M, Tuschl T (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141(1):129–141
23. Patel VL, Mitra S, Harris R, Buxbaum AR, Lionnet T, Brenowitz M, Girvin M, Levy M, Almo SC, Singer RH, Chao JA (2012) Spatial arrangement of an RNA zipcode identifies mRNAs under post-transcriptional control. Genes Dev 26(1):43–53
24. Git A, Standart N (2002) The KH domains of *Xenopus* Vg1RBP mediate RNA binding and self-association. RNA 8(10):1319–1333
25. Hansen TV, Hammer NA, Nielsen J, Madsen M, Dalbaeck C, Wewer UM, Christiansen J, Nielsen FC (2004) Dwarfism and impaired gut development in insulin-like growth factor II mRNA-binding protein 1-deficient mice. Mol Cell Biol 24(10):4448–4464
26. Hammer NA, Hansen TO, Byskov AG, Rajpert-De Meyts E, Grondahl ML, Bredkjaer HE, Wewer UM, Christiansen J, Nielsen FC (2005) Expression of IGF-II mRNA-binding proteins (IMPs) in gonads and testicular cancer. Reproduction 130(2):203–212
27. Yaniv K, Fainsod A, Kalcheim C, Yisraeli JK (2003) The RNA-binding protein Vg1 RBP is required for cell migration during early neural development. Development 130(23):5649–5661
28. Yaniv K, Yisraeli JK (2002) The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. Gene 287(1–2):49–54
29. Yisraeli JK (2005) VICKZ proteins: a multi-talented family of regulatory RNA-binding proteins. Biol Cell 97(1):87–96
30. Christiansen J, Kolte AM, Hansen TO, Nielsen FC (2009) IGF2 mRNA-binding protein 2: biological function and putative role in type 2 diabetes. J Mol Endocrinol 43(5):187–195
31. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC (1999) A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 19(2):1262–1270
32. Boylan KL, Mische S, Li M, Marques G, Morin X, Chia W, Hays TS (2008) Motility screen identifies *Drosophila* IGF-II mRNA-binding protein–zipcode-binding protein acting in oogenesis and synaptogenesis. PLoS Genet 4(2):e36
33. Munro TP, Kwon S, Schnapp BJ, St Johnston D (2006) A repeated IMP-binding motif controls oskar mRNA translation and anchoring independently of *Drosophila melanogaster* IMP. J Cell Biol 172(4):577–588
34. Geng C, Macdonald PM (2006) Imp associates with squid and Hrp48 and contributes to localized expression of gurken in the oocyte. Mol Cell Biol 26(24):9508–9516
35. Adolph SK, DeLotto R, Nielsen FC, Christiansen J (2009) Embryonic expression of *Drosophila* IMP in the developing CNS and PNS. Gene Expr Patterns 9(3):138–143
36. Fabrizio JJ, Hickey CA, Stabrawa C, Meytes V, Hutter JA, Talbert C, Regis N (2008) Imp (IGF-II mRNA-binding protein)

is expressed during spermatogenesis in *Drosophila melanogaster*. Fly (Austin) 2(1):47–52
37. Nielsen J, Cilius Nielsen F, Kragh Jakobsen R, Christiansen J (2000) The biphasic expression of IMP/Vg1-RBP is conserved between vertebrates and *Drosophila*. Mech Dev 96(1):129–132
38. Toledano H, D'Alterio C, Czech B, Levine E, Jones DL (2012) The let-7-Imp axis regulates ageing of the *Drosophila* testis stem-cell niche. Nature 485(7400):605–610
39. Jung H, Yoon BC, Holt CE (2012) Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. Nat Rev Neurosci 13(5):308–324
40. Doyle M, Kiebler MA (2011) Mechanisms of dendritic mRNA transport and its role in synaptic tagging. EMBO J 30(17):3540–3552
41. Dahm R, Kiebler M (2005) Cell biology: silenced RNA on the move. Nature 438(7067):432–435
42. Eom T, Antar LN, Singer RH, Bassell GJ (2003) Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. J Neurosci 23(32):10433–10444
43. Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ (2006) An essential role for beta-actin mRNA localization and translation in Ca^{2+}-dependent growth cone guidance. Nat Neurosci 9(10):1265–1273
44. Leung KM, van Horck FP, Lin AC, Allison R, Standart N, Holt CE (2006) Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. Nat Neurosci 9(10):1247–1256
45. Welshhans K, Bassell GJ (2011) Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. J Neurosci 31(27):9800–9813
46. Perycz M, Urbanska AS, Krawczyk PS, Parobczak K, Jaworski J (2011) Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons. J Neurosci 31(14):5271–5285
47. Donnelly CJ, Willis DE, Xu M, Tep C, Jiang C, Yoo S, Schanen NC, Kirn-Safran CB, van Minnen J, English A, Yoon SO, Bassell GJ, Twiss JL (2011) Limited availability of ZBP1 restricts axonal mRNA localization and nerve regeneration capacity. EMBO J 30(22):4665–4677
48. Mayr C, Bartel DP (2009) Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. Cell 138(4):673–684
49. Noubissi FK, Nikiforov MA, Colburn N, Spiegelman VS (2010) Transcriptional regulation of CRD-BP by c-myc: implications for c-myc functions. Genes Cancer 1(10):1074–1082
50. Gu W, Wells AL, Pan F, Singer RH (2008) Feedback regulation between zipcode binding protein 1 and beta-catenin mRNAs in breast cancer cells. Mol Cell Biol 28(16):4963–4974
51. Ross J, Lemm I, Berberet B (2001) Overexpression of an mRNA-binding protein in human colorectal cancer. Oncogene 20(45):6544–6550
52. Dimitriadis E, Trangas T, Milatos S, Foukas PG, Gioulbasanis I, Courtis N, Nielsen FC, Pandis N, Dafni U, Bardi G, Ioannidis P (2007) Expression of oncofetal RNA-binding protein CRD-BP/IMP1 predicts clinical outcome in colon cancer. Int J Cancer 121(3):486–494
53. Brants JR, Ayoubi TA, Chada K, Marchal K, Van de Ven WJ, Petit MM (2004) Differential regulation of the insulin-like growth factor II mRNA-binding protein genes by architectural transcription factor HMGA2. FEBS Lett 569(1–3):277–283
54. Cleynen I, Brants JR, Peeters K, Deckers R, Debiec-Rychter M, Sciot R, Van de Ven WJ, Petit MM (2007) HMGA2 regulates transcription of the Imp2 gene via an intronic regulatory element in cooperation with nuclear factor-kappaB. Mol Cancer Res 5(4):363–372
55. Boyerinas B, Park SM, Shomron N, Hedegaard MM, Vinther J, Andersen JS, Feig C, Xu J, Burge CB, Peter ME (2008) Identification of let-7-regulated oncofetal genes. Cancer Res 68(8):2587–2591
56. Boyerinas B, Park SM, Murmann AE, Gwin K, Montag AG, Zillhardt M, Hua YJ, Lengyel E, Peter ME (2012) Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer 130(8):1787–1797
57. Mongroo PS, Noubissi FK, Cuatrecasas M, Kalabis J, King CE, Johnstone CN, Bowser MJ, Castells A, Spiegelman VS, Rustgi AK (2011) IMP-1 displays cross-talk with K-Ras and modulates colon cancer cell survival through the novel proapoptotic protein CYFIP2. Cancer Res 71(6):2172–2182
58. Le HT, Sorrell AM, Siddle K (2012) Two isoforms of the mRNA binding protein IGF2BP2 are generated by alternative translational initiation. PLoS One 7(3):e33140
59. Zhang L, Liu Y, Hao S, Woda BA, Lu D (2011) IMP2 expression distinguishes endometrioid from serous endometrial adenocarcinomas. Am J Surg Pathol 35(6):868–872
60. Lu M, Nakamura RM, Dent ED, Zhang JY, Nielsen FC, Christiansen J, Chan EK, Tan EM (2001) Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. Am J Pathol 159(3):945–953
61. Vainer G, Vainer-Mosse E, Pikarsky A, Shenoy SM, Oberman F, Yeffet A, Singer RH, Pikarsky E, Yisraeli JK (2008) A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. J Pathol 215(4):445–456
62. Tessier CR, Doyle GA, Clark BA, Pitot HC, Ross J (2004) Mammary tumor induction in transgenic mice expressing an RNA-binding protein. Cancer Res 64(1):209–214
63. Ratajczak MZ (2012) Igf2-H19, an imprinted tandem gene, is an important regulator of embryonic development, a guardian of proliferation of adult pluripotent stem cells, a regulator of longevity, and a 'passkey' to cancerogenesis. Folia Histochem Cytobiol 50(2):19347
64. Gabory A, Jammes H, Dandolo L (2010) The H19 locus: role of an imprinted non-coding RNA in growth and development. Bioessays 32(6):473–480
65. Bernstein PL, Herrick DJ, Prokipcak RD, Ross J (1992) Control of c-myc mRNA half-life in vitro by a protein capable of binding to a coding region stability determinant. Genes Dev 6(4):642–654
66. Ioannidis P, Mahaira LG, Perez SA, Gritzapis AD, Sotiropoulou PA, Kavalakis GJ, Antsaklis AI, Baxevanis CN, Papamichail M (2005) CRD-BP/IMP1 expression characterizes cord blood CD34+ stem cells and affects c-myc and IGF-II expression in MCF-7 cancer cells. J Biol Chem 280(20):20086–20093
67. Bergstrom K, Urquhart JC, Tafech A, Doyle E, Lee CH (2006) Purification and characterization of a novel mammalian endoribonuclease. J Cell Biochem 98(3):519–537
68. Kapoor S (2008) IMP3: a new and important biomarker of systemic malignancies. Clin Cancer Res 14(17):5640 (author reply 5640–5641)
69. Findeis-Hosey JJ, Xu H (2011) The use of insulin like-growth factor II messenger RNA binding protein-3 in diagnostic pathology. Hum Pathol 42(3):303–314
70. Liao B, Hu Y, Herrick DJ, Brewer G (2005) The RNA-binding protein IMP-3 is a translational activator of insulin-like growth factor II leader-3 mRNA during proliferation of human K562 leukemia cells. J Biol Chem 280(18):18517–18524
71. Suvasini R, Shruti B, Thota B, Shinde SV, Friedmann-Morvinski D, Nawaz Z, Prasanna KV, Thennarasu K, Hegde AS, Arivazhagan A, Chandramouli BA, Santosh V, Somasundaram K (2011) Insulin growth factor-2 binding protein 3 (IGF2BP3) is a glioblastoma-specific marker that activates

phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3 K/MAPK) pathways by modulating IGF-2. J Biol Chem 286(29):25882–25890
72. Liao B, Hu Y, Brewer G (2011) RNA-binding protein insulin-like growth factor mRNA-binding protein 3 (IMP-3) promotes cell survival via insulin-like growth factor II signaling after ionizing radiation. J Biol Chem 286(36):31145–31152
73. Samanta S, Sharma VM, Khan A, Mercurio AM (2012) Regulation of IMP3 by EGFR signaling and repression by ERbeta: implications for triple-negative breast cancer. Oncogene (in press)
74. Zheng W, Yi X, Fadare O, Liang SX, Martel M, Schwartz PE, Jiang Z (2008) The oncofetal protein IMP3: a novel biomarker for endometrial serous carcinoma. Am J Surg Pathol 32(2):304–315
75. Li C, Rock KL, Woda BA, Jiang Z, Fraire AE, Dresser K (2007) IMP3 is a novel biomarker for adenocarcinoma in situ of the uterine cervix: an immunohistochemical study in comparison with p16(INK4a) expression. Mod Pathol 20(2):242–247
76. Noske A, Faggad A, Wirtz R, Darb-Esfahani S, Sehouli J, Sinn B, Nielsen FC, Weichert W, Buckendahl AC, Roske A, Muller B, Dietel M, Denkert C (2009) IMP3 expression in human ovarian cancer is associated with improved survival. Int J Gynecol Pathol 28(3):203–210
77. King RL, Pasha T, Roullet MR, Zhang PJ, Bagg A (2009) IMP-3 is differentially expressed in normal and neoplastic lymphoid tissue. Hum Pathol 40(12):1699–1705
78. Natkunam Y, Vainer G, Chen J, Zhao S, Marinelli RJ, Hammer AS, Hamilton-Dutoit S, Pikarsky E, Amir G, Levy R, Yisraeli JK, Lossos IS (2007) Expression of the RNA-binding protein VICKZ in normal hematopoietic tissues and neoplasms. Haematologica 92(2):176–183
79. Wachter DL, Kristiansen G, Soll C, Hellerbrand C, Breuhahn K, Fritzsche F, Agaimy A, Hartmann A, Riener MO (2012) Insulin-like growth factor II mRNA-binding protein 3 (IMP3) expression in hepatocellular carcinoma. A clinicopathological analysis with emphasis on diagnostic value. Histopathology 60(2):278–286
80. Pryor JG, Bourne PA, Yang Q, Spaulding BO, Scott GA, Xu H (2008) IMP-3 is a novel progression marker in malignant melanoma. Mod Pathol 21(4):431–437
81. Chen ST, Jeng YM, Chang CC, Chang HH, Huang MC, Juan HF, Hsu CH, Lee H, Liao YF, Lee YL, Hsu WM, Lai HS (2011) Insulin-like growth factor II mRNA-binding protein 3 expression predicts unfavorable prognosis in patients with neuroblastoma. Cancer Sci 102(12):2191–2198
82. Tang XH, Gudas LJ (2011) Retinoids, retinoic acid receptors, and cancer. Annu Rev Pathol 6:345–364
83. Craig EA, Spiegelman VS (2012) Inhibition of coding region determinant binding protein sensitizes melanoma cells to chemotherapeutic agents. Pigment Cell Melanoma Res 25(1):83–87
84. Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH (1997) Characterization of a beta-actin mRNA zipcode-binding protein. Mol Cell Biol 17(4):2158–2165
85. Lapidus K, Wyckoff J, Mouneimne G, Lorenz M, Soon L, Condeelis JS, Singer RH (2007) ZBP1 enhances cell polarity and reduces chemotaxis. J Cell Sci 120(Pt 18):3173–3178
86. Oberman F, Rand K, Maizels Y, Rubinstein AM, Yisraeli JK (2007) VICKZ proteins mediate cell migration via their RNA binding activity. RNA 13(9):1558–1569
87. Park HY, Trcek T, Wells AL, Chao JA, Singer RH (2012) An unbiased analysis method to quantify mRNA localization reveals its correlation with cell motility. Cell Rep 1(2):179–184
88. Stöhr N, Hüttelmaier S (2012) IGF2BP1: a post-transcriptional "driver" of tumor cell migration. Cell Adh Migr 6(4) (in press)
89. Schaeffer V, Hansen KM, Morris DR, Leboeuf RC, Abrass CK (2012) RNA-binding protein IGF2BP2/IMP2 is required for laminin-beta2 mRNA translation and is modulated by glucose concentration. Am J Physiol Renal Physiol 303(1):F75–82
90. Boudoukha S, Cuvellier S, Polesskaya A (2010) Role of the RNA-binding protein IMP-2 in muscle cell motility. Mol Cell Biol 30(24):5710–5725
91. Manieri NA, Drylewicz MR, Miyoshi H, Stappenbeck TS (2012) Igf2bp1 is required for full induction of Ptgs2 mRNA in colonic mesenchymal stem cells in mice. Gastroenterology 143(1):110–121 (e110)
92. Riss J, Khanna C, Koo S, Chandramouli GV, Yang HH, Hu Y, Kleiner DE, Rosenwald A, Schaefer CF, Ben-Sasson SA, Yang L, Powell J, Kane DW, Star RA, Aprelikova O, Bauer K, Vasselli JR, Maranchie JK, Kohn KW, Buetow KH, Linehan WM, Weinstein JN, Lee MP, Klausner RD, Barrett JC (2006) Cancers as wounds that do not heal: differences and similarities between renal regeneration/repair and renal cell carcinoma. Cancer Res 66(14):7216–7224
93. Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, Wang F, Guo A, Walczysko P, Gu Y, Sasaki T, Suzuki A, Forrester JV, Bourne HR, Devreotes PN, McCaig CD, Penninger JM (2006) Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature 442(7101):457–460
94. Spagnoli FM, Brivanlou AH (2006) The RNA-binding protein, Vg1RBP, is required for pancreatic fate specification. Dev Biol 292(2):442–456
95. Butter F, Scheibe M, Morl M, Mann M (2009) Unbiased RNA-protein interaction screen by quantitative proteomics. Proc Natl Acad Sci USA 106(26):10626–10631
96. Liao B, Patel M, Hu Y, Charles S, Herrick DJ, Brewer G (2004) Targeted knockdown of the RNA-binding protein CRD-BP promotes cell proliferation via an insulin-like growth factor II-dependent pathway in human K562 leukemia cells. J Biol Chem 279(47):48716–48724
97. Kohn M, Lederer M, Wachter K, Huttelmaier S (2010) Near-infrared (NIR) dye-labeled RNAs identify binding of ZBP1 to the noncoding Y3-RNA. RNA 16(7):1420–1428
98. Noubissi FK, Goswami S, Sanek NA, Kawakami K, Minamoto T, Moser A, Grinblat Y, Spiegelman VS (2009) Wnt signaling stimulates transcriptional outcome of the Hedgehog pathway by stabilizing GLI1 mRNA. Cancer Res 69(22):8572–8578
99. Deshler JO, Highett MI, Schnapp BJ (1997) Localization of *Xenopus* Vg1 mRNA by Vera protein and the endoplasmic reticulum. Science 276(5315):1128–1131
100. Elisha Z, Havin L, Ringel I, Yisraeli JK (1995) Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in Xenopus oocytes. EMBO J 14(20):5109–5114
101. Schwartz SP, Aisenthal L, Elisha Z, Oberman F, Yisraeli JK (1992) A 69-kDa RNA-binding protein from Xenopus oocytes recognizes a common motif in two vegetally localized maternal mRNAs. Proc Natl Acad Sci USA 89(24):11895–11899
102. Weinlich S, Huttelmaier S, Schierhorn A, Behrens SE, Ostareck-Lederer A, Ostareck DH (2009) IGF2BP1 enhances HCV IRES-mediated translation initiation via the 3′UTR. RNA 15(8):1528–1542
103. Gu W, Katz Z, Wu B, Park HY, Li D, Lin S, Wells AL, Singer RH (2012) Regulation of local expression of cell adhesion and motility-related mRNAs in breast cancer cells by IMP1/ZBP1. J Cell Sci 125(Pt 1):81–91
104. Runge S, Nielsen FC, Nielsen J, Lykke-Andersen J, Wewer UM, Christiansen J (2000) H19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein. J Biol Chem 275(38):29562–29569
105. Atlas R, Behar L, Sapoznik S, Ginzburg I (2007) Dynamic association with polysomes during P19 neuronal differentiation and an untranslated-region-dependent translation regulation of

the tau mRNA by the tau mRNA-associated proteins IMP1, HuD, and G3BP1. J Neurosci Res 85(1):173–183
106. Atlas R, Behar L, Elliott E, Ginzburg I (2004) The insulin-like growth factor mRNA binding-protein IMP-1 and the Ras-regulatory protein G3BP associate with tau mRNA and HuD protein in differentiated P19 neuronal cells. J Neurochem 89(3):613–626
107. Patel GP, Ma S, Bag J (2005) The autoregulatory translational control element of poly(A)-binding protein mRNA forms a heteromeric ribonucleoprotein complex. Nucleic Acids Res 33(22):7074–7089
108. Sim S, Yao J, Weinberg DE, Niessen S, Yates JR 3rd, Wolin SL (2012) The zipcode-binding protein ZBP1 influences the subcellular location of the Ro 60-kDa autoantigen and the noncoding Y3 RNA. RNA 18(1):100–110
109. Ioannidis P, Mahaira L, Papadopoulou A, Teixeira MR, Heim S, Andersen JA, Evangelou E, Dafni U, Pandis N, Trangas T (2003) 8q24 Copy number gains and expression of the c-myc mRNA stabilizing protein CRD-BP in primary breast carcinomas. Int J Cancer 104(1):54–59
110. Ioannidis P, Kottaridi C, Dimitriadis E, Courtis N, Mahaira L, Talieri M, Giannopoulos A, Iliadis K, Papaioannou D, Nasioulas G, Trangas T (2004) Expression of the RNA-binding protein CRD-BP in brain and non-small cell lung tumors. Cancer Lett 209(2):245–250
111. Elcheva I, Tarapore RS, Bhatia N, Spiegelman VS (2008) Overexpression of mRNA-binding protein CRD-BP in malignant melanomas. Oncogene 27(37):5069–5074
112. Mueller-Pillasch F, Lacher U, Wallrapp C, Micha A, Zimmerhackl F, Hameister H, Varga G, Friess H, Buchler M, Beger HG, Vila MR, Adler G, Gress TM (1997) Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. Oncogene 14(22):2729–2733
113. Ioannidis P, Trangas T, Dimitriadis E, Samiotaki M, Kyriazoglou I, Tsiapalis CM, Kittas C, Agnantis N, Nielsen FC, Nielsen J, Christiansen J, Pandis N (2001) C-MYC and IGF-II mRNA-binding protein (CRD-BP/IMP-1) in benign and malignant mesenchymal tumors. Int J Cancer 94(4):480–484
114. Yantiss RK, Woda BA, Fanger GR, Kalos M, Whalen GF, Tada H, Andersen DK, Rock KL, Dresser K (2005) KOC (K homology domain containing protein overexpressed in cancer): a novel molecular marker that distinguishes between benign and malignant lesions of the pancreas. Am J Surg Pathol 29(2):188–195
115. Schaeffer DF, Owen DR, Lim HJ, Buczkowski AK, Chung SW, Scudamore CH, Huntsman DG, Ng SS, Owen DA (2010) Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) overexpression in pancreatic ductal adenocarcinoma correlates with poor survival. BMC Cancer 10:59
116. Zhao H, Mandich D, Cartun RW, Ligato S (2007) Expression of K homology domain containing protein overexpressed in cancer in pancreatic FNA for diagnosing adenocarcinoma of pancreas. Diagn Cytopathol 35(11):700–704
117. Yantiss RK, Cosar E, Fischer AH (2008) Use of IMP3 in identification of carcinoma in fine needle aspiration biopsies of pancreas. Acta Cytol 52(2):133–138
118. Ligato S, Zhao H, Mandich D, Cartun RW (2008) KOC (K homology domain containing protein overexpressed in cancer) and S100A4-protein immunoreactivity improves the diagnostic sensitivity of biliary brushing cytology for diagnosing pancreaticobiliary malignancies. Diagn Cytopathol 36(8):561–567
119. Levy M, Lin F, Xu H, Dhall D, Spaulding BO, Wang HL (2010) S100P, von Hippel-Lindau gene product, and IMP3 serve as a useful immunohistochemical panel in the diagnosis of adenocarcinoma on endoscopic bile duct biopsy. Hum Pathol 41(9):1210–1219
120. Lu D, Vohra P, Chu PG, Woda B, Rock KL, Jiang Z (2009) An oncofetal protein IMP3: a new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. Am J Surg Pathol 33(4):521–525
121. Feng W, Zhou Z, Peters JH, Khoury T, Zhai Q, Wei Q, Truong CD, Song SW, Tan D (2011) Expression of insulin-like growth factor II mRNA-binding protein 3 in human esophageal adenocarcinoma and its precursor lesions. Arch Pathol Lab Med 135(8):1024–1031
122. Jeng YM, Wang TH, Lu SH, Yuan RH, Hsu HC (2009) Prognostic significance of insulin-like growth factor II mRNA-binding protein 3 expression in gastric adenocarcinoma. Br J Surg 96(1):66–73
123. Li D, Yan D, Tang H, Zhou C, Fan J, Li S, Wang X, Xia J, Huang F, Qiu G, Peng Z (2009) IMP3 is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. Ann Surg Oncol 16(12):3499–3506
124. Yuan RH, Wang CC, Chou CC, Chang KJ, Lee PH, Jeng YM (2009) Diffuse expression of RNA-binding protein IMP3 predicts high-stage lymph node metastasis and poor prognosis in colorectal adenocarcinoma. Ann Surg Oncol 16(6):1711–1719
125. Jeng YM, Chang CC, Hu FC, Chou HY, Kao HL, Wang TH, Hsu HC (2008) RNA-binding protein insulin-like growth factor II mRNA-binding protein 3 expression promotes tumor invasion and predicts early recurrence and poor prognosis in hepatocellular carcinoma. Hepatology 48(4):1118–1127
126. Riener MO, Fritzsche FR, Clavien PA, Pestalozzi BC, Probst-Hensch N, Jochum W, Kristiansen G (2009) IMP3 expression in lesions of the biliary tract: a marker for high-grade dysplasia and an independent prognostic factor in bile duct carcinomas. Hum Pathol 40(10):1377–1383
127. Li C, Zota V, Woda BA, Rock KL, Fraire AE, Jiang Z, Lu D, Xu B, Dresser K, Lutman CV, Fischer AH (2007) Expression of a novel oncofetal mRNA-binding protein IMP3 in endometrial carcinomas: diagnostic significance and clinicopathologic correlations. Mod Pathol 20(12):1263–1268
128. Lu D, Yang X, Jiang NY, Woda BA, Liu Q, Dresser K, Mercurio AM, Rock KL, Jiang Z (2011) IMP3, a new biomarker to predict progression of cervical intraepithelial neoplasia into invasive cancer. Am J Surg Pathol 35(11):1638–1645
129. Kobel M, Xu H, Bourne PA, Spaulding BO, Shih Ie M, Mao TL, Soslow RA, Ewanowich CA, Kalloger SE, Mehl E, Lee CH, Huntsman D, Gilks CB (2009) IGF2BP3 (IMP3) expression is a marker of unfavorable prognosis in ovarian carcinoma of clear cell subtype. Mod Pathol 22(3):469–475
130. Bellezza G, Cavaliere A, Sidoni A (2009) IMP3 expression in non-small cell lung cancer. Hum Pathol 40(8):1205–1206
131. Wang T, Fan L, Watanabe Y, McNeill PD, Moulton GG, Bangur C, Fanger GR, Okada M, Inoue Y, Persing DH, Reed SG (2003) L523S, an RNA-binding protein as a potential therapeutic target for lung cancer. Br J Cancer 88(6):887–894
132. Findeis-Hosey JJ, Yang Q, Spaulding BO, Wang HL, Xu H (2010) IMP3 expression is correlated with histologic grade of lung adenocarcinoma. Hum Pathol 41(4):477–484
133. Ikeda K, Tate G, Suzuki T, Kitamura T, Mitsuya T (2010) IMP3/L523S, a novel immunocytochemical marker that distinguishes benign and malignant cells: the expression profiles of IMP3/L523S in effusion cytology. Hum Pathol 41(5):745–750
134. Hanley KZ, Facik MS, Bourne PA, Yang Q, Spaulding BO, Bonfiglio TA, Xu H (2008) Utility of anti-L523S antibody in the diagnosis of benign and malignant serous effusions. Cancer 114(1):49–56
135. Yu L, Xu H, Wasco MJ, Bourne PA, Ma L (2010) IMP-3 expression in melanocytic lesions. J Cutan Pathol 37(3):316–322
136. Mentrikoski MJ, Ma L, Pryor JG, McMahon LA, Yang Q, Spaulding BO, Scott GA, Wang HL, Xu H (2009) Diagnostic

utility of IMP3 in segregating metastatic melanoma from benign nevi in lymph nodes. Mod Pathol 22(12):1582–1587
137. Pryor JG, Simon RA, Bourne PA, Spaulding BO, Scott GA, Xu H (2009) Merkel cell carcinoma expresses K homology domain-containing protein overexpressed in cancer similar to other high-grade neuroendocrine carcinomas. Hum Pathol 40(2):238–243
138. Slosar M, Vohra P, Prasad M, Fischer A, Quinlan R, Khan A (2009) Insulin-like growth factor mRNA binding protein 3 (IMP3) is differentially expressed in benign and malignant follicular patterned thyroid tumors. Endocr Pathol 20(3):149–157
139. Jin L, Seys AR, Zhang S, Erickson-Johnson MR, Roth CW, Evers BR, Oliveira AM, Lloyd RV (2010) Diagnostic utility of IMP3 expression in thyroid neoplasms: a quantitative RT-PCR study. Diagn Mol Pathol 19(2):63–69
140. Asioli S, Erickson LA, Righi A, Jin L, Volante M, Jenkins S, Papotti M, Bussolati G, Lloyd RV (2010) Poorly differentiated carcinoma of the thyroid: validation of the Turin proposal and analysis of IMP3 expression. Mod Pathol 23(9):1269–1278
141. Hao S, Smith TW, Chu PG, Liu Q, Ok CY, Woda BA, Lu D, Lin P, Wang SA, Dresser K, Rock KL, Jiang Z (2011) The oncofetal protein IMP3: a novel molecular marker to predict aggressive meningioma. Arch Pathol Lab Med 135(8):1032–1036
142. Righi A, Zhang S, Jin L, Scheithauer BW, Kovacs K, Kovacs G, Goth MI, Korbonits M, Lloyd RV (2010) Analysis of IMP3 expression in normal and neoplastic human pituitary tissues. Endocr Pathol 21(1):25–31
143. Jiang Z, Chu PG, Woda BA, Rock KL, Liu Q, Hsieh CC, Li C, Chen W, Duan HO, McDougal S, Wu CL (2006) Analysis of RNA-binding protein IMP3 to predict metastasis and prognosis of renal-cell carcinoma: a retrospective study. Lancet Oncol 7(7):556–564
144. Jiang Z, Chu PG, Woda BA, Liu Q, Balaji KC, Rock KL, Wu CL (2008) Combination of quantitative IMP3 and tumor stage: a new system to predict metastasis for patients with localized renal cell carcinomas. Clin Cancer Res 14(17):5579–5584
145. Jiang Z, Lohse CM, Chu PG, Wu CL, Woda BA, Rock KL, Kwon ED (2008) Oncofetal protein IMP3: a novel molecular marker that predicts metastasis of papillary and chromophobe renal cell carcinomas. Cancer 112(12):2676–2682
146. Li L, Xu H, Spaulding BO, Cheng L, Simon R, Yao JL, di Sant'Agnese PA, Bourne PA, Huang J (2008) Expression of RNA-binding protein IMP3 (KOC) in benign urothelium and urothelial tumors. Hum Pathol 39(8):1205–1211
147. Sitnikova L, Mendese G, Liu Q, Woda BA, Lu D, Dresser K, Mohanty S, Rock KL, Jiang Z (2008) IMP3 predicts aggressive superficial urothelial carcinoma of the bladder. Clin Cancer Res 14(6):1701–1706
148. Walter O, Prasad M, Lu S, Quinlan RM, Edmiston KL, Khan A (2009) IMP3 is a novel biomarker for triple negative invasive mammary carcinoma associated with a more aggressive phenotype. Hum Pathol 40(11):1528–1533
149. Sidoni A, Cartaginese F (2010) IMP3 expression in triple-negative breast carcinoma. Hum Pathol 41(9):1355–1356 (author reply 1356–1357)
150. Vranic S, Gurjeva O, Frkovic-Grazio S, Palazzo J, Tawfik O, Gatalica Z (2011) IMP3, a proposed novel basal phenotype marker, is commonly overexpressed in adenoid cystic carcinomas but not in apocrine carcinomas of the breast. Appl Immunohistochem Mol Morphol 19(5):413–416
151. Simon R, Bourne PA, Yang Q, Spaulding BO, di Sant'Agnese PA, Wang HL, Xu H (2007) Extrapulmonary small cell carcinomas express K homology domain containing protein overexpressed in cancer, but carcinoid tumors do not. Hum Pathol 38(8):1178–1183
152. Shi M, Fraire AE, Chu P, Cornejo K, Woda BA, Dresser K, Rock KL, Jiang Z (2011) Oncofetal protein IMP3, a new diagnostic biomarker to distinguish malignant mesothelioma from reactive mesothelial proliferation. Am J Surg Pathol 35(6):878–882
153. Do SI, Kim YW, Park HR, Park YK (2008) Expression of insulin-like growth factor-II mRNA binding protein 3 (IMP3) in osteosarcoma. Oncol Res 17(6):269–272
154. Li KH, Huang YP, Zhang J, Li GJ, Li SH (2009) Expression of IMP3 in osteosarcoma and its clinical significance. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 25(5):426–427

Manuskript 2

Gutschner T*, Hämmerle M*, Pazaitis N*, Bley N, Fiskin E, Uckelmann H, Heim A, Gross M, Hofmann N, Geffers R, Skawran B, Longerich T, Breuhahn K, Schirmacher P, Mühleck B, Hüttelmaier S, Diederichs S.

Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma.

Hepatology
May 2014, 59:1900-11.

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Insulin-Like Growth Factor 2 mRNA-Binding Protein 1 (IGF2BP1) Is an Important Protumorigenic Factor in Hepatocellular Carcinoma

Tony Gutschner,[1*] Monika Hämmerle,[1*] Nikolaos Pazaitis,[2*] Nadine Bley,[2] Evgenij Fiskin,[1] Hannah Uckelmann,[1] Andreas Heim,[1] Matthias Groβ,[1] Nina Hofmann,[1] Robert Geffers,[3] Britta Skawran,[4] Thomas Longerich,[5] Kai Breuhahn,[5] Peter Schirmacher,[5] Britta Mühleck,[2] Stefan Hüttelmaier,[2**] and Sven Diederichs[1**]

Hepatocarcinogenesis is a stepwise process. It involves several genetic and epigenetic alterations, e.g., loss of tumor suppressor gene expression (*TP53, PTEN, RB*) as well as activation of oncogenes (c-*MYC, MET, BRAF, RAS*). However, the role of RNA-binding proteins (RBPs), which regulate tumor suppressor and oncogene expression at the post-transcriptional level, are not well understood in hepatocellular carcinoma (HCC). Here we analyzed RBPs induced in human liver cancer, revealing 116 RBPs with a significant and more than 2-fold higher expression in HCC compared to normal liver tissue. We focused our subsequent analyses on the Insulin-like growth factor 2 messenger RNA (mRNA)-binding protein 1 (IGF2BP1) representing the most strongly up-regulated RBP in HCC in our cohort. Depletion of IGF2BP1 from multiple liver cancer cell lines inhibits proliferation and induces apoptosis *in vitro*. Accordingly, murine xenograft assays after stable depletion of IGF2BP1 reveal that tumor growth, but not tumor initiation, strongly depends on IGF2BP1 *in vivo*. At the molecular level, IGF2BP1 binds to and stabilizes the c-MYC and MKI67 mRNAs and increases c-Myc and Ki-67 protein expression, two potent regulators of cell proliferation and apoptosis. These substrates likely mediate the impact of IGF2BP1 in human liver cancer, but certainly additional target genes contribute to its function. *Conclusion*: The RNA-binding protein IGF2BP1 is an important protumorigenic factor in liver carcinogenesis. Hence, therapeutic targeting of IGF2BP1 may offer options for intervention in human HCC. (HEPATOLOGY 2014;59:1900-1911)

Hepatocellular carcinoma (HCC) is the fifth most common cancer and accounts for an estimated 695,900 deaths per year worldwide, representing the second most frequent cause of cancer-related death.[1-3] HCC accounts for 90% of all primary liver neoplasia and its incidence rate is increasing.[4] Long-term liver injuries caused by infection with hepatitis B or C virus, alcoholic liver disease, aflatoxin

Abbreviations: c-MYC, v-myc myelocytomatosis viral oncogene homolog (avian); FC, fold change; GFP, green fluorescent protein; HCC, hepatocellular carcinoma; IGF2BP, IGF2 mRNA-binding protein; iRFP, near-infrared fluorescent protein; NAFLD, nonalcoholic fatty liver disease; qRT-PCR, quantitative reverse-transcription, polymerase chain reaction; RBP, RNA-binding protein; shRNA, short hairpin RNA; siRNA, small interfering RNA.

From the [1]Helmholtz-University-Group "Molecular RNA Biology & Cancer," German Cancer Research Center DKFZ & Institute of Pathology, University Hospital Heidelberg, Germany; [2]Institute of Molecular Medicine, Department of Molecular Cell Biology, Martin-Luther-University, Halle, Germany; [3]Genome Analytics Research Group, Helmholtz Center for Infection Research, Braunschweig, Germany; [4]Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany; [5]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany.

Received July 9, 2013; accepted January 3, 2014.

Supported by the German Research Foundation (DFG Transregio TRR77, TP B3, B2, B4, B5, B7), the Excellence Cluster CellNetworks, the Helmholtz Society (VH-NG-504), and the Virtual Helmholtz Institute for Resistance in Leukemia. M.H. was supported by a Gerok stipend of the TRR77. N.P. was supported by a stipend of the GRK1591. Funding to S.H. was provided by ProNet-T3 (BMBF) and the GRK1591 (DFG).

Current address for Tony Gutschner: Department of Genomic Medicine, UT M.D. Anderson Cancer Center, Houston, TX 77054.

Current address for Evgenij Fiskin: Institute of Biochemistry II, Goethe University School of Medicine, Frankfurt am Main, Germany.

Current address for Hannah Uckelmann: Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ) and Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), Heidelberg, Germany.

**These authors contributed equally to this work.*

***These authors are joint senior authors.*

exposure, or inherited metabolic diseases contribute to the onset of HCC.[4] Hepatocarcinogenesis represents a multistep process in which tumor suppressor genes (*TP53, PTEN, RB*), oncogenes (*c-MYC, RAS, MET, BRAF*), developmental pathways (Wnt/β-catenin, Hedgehog), or growth factors and their receptors (IGF2, TGFB1, FGFR) are altered.[5,6] Next to genetic aberrations, regulators of all layers of gene expression may control HCC-related genes and contribute to tumorigenesis.

RNA-binding proteins (RBPs) represent a large and diverse class of posttranscriptional regulators.[7] Through direct interaction, RBPs control the localization, stability, or translation of their target RNAs.[8] The insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), also known as IMP-1 or CRD-BP (coding region stability determinant-binding protein), belongs to a conserved family of RNA-binding, oncofetal proteins, which includes IGF2BP2 and IGF2BP3.[9] Several target RNAs of IGF2BP1 are known and often encode proteins that have exceptional roles in developmental processes and neoplastic transformation. For example, IGF2BP1 binds to the IGF2 mRNA and controls the translation of this growth factor.[10,11] Binding of IGF2BP1 to ACTB messenger RNA (mRNA) regulates the spatiotemporal expression of ACTB in developing axons and dendrites.[12,13] Interaction of IGF2BP1 with c-MYC and MDR1 mRNAs inhibits the endonucleolytic cleavage of these transcripts, prolonging mRNA half-life.[14-18] IGF2BP1 binds to the long noncoding RNA *HULC* and facilitates its degradation by way of interaction with CNOT1, a component of the deadenylation complex.[19] The expression of IGF2BP1 has been implicated in various cancers, e.g., breast, ovarian, brain, lung, pancreas, colon, and skin cancer as well as Hodgkin lymphoma and B cell lymphomas.[9] Only recently, the family member IGF2BP2 was reported to be up-regulated in liver cancer,[20] but neither the regulation nor the specific functional role of IGF2BP1 in HCC has been studied.

Here we show that IGF2BP1 is the most strongly up-regulated RNA-binding protein in human HCC compared to normal liver. Its depletion in several liver cancer cell lines inhibits proliferation and induces apoptosis, partially by way of down-regulating c-MYC mRNA and protein levels. As a novel target for IGF2BP1, we identify the proliferation marker protein MKI67 (Ki-67). Importantly, a stable reduction of IGF2BP1 in HepG2 cells impairs tumor growth *in vivo* in a murine xenograft model. Thus, our study identifies IGF2BP1 as a novel potential target in HCC treatment.

Materials and Methods

RNA Isolation, cDNA and Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR) Analysis. Trizol lysis and RNA isolation was done as described previously.[21] For cDNA synthesis, equal amounts of RNA were transcribed and random primers (Life Technologies) were used for reverse transcription as described previously.[22] Gene expression was measured on an ABI StepOnePlus using SYBRGreen (Life Technologies). The housekeeping gene *GAPDH* was used as reference gene in all qRT-PCR analyses. All qRT-PCR primers are shown in Supporting Table 2.

RNA Interference. RNA interference (RNAi) was essentially performed as described previously with minor modifications.[19] In brief, for siRNA-mediated gene knockdown, 1×10^5 (HLE, HLF, Huh6) or 2×10^5 cells (HepG2, Huh7, Hep3B) were reverse transfected with 5 μL small interfering RNA (siRNA) (20 μM) and 5 μL RNAiMAX (HepG2, Huh7, Hep3B) or 10 μL siRNA (20 μM) and 10 μL RNAiMax (Life Technologies) in a 6-well plate according to the manufacturer's recommendations. Cells were harvested 48 hours or 72 hours after transfection for subsequent gene expression analysis (protein or RNA).

Sequences of the individual siRNAs can be found in Supporting Table 3, shRNA sequences for lentiviral transduction in Supporting Table 4. As nontargeting siRNA control, siAllStars Negative Control siRNA from Qiagen (Hilden, Germany) was used.

Proliferation Assays. Proliferation was analyzed with the bromodeoxyuridine (BrdU) Cell Proliferation ELISA kit (Roche, Basel, Switzerland) as described previously[23] or by monitoring cell numbers by counting at different timepoints (TC-20 Cell Counter, Bio-Rad).

Results

IGF2BP1 Is the Most Strongly Up-Regulated RNA-Binding Protein in HCC. Unbiased microarray analysis of 60 human HCC (Supporting Table 1) and

Address reprint requests to: Dr. Sven Diederichs, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280 (B150), D-69120 Heidelberg, Germany. E-mail: s.diederichs@dkfz.de; or Dr. Tony Gutschner, Department of Genomic Medicine, UT M.D. Anderson Cancer Center, 1901 East Road, Houston, TX 77054. E-mail: tgutschner@mdanderson.org.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.26997
Potential conflict of interest: Nothing to report.

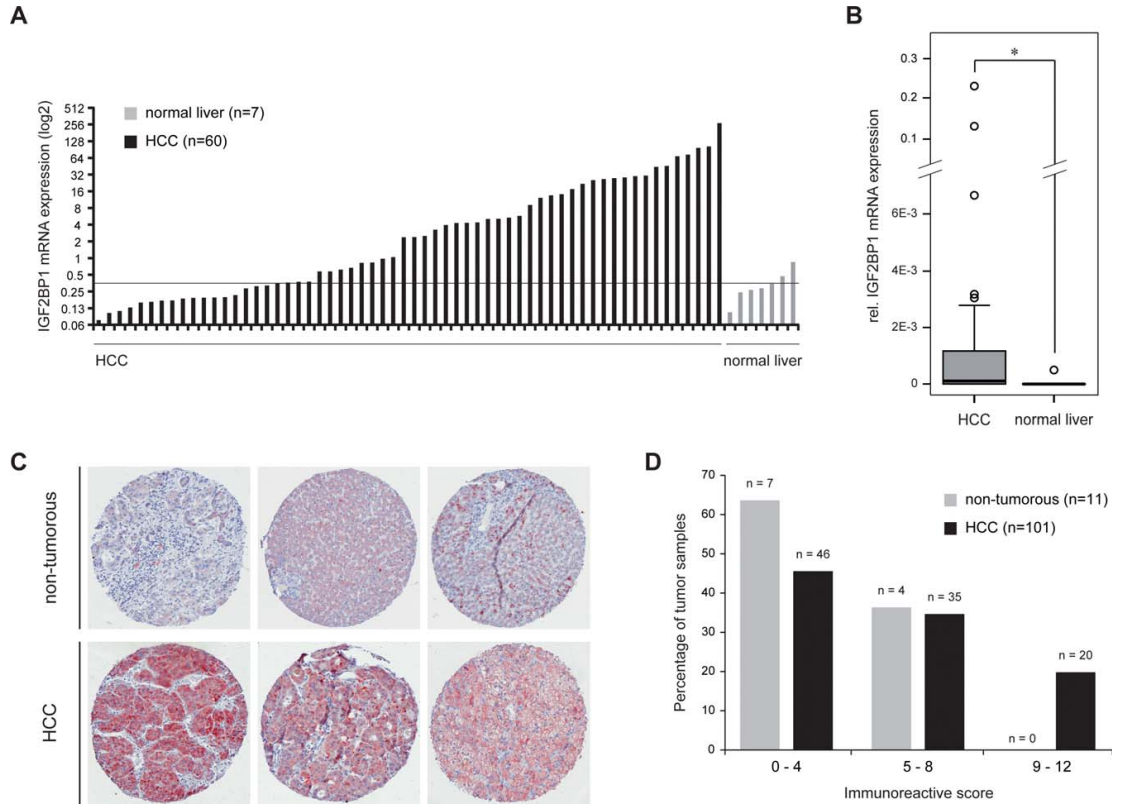


Fig. 1. Differential expression of IGF2BP1 in HCC and normal liver patient samples. (A) Microarray analysis of 60 HCC and 7 normal liver samples showing significantly increased IGF2BP1 mRNA expression in HCC. The horizontal line represents the mean of nontumor samples. (B) Validation of differential IGF2BP1 expression using qRT-PCR. *GAPDH* was used as reference gene. The boxplot shows a significantly higher expression of IGF2BP1 mRNA in tumor samples ($P = 0.03$). (C) Confirmation of IGF2BP1 up-regulation at the protein level. Tissue microarray staining was performed using a custom-made anti-IGF2BP1 antibody (clone 6A9). A strong cytoplasmic staining in liver cancer samples is detected (lower panel). (D) Analysis of protein expression using the immunoreactive score reveals higher IGF2BP1 protein levels in HCCs.

seven normal liver samples was performed previously using the Agilent SurePrint G3 Human Gene Expression array.[24] Here we focused our analysis on RBPs in human HCCs as defined by the Gene Ontology terms "RNA binding" (GO:0003723) and "mRNA binding" (GO:0003729). This yielded a list of 116 significantly up-regulated (fold change [FC] ≥ 2.0; $P \leq 0.05$) RBPs and identified IGF2BP1 as the most highly up-regulated RBP in HCC (FC = 6.8, $P = 7.8 \times 10^{-3}$, *t* test) (Fig. 1A; Supporting Table 5). We confirmed the overexpression of IGF2BP1 mRNA in HCC with qRT-PCR (Fig. 1B). Interestingly, IGF2BP1 expression positively correlated with tumor size ($P = 0.038$; correlation coefficient [Spearman-Rho]: 0.276). We also tested for correlation with other clinical parameters (etiology, staging, grading, vascular invasion, age, and sex), but only detected weak but significant correlations with advanced stages and grading of poor differentiation (Supporting Fig. 1, Supporting Table 1).

In addition to our microarray data, we confirmed IGF2BP1 induction in the Oncomine database (www.oncomine.org) (Supporting Fig. 2A,B). Analysis of the Chen et al. liver dataset[25] confirmed a higher expression of IGF2BP1 in HCC compared to normal liver tissue and precursor lesions. The comparison of ~900 human cancer cell lines of different origin[26] revealed the highest expression level of IGF2BP1 in liver cancer cells, implicating a specific importance of IGF2BP1 in this entity (Supporting Fig. 2C).

To confirm the induction of IGF2BP1 at the protein level, we performed tissue microarray analyses and stained 101 HCCs and 11 nontumorous liver samples (Fig. 1C,D). We observed a stronger cytoplasmic

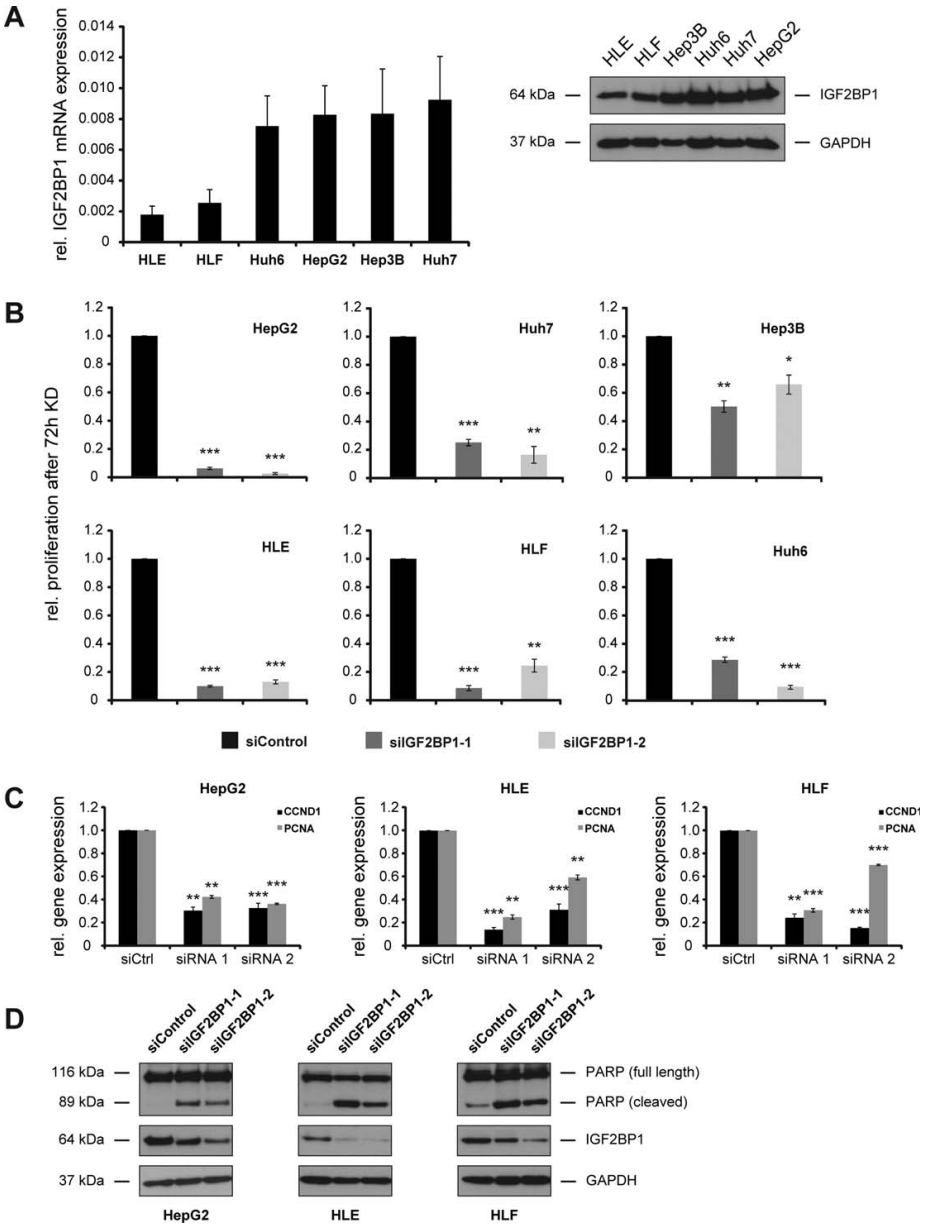


Fig. 2. Reduced proliferation and enhanced apoptosis after IGF2BP1 depletion. (A) IGF2BP1 mRNA and protein expression were analyzed in six human liver cancer cell lines. *GAPDH* was used as reference gene in qRT-PCR analysis as well as loading control for western blot. (B) A BrdU incorporation assay was used to analyze cellular proliferation 72 hours after IGF2BP1 knockdown with either of two siRNAs. The proliferation rate was normalized to siControl for each cell line. (C) Expression analysis of proliferation markers *CCND1* and *PCNA* after 48 hours or 72 hours of IGF2BP1 depletion. The expression was normalized to siControl and *GAPDH* was used as reference gene. (D) Analysis of apoptosis induction 72 hours after IGF2BP1 knockdown. Total protein lysates were analyzed for enhanced PARP cleavage as a marker for apoptosis by western blotting. Efficient knockdown of IGF2BP1 was accomplished in all cell lines. Representative western blots are shown. All experiments were done in biological replicates (n=3). Given is the mean and the respective standard error of the mean (±SEM).

staining for IGF2BP1 in the HCC samples compared to the nontumorous sections recapitulating the upregulation of IGF2BP1 mRNA also at the protein level. The IGF2BP1 antibody used was custom-made and specifically recognized human IGF2BP1 in western blotting (Supporting Fig. 3).[9]

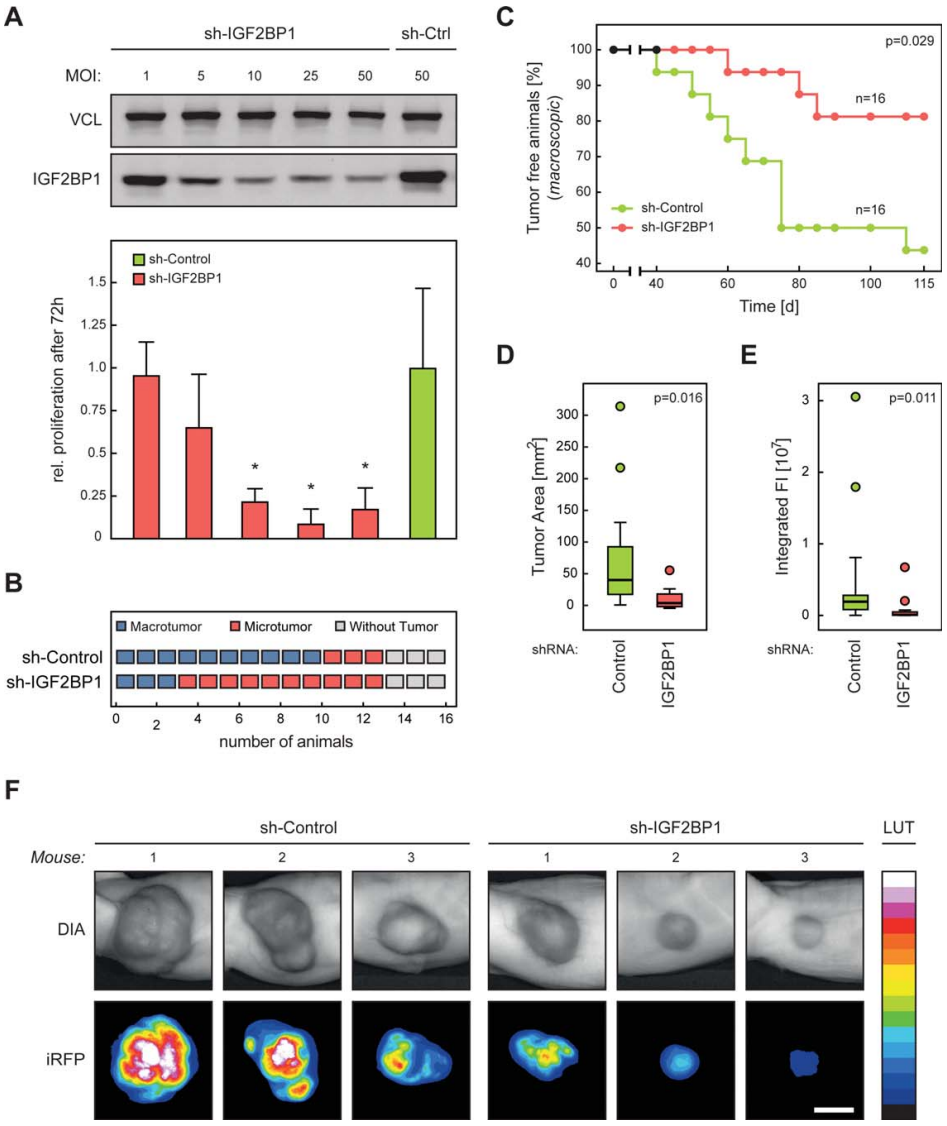


Fig. 3. IGF2BP1 is important for tumor growth *in vivo*. IGF2BP1 depletion impairs the growth of heterologous *xenograft* tumors. (A) HepG2 cells were transduced with the indicated multiplicity of infection (MOI) of control or IGF2BP1-directed shRNA encoding lentiviral vectors for 72 hours. IGF2BP1 depletion was monitored by western blotting using the indicated antibodies (upper panel). VCL served as a loading control. Cellular proliferation relative to controls transduced with control shRNA (sh-Control) was determined by cell counting (lower panel). Statistical significance was determined by Levene's F- and Student *t* test. (B-F) Transduced HepG2 were harvested 48 hours after transduction and suspended in serum-free media containing Matrigel before subcutaneous injection (4-8 $\times$ 10^5 cells per animal). For each cohort, control knockdown (sh-Control) and IGF2BP1 depletion, 16 animals were injected. Tumor growth was monitored at 5-day intervals. The number of macroscopic palpable tumors as well as microtumors was determined 115 days (endpoint) after injection by NIR-imaging (B). Time resolved macroscopic tumor burden is depicted by a Kaplan-Meier plot (C). The area (D) and integrated fluorescence intensity (E) of macro- and microtumors identified in both cohorts is depicted by boxplots. Images of three macroscopic tumors acquired by brightfield (DIA) or NIR-imaging (iRFP) are shown in (F). Bar = 10 mm; LUT, fluorescence intensity scale (white = highest / black = lowest). Statistical significance was determined by logrank test (C) or Mann-Whitney *U* tests (E,F).

Depletion of IGF2BP1 Inhibits Proliferation and Induces Apoptosis. IGF2BP1 was highly expressed in a panel of six human liver cancer cell lines, both at the mRNA and protein level (Fig. 2A). Only minor expression differences were detected. Next we analyzed the cellular loss-of-function phenotype by way of

siRNA-mediated depletion of IGF2BP1 with two independent siRNAs. First, the proliferation of the six cell lines was measured using a BrdU incorporation assay. Loss of IGF2BP1 significantly decreased proliferation in all cell lines (Fig. 2B). The effect was most pronounced in HepG2 cells (~10-fold), while Hep3B cells showed only a 2-fold reduction in proliferation. The reduced proliferation rate was accompanied by a significant reduction of the proliferation markers *CCND1* and *PCNA* in all six cell lines as determined by qRT-PCR (Fig. 2C; Supporting Fig. 4A). Moreover, depletion of IGF2BP1 efficiently induced apoptosis in all six cell lines as indicated by enhanced poly (ADP-ribose) polymerase (PARP) cleavage (Fig. 2D; Supporting Fig. 4B).

Knockdown of IGF2BP1 Impairs Tumor Growth In Vivo. As knockdown of IGF2BP1 strongly reduced cancer cell proliferation and survival *in vitro*, we tested whether depletion of IGF2BP1 also prevented tumor growth *in vivo*. HepG2 cells were transduced with lentiviral vectors encoding IGF2BP1-directed shRNA and an iRFP expression cassette to trace transduced cells. Efficient IGF2BP1 knockdown was confirmed by western blot (Fig. 3A, upper panel). Similar to transient IGF2BP1 depletion, shRNA-mediated knockdown of IGF2BP1 correlated with reduced cell proliferation (Fig. 3A, lower panel). Subcutaneous injection of 4-8 $\times$ 10^5 HepG2 cells transduced with the control shRNA vector (sh-Control) led to the formation of macroscopic tumors in 10 out of 16 animals (Fig. 3B). In contrast, only 3 out of 16 animals showed macroscopic tumors when injected with HepG2 cells depleted of IGF2BP1 (sh-IGF2BP1). This was analyzed in further detail by near-infrared (NIR) *in vivo* imaging of iRFP-labeled tumor cells. Surprisingly, the *in vivo* imaging revealed that the total number of micro- and macrotumors was the same in both populations with 13 out of 16 tumors (Fig. 3B). However, in the IGF2BP1 knockdown cohort, the number of microscopic and thus nonpalpable tumors was significantly increased. In agreement, Kaplan-Meier analyses confirmed a significantly delayed formation of macroscopic tumors (Fig. 3C). This was further supported by the quantitative assessment and significant decrease of endpoint tumor area and integrated fluorescence intensities (FI) as determined by NIR-imaging (Fig. 3D-F). These findings provide the first evidence that IGF2BP1 depletion impairs tumor growth *in vivo* and support *in vitro* observations indicating that the protein promotes tumor cell proliferation and survival.

IGF2BP1 Regulates c-MYC Expression at the Posttranscriptional Level. To gain further insights into the molecular mechanisms underlying IGF2BP1's cellular functions, we hypothesized that IGF2BP1 may posttranscriptionally regulate the expression of oncogenes or tumor suppressors that are known to have pivotal roles in hepatocarcinogenesis. Among the known IGF2BP1 target genes, e.g., *ACTB*,[13] *IGF2*,[10,11] *MYC*,[14,15] or *MDR1*,[17] *MYC* represented the most interesting target due to its established role as regulator of proliferation and apoptosis in diverse human cancers including HCC.[27-30] Notably, previous studies revealed that IGF2BP1 promoted the proliferation of ovarian cancer cells by enhancing the expression of c-MYC mRNA and protein. This was correlated with a severe up-regulation of IGF2BP1 expression in serous ovarian carcinomas.[31] Thus, we analyzed the expression of c-MYC after IGF2BP1 depletion in HepG2 cells. The mRNA and protein levels of c-MYC were significantly down-regulated upon IGF2BP1 knockdown (Fig. 4A,B). This was also observed in Huh6 cells (Supporting Fig. 5A,B). To confirm that IGF2BP1-dependent enhancement of c-MYC expression was correlated with a direct association of IGF2BP1 with the c-MYC mRNA, we performed RNA immunoprecipitation (RIP) experiments. FLAG-tagged IGF2BP1 or GFP (green fluorescent protein; negative control) were transiently overexpressed in HepG2 cells and immunoprecipitated with an anti-FLAG antibody (Fig. 4C, left panel). After isolation of the copurifying RNA, the enrichment of selected transcripts was determined by way of qRT-PCR. This confirmed selective enrichment of the c-MYC mRNA as well as other previously identified IGF2BP1 target transcripts, i.e., the IGF2 mRNA and the long noncoding RNA *HULC* (Fig. 4C, right panel). No enrichment of c-MYC mRNA was seen in GFP controls. The highly abundant 5.8S rRNA or lysine-tRNA served as negative controls and were not enriched in any of the purifications. Thus, we confirmed selective association of IGF2BP1 with the c-MYC mRNA in human liver cancer cells.

Since IGF2BP1 could stabilize, destabilize, or control the translation of its target RNAs, we tested whether its knockdown affected c-MYC mRNA stability in liver cancer cells as previously shown in other tumor entities.[18] Upon siRNA-directed IGF2BP1 depletion, HepG2 cells were treated with Actinomycin D to inhibit transcription. Indeed, we observed an enhanced decay and a significantly reduced half-life (siControl: ~43 minutes versus siIGF2BP1: ~32 minutes) of c-MYC mRNA (Fig. 4D). Hence,

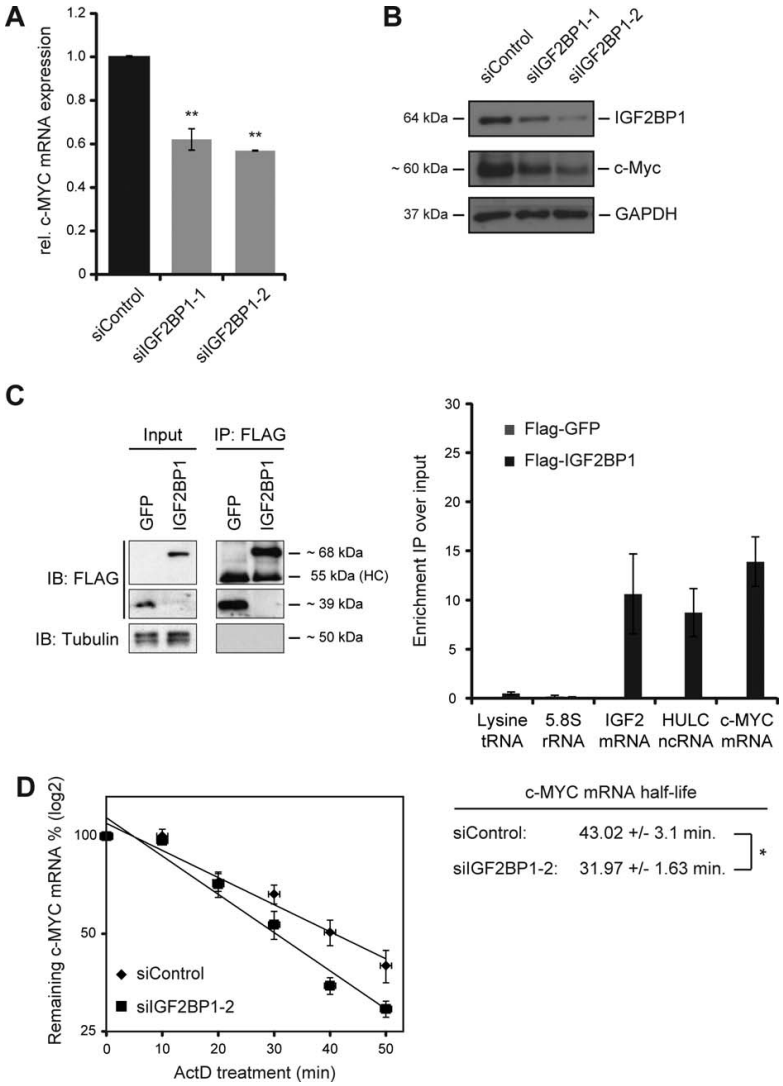


Fig. 4. Impact of IGF2BP1 depletion on c-MYC expression and mRNA stability. (A) Analysis of c-MYC mRNA 48 hours after IGF2BP1 depletion in HepG2 cells. *GAPDH* was used as reference gene in qRT-PCR. Depicted is the remaining c-MYC expression relative to its level in cells transfected with control siRNA (mean of at least three independent experiments ±SEM). (B) A representative western blot shows the reduction of c-Myc protein after IGF2BP1 depletion. (C) Representative western blot analysis after FLAG-immunoprecipitation. HepG2 cells were transiently transfected for 72 hours with FLAG-tagged GFP (negative control) or IGF2BP1, respectively (left panel). Analysis of copurified RNA and respective enrichment as determined by qRT-PCR after anti-FLAG immunoprecipitation showing the specific binding of c-MYC mRNA to IGF2BP1 (right panel). All immunoprecipitation experiments were done in biological replicates (n = 3). Given is the mean and the respective standard error of the mean (±SEM). HC, heavy chain. (D) c-MYC mRNA stability analysis in HepG2 cells after actinomycin D (ActD) treatment. Cells were transfected with siRNAs against IGF2BP1 or a control siRNA and 48 hours later a time course for RNA stability was started by adding the transcription inhibitor. Cells were harvested at the indicated timepoints. Expression levels were normalized to "0 h" and *GAPDH* was used as reference gene. Shown is the mean of at least three independent experiments (±SEM).

IGF2BP1 directly associated with the c-MYC mRNA, stabilized this target transcript, and gave rise to an enhanced c-MYC expression.

Since the c-MYC oncogene is a key player associated with malignant transformation by regulating cell cycle progression, differentiation, cell growth, apoptosis, and other tumor traits,[28] we tested whether c-MYC down-regulation could contribute to the observed cellular phenotypes associated with IGF2BP1 depletion. Two different siRNAs efficiently reduced

c-MYC at the mRNA and protein level (Supporting Fig. 6A,B). At the cellular level, c-MYC knockdown reduced proliferation of HepG2 cells, which correlated with the knockdown efficiency (Supporting Fig. 6C). In addition, the depletion of c-MYC slightly induced apoptosis in a dose-dependent manner in HepG2 cells, as indicated by enhanced PARP cleavage (Supporting Fig. 6B). Knockdown of c-MYC with the second siRNA was apparently not sufficient to induce apoptosis, but consistently affected cell proliferation (Supporting Fig. 6C). A similar decrease in proliferation upon c-MYC knockdown was obtained in Huh6 cells (Supporting Fig. 6D-F).

To determine whether c-MYC could, at least partially, rescue the cell growth phenotype observed upon IGF2BP1 depletion, we overexpressed c-MYC at moderate levels in IGF2BP1-depleted cells (Supporting Fig. 5C). Consistent with our previous findings, IGF2BP1 knockdown strongly impaired cell growth. The latter was substantially restored by the overexpression of Flag-tagged c-MYC protein, suggesting that IGF2BP1 promotes tumor cell growth and survival partially by way of c-MYC (Supporting Fig. 5D). Notably, IGF2BP1 was modestly up-regulated by the overexpression of c-MYC even in the presence of IGF2BP1 targeting siRNAs. This suggested that c-MYC could induce IGF2BP1 expression. Depletion of c-MYC in HepG2 and Huh6 cells indeed reduced IGF2BP1 mRNA and protein expression and its overexpression strengthened IGF2BP1 promoter activity (Supporting Fig. 7), supporting previous studies suggesting that c-MYC promotes IGF2BP1 mRNA synthesis.[32] This might point towards a positive feedback loop and further underscores the functional interplay between these two factors in liver cancer.

Finally, we tested whether IGF2BP1-directed enhancement of c-MYC expression was also detectable in human HCCs. We correlated IGF2BP1 and c-MYC mRNA expression in HCC patient samples. Consistent with IGF2BP1-dependent control of c-MYC expression *in vitro*, we observed a modest, but significant, positive correlation between the expression of IGF2BP1 and c-MYC mRNA in our microarray dataset ($P = 0.029$; correlation coefficient [Spearman-Rho]: 0.282) (Supporting Fig. 8A). However, this analysis revealed two distinct groups of HCC with different interdependencies between IGF2BP1 and c-MYC (Supporting Fig. 8B,C): A subset of HCCs with well detectable IGF2BP1 expression (group A, red dots) showed a strong and significant correlation ($P = 0.013$; correlation coefficient 0.558). In contrast, group B HCCs (gray dots) with marginal expression of IGF2BP1 did not show a significant correlation ($P = 0.446$; correlation coefficient 0.122). Hence, in HCCs with relevant expression of IGF2BP1, its expression was positively correlated with c-MYC expression. We compared the gene expression profiles between the two groups (A, B) and analyzed pathway activation using the DAVID v. 6.7 analysis tool (http://david.abcc.ncifcrf.gov/)[33] for genes that showed a significant ($P < 0.05$) and at least a 1.5-fold increase in group A versus group B. Pathways overrepresented in group A were most prominently linked to the cell cycle (Supporting Fig. 8D). These processes might thus be distinct between the two groups of HCCs with high versus marginal IGF2BP1 expression and could be causally linked to IGF2BP1 and c-MYC expression.

Ki-67 Is a Novel Target for IGF2BP1 in Liver Cancer. While c-MYC is undoubtedly an important target of IGF2BP1 with a strong link to cancer, a direct comparison of the proliferative and apoptotic effects after IGF2BP1 and c-MYC silencing (Fig. 2B; Supporting Fig. 6) revealed a stronger impact of IGF2BP1, which implicates additional targets in its function in liver cancer cells. Hence, we aimed to explore further targets of IGF2BP1 in HCC. We identified mRNAs positively correlated with IGF2BP1 in our HCC microarrays and compared these to the mRNAs interacting with IGF2BP1 in public datasets.[34] Thereby, we identified the proliferation marker MKI67 (Ki-67)[35] as a novel client mRNA of IGF2BP1. Ki-67 mRNA was strongly up-regulated in HCC compared to normal liver (Fig. 5A) and positively correlated with IGF2BP1 expression (Fig. 5B). Additionally, we found a positive, but not statistically significant, correlation between IGF2BP1 protein expression and the number nuclei positive for Ki-67 protein in the HCC TMA ($P = 0.09$, correlation coefficient [Spearman-Rho]: 0.345). Using RIP-qPCR, the interaction of Ki-67 mRNA with IGF2BP1 protein was validated (Fig. 5C). Upon IGF2BP1 silencing, Ki-67 mRNA as well as protein was reduced (Fig. 5D-F). At the mechanistic level, depletion of IGF2BP1 significantly decreased the half-life of Ki-67 mRNA (Fig. 5G).

Taken together, we propose the following model of IGF2BP1 function in human HCC (Supporting Fig. 9): The RNA-binding protein interacts with several protein-coding mRNAs, including c-MYC and MKI67, or long noncoding RNAs and regulates their fate, i.e., degradation, translation, and/or localization. Interaction with c-MYC and MKI67 mRNAs leads to a stabilization and increased expression in liver cancer.

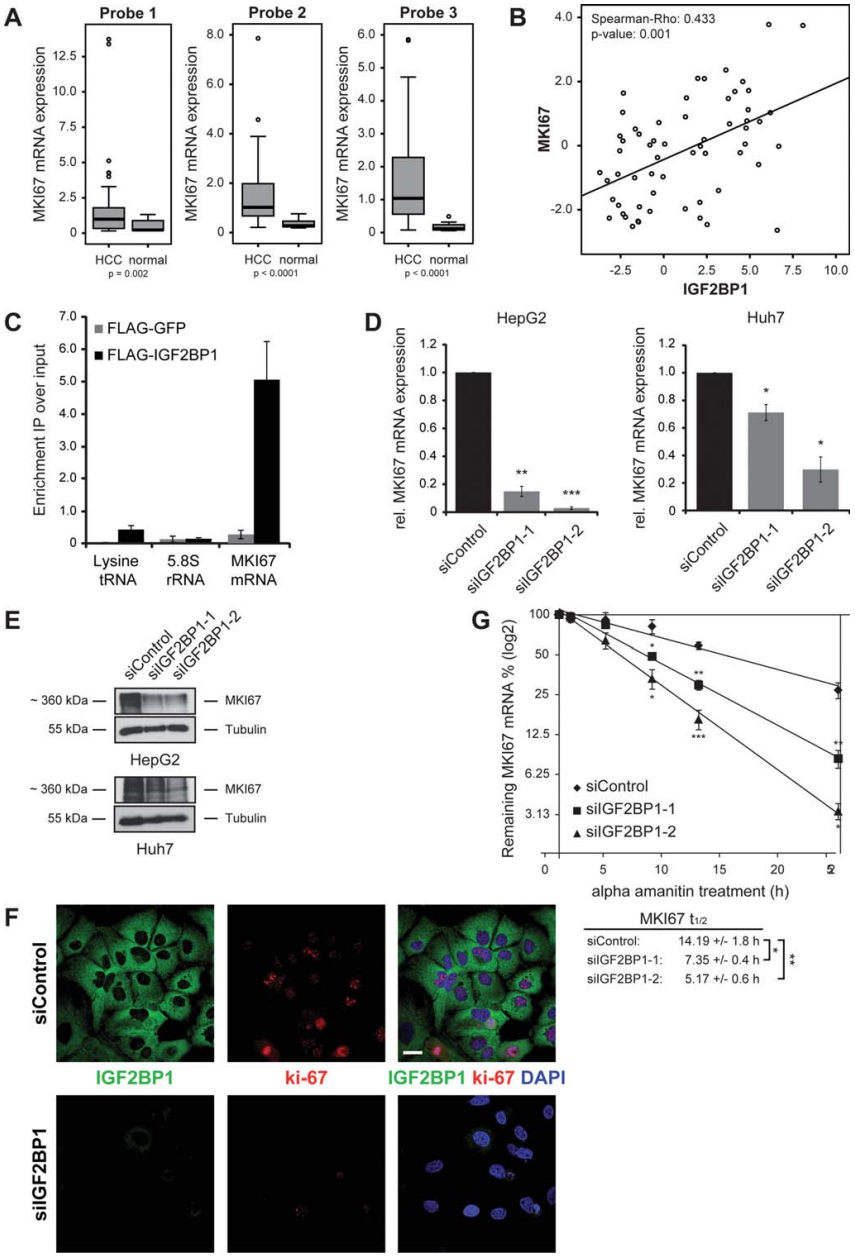


Fig. 5. Impact of IGF2BP1 depletion on MKI67 expression and mRNA stability. (A) MKI67 mRNA levels (represented by three different probes) were significantly increased in HCC tissue compared to normal livers as detected by microarray analysis of 60 HCC and 7 normal liver samples. (B) Positive and strong correlation of IGF2BP1 and MKI67 mRNA in 60 HCC samples. (C) Analysis of copurified RNA and respective enrichment as determined by qRT-PCR after anti-FLAG immunoprecipitation showing the specific binding of MKI67 mRNA to IGF2BP1. (D) MKI67 mRNA levels were significantly reduced in HepG2 and Huh7 cells with two independent siRNAs as analyzed by qRT-PCR. (E) The reduced MKI67 protein was analyzed by western blotting. Tubulin was used as reference gene. All experiments were done in three biological replicates and representative blots are shown. (F) IGF2BP1 (green) and MKI67 (red) protein expression were determined by immunofluorescence in Huh7 cells after knockdown of IGF2BP1 (blue: DAPI; scale bar = 25 μm). (G) MKI67 mRNA stability analysis in HepG2 cells after alpha-amanitin treatment. Cells were transfected with siRNAs against IGF2BP1 or a control siRNA and 48 hours later a time course for RNA stability was started by adding the transcription inhibitor. Cells were harvested at the indicated timepoints. Expression levels were normalized to "0 h" and *GAPDH* was used as reference mRNA. Shown is the mean of at least three independent experiments (±SEM). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Altogether, these regulatory circuits that are controlled by IGF2BP1 act in concert to allow tumor cell proliferation, tumor growth, and prevent apoptosis in liver cancer.

Discussion

Large-scale genomic approaches have identified genetic abnormalities in putative "drivers" of transformation in liver cancer.[36-39] HCC appears as a multifactorial disease emerging from a stepwise process including loss of tumor suppressors on chromosome 1, 3, and 8p, and gain of oncogenes such as *c-MYC* on 8q.[5,40,41] Nonetheless, the molecular mechanisms underlying neoplastic transformation are incompletely understood. Oncogenic drivers without chromosomal alteration or mutation are missed in these analyses, although their expression may be deregulated. Moreover, cellular processes are dynamically regulated also posttranscriptionally and posttranslationally. MicroRNAs are well established as posttranscriptional regulators in HCC.[42] However, surprisingly little is known about RNA-binding proteins and their function in liver carcinogenesis.

RNA-binding proteins can influence gene expression by their direct interaction with specific sequence motifs or structural elements present in the coding or 5′- or 3′-untranslated region of their target RNA[7,8] controlling RNA localization, stability, or translation. So far, only a small number of RBPs have been studied in human liver diseases, e.g., HuR,[43] CUGBP1,[44] and Lin28.[45]

In this study we determined the role of IGF2BP1 in liver cancer. In our microarray profiling, IGF2BP1 is the most highly up-regulated RBP in human HCCs (Fig. 1). The precise mechanism of regulation remains elusive. *IGF2BP1* gene amplifications have been found in human breast cancer.[46] However, no evidence of IGF2BP1 genomic alterations (17q21.32) in liver cancer is found in public databases (COSMIC, CONAN, Oncomine), suggesting a transcriptional induction. Notably, the IGF2BP2 family member is not strongly induced in our patient cohort in contrast to recent observations.[20] This finding is consistent with reports that IGF2BP2 rather plays a role in metabolic control than in carcinogenesis.[9,47] Also, while IGF2BP2 silencing has been published to reduce ERK phosphorylation as its main mechanism of action,[20] we do not observe this effect for IGF2BP1 silencing (Gutschner, Hämmerle, Diederichs, unpublished). This further underlines the functional distinctness of these family members.

IGF2BP1 is a known oncofetal protein linked to several human cancers: Its expression is induced in human malignant melanomas or colorectal carcinomas with activated WNT/β-catenin/TCF signaling.[16,48] High IGF2BP1 expression is a poor prognostic marker in ovarian and lung cancer.[31,49,50] Here we show that IGF2BP1 expression in HCC patients is associated with enhanced tumor growth and identify IGF2BP1 as a critical regulator of liver cancer cell proliferation and survival (Fig. 2). Our xenograft assay further supports the notion that IGF2BP1 is important for liver cancer growth *in vivo* (Fig. 3). The correlation of IGF2BP1 expression with tumor size and the formation of microtumors in the xenograft mouse model might indicate that IGF2BP1 is important for liver cancer progression and tumor growth rather than initiation.

IGF2BP1 may contribute to liver carcinogenesis in part by regulating the expression of the oncogene c-MYC. IGF2BP1 directly interacts with the c-MYC mRNA, stabilizes the transcript, and leads to higher c-MYC protein expression (Fig. 4). These findings corroborate previous findings from other cell types in liver cancer.[18,31]

c-MYC acts as a transcription factor and controls the expression of pro-oncogenic factors that drive cell proliferation.[28] Interestingly, c-MYC also seems to moderately induce IGF2BP1 expression and promoter activity in HepG2 and Huh6 cells (Supporting Fig. 7), recapitulating findings in HeLa and HEK293T cells[32] in a liver cancer model. Chromatin immunoprecipitation data deposited in the UCSC genome browser (www.genome.ucsc.edu) provide evidence for direct binding of the c-Myc/Max dimer to the promoter region of *IGF2BP1* in HepG2 cells, further supporting the idea of a self-amplifying loop. This feedforward loop may contribute to the maintenance of high c-MYC levels in liver cancer cells.

Depletion of c-MYC leads to decreased cell proliferation and induced apoptosis (Supporting Fig. 6). While this might seem unexpected at first, given that c-MYC overexpression is also known to induce apoptosis, our finding matches previous observations in liver cancer cells.[29] Loss of c-MYC or IGF2BP1 give rise to similar phenotypes, but our data also suggest that c-MYC is not the sole mediator of IGF2BP1 function. Concordantly, IGF2BP1 depletion reduces c-MYC expression in HepG2, Huh6, and Huh7 cells, but not in other liver cancer cell lines tested (data not shown), although these are highly responsive to IGF2BP1 knockdown. This could be explained by the heterogeneity and mutational diversity of the different cell lines or additional factors that control c-MYC

expression levels and render it largely insensitive towards changes in IGF2BP1 expression. Thus, besides its striking effect on c-MYC expression in a subset of liver cancer cells, IGF2BP1 likely achieves its full oncogenic potential by the pleiotropic regulation of multiple target genes. Here we identify one additional substrate mRNA for IGF2BP1: MKI67 (Ki-67). The Ki-67 mRNA is induced in HCC and positively correlated with IGF2BP1, it interacts with IGF2BP1, its expression is reduced, and its mRNA destabilized upon IGF2BP1 knockdown. Ki-67 has been linked to proliferation, one of the hallmarks of cancer, providing a further important link between IGF2BP1 and its oncogenic phenotypes.

Future studies will elucidate the full RNA-IGF2BP1 network that promotes HCC development.

In summary, our study unravels a striking example that RNA-binding proteins represent important functional regulators of liver carcinogenesis by defining IGF2BP1 as an important player in human HCC with a broad impact on proliferation, survival, and tumor growth. Hence, inhibition of IGF2BP1 expression or blocking the interaction with its target RNAs may represent a promising and innovative approach for clinical intervention.

Acknowledgment: The authors thank Dr. Georg Stoecklin for helpful discussions and Dr. Vladimir Spiegelman for IGF2BP1 promoter luciferase constructs. We thank Karin Rebholz for excellent technical assistance with immunohistochemical stainings and Dr. Bernhard Hiebl for mouse injections. This project was supported by the tissue bank of the National Center for Tumor Diseases (NCT) of Heidelberg.

Author Contributions: T.G. and M.H. conceived the study, designed and executed the *in vitro* experiments, analyzed and interpreted the data, and wrote the article. N.P. designed, executed, and analyzed *in vivo* xenograft experiments and helped write the article. N.B., E.F., H.U., A.H., M.G., N.H., B.M. performed experiments and analyzed data. B.S. and R.G. performed microarray expression analysis. T.L., K.B., and P.S. provided tissue samples and helped with data interpretation. S.H. and S.D. contributed to the design of experimentation and data interpretation and wrote the article.

References

1. Breuhahn K, Gores G, Schirmacher P. Strategies for hepatocellular carcinoma therapy and diagnostics: lessons learned from high throughput and profiling approaches. Hepatology 2011;53:2112-2121.
2. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365: 1118-1127.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
4. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 2002;31:339-346.
5. Shiraha H, Yamamoto K, Namba M. Human hepatocyte carcinogenesis (review). Int J Oncol 2013;42:1133-1138.
6. Breuhahn K, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene 2006;25: 3787-3800.
7. Muller-McNicoll M, Neugebauer KM. How cells get the message: dynamic assembly and function of mRNA-protein complexes. Nat Rev Genet 2013;14:275-287.
8. Mignone F, Gissi C, Liuni S, Pesole G. Untranslated regions of mRNAs. Genome Biol 2002;3:REVIEWS0004.
9. Bell JL, Wachter K, Muhleck B, Pazaitis N, Kohn M, Lederer M, et al. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? Cell Mol Life Sci 2013; 70:2657-2675.
10. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 1999;19:1262-1270.
11. Dai N, Christiansen J, Nielsen FC, Avruch J. mTOR complex 2 phosphorylates IMP1 cotranslationally to promote IGF2 production and the proliferation of mouse embryonic fibroblasts. Genes Dev 2013;27:301-312.
12. Farina KL, Huttelmaier S, Musunuru K, Darnell R, Singer RH. Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. J Cell Biol 2003;160:77-87.
13. Hüttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, Meng X, et al. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 2005;438:512-515.
14. Bernstein PL, Herrick DJ, Prokipcak RD, Ross J. Control of c-myc mRNA half-life in vitro by a protein capable of binding to a coding region stability determinant. Genes Dev 1992;6:642-654.
15. Ioannidis P, Mahaira LG, Perez SA, Gritzapis AD, Sotiropoulou PA, Kavalakis GJ, et al. CRD-BP/IMP1 expression characterizes cord blood CD34+ stem cells and affects c-myc and IGF-II expression in MCF-7 cancer cells. J Biol Chem 2005;280:20086-20093.
16. Noubissi FK, Elcheva I, Bhatia N, Shakoori A, Ougolkov A, Liu J, et al. CRD-BP mediates stabilization of betaTrCP1 and c-myc mRNA in response to beta-catenin signalling. Nature 2006;441:898-901.
17. Sparanese D, Lee CH. CRD-BP shields c-myc and MDR-1 RNA from endonucleolytic attack by a mammalian endoribonuclease. Nucleic Acids Res 2007;35:1209-1221.
18. Weidensdorfer D, Stohr N, Baude A, Lederer M, Kohn M, Schierhorn A, et al. Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. RNA 2009;15:104-115.
19. Hämmerle M, Gutschner T, Uckelmann H, Ozgur S, Fiskin E, Gross M, et al. Post-transcriptional destabilization of the liver-specific long non-coding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). Hepatology 2013;58:1703-1712.
20. Kessler SM, Pokorny J, Zimmer V, Laggai S, Lammert F, Bohle RM, et al. IGF2 mRNA binding protein p62/IMP2-2 in hepatocellular carcinoma: antiapoptotic action is independent of IGF2/PI3K signaling. Am J Physiol Gastrointest Liver Physiol 2013;304:G328-336.
21. Gutschner T, Baas M, Diederichs S. Noncoding RNA gene silencing through genomic integration of RNA destabilizing elements using zinc finger nucleases. Genome Res 2011;21:1944-1954.
22. Eissmann M, Gutschner T, Hammerle M, Gunther S, Caudron-Herger M, Gross M, et al. Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. RNA Biol 2012;9: 1076-1087.
23. Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 2013;73:1180-1189.

24. Neumann O, Kesselmeier M, Geffers R, Pellegrino R, Radlwimmer B, Hoffmann K, et al. Methylome analysis and integrative profiling of human HCCs identify novel protumorigenic factors. HEPATOLOGY 2012;56:1817-1827.
25. Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, et al. Gene expression patterns in human liver cancers. Mol Biol Cell 2002;13: 1929-1939.
26. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603-607.
27. Calvisi DF, Thorgeirsson SS. Molecular mechanisms of hepatocarcinogenesis in transgenic mouse models of liver cancer. Toxicol Pathol 2005;33:181-184.
28. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008;8:976-990.
29. Ebinuma H, Saito H, Kosuga M, Wakabayashi K, Saito Y, Takagi T, et al. Reduction of c-myc expression by an antisense approach under Cre/loxP switching induces apoptosis in human liver cancer cells. J Cell Physiol 2001;188:56-66.
30. Simile MM, De Miglio MR, Muroni MR, Frau M, Asara G, Serra S, et al. Down-regulation of c-myc and Cyclin D1 genes by antisense oligodeoxy nucleotides inhibits the expression of E2F1 and in vitro growth of HepG2 and Morris 5123 liver cancer cells. Carcinogenesis 2004;25:333-341.
31. Kobel M, Weidensdorfer D, Reinke C, Lederer M, Schmitt WD, Zeng K, et al. Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. Oncogene 2007;26:7584-7589.
32. Noubissi FK, Nikiforov MA, Colburn N, Spiegelman VS. Transcriptional Regulation of CRD-BP by c-myc: Implications for c-myc Functions. Genes Cancer 2010;1:1074-1082.
33. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44-57.
34. Hafner M, Landthaler M, Burger L, Khorshid M, Hausser J, Berninger P, et al. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 2010;141:129-141.
35. Guzman G, Alagiozian-Angelova V, Layden-Almer JE, Layden TJ, Testa G, Benedetti E, et al. p53, Ki-67, and serum alpha feto-protein as predictors of hepatocellular carcinoma recurrence in liver transplant patients. Mod Pathol 2005;18:1498-1503.
36. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694-698.
37. Nakagawa H, Shibata T. Comprehensive genome sequencing of the liver cancer genome. Cancer Lett 2013;340:234-240.
38. Tao Y, Ruan J, Yeh SH, Lu X, Wang Y, Zhai W, et al. Rapid growth of a hepatocellular carcinoma and the driving mutations revealed by cell-population genetic analysis of whole-genome data. Proc Natl Acad Sci U S A 2011;108:12042-12047.
39. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, Ishikawa S, et al. High-resolution characterization of a hepatocellular carcinoma genome. Nat Genet 2011;43:464-469.
40. Roessler S, Long EL, Budhu A, Chen Y, Zhao X, Ji J, et al. Integrative genomic identification of genes on 8p associated with hepatocellular carcinoma progression and patient survival. Gastroenterology 2012; 142:957-966 e912.
41. Xue W, Kitzing T, Roessler S, Zuber J, Krasnitz A, Schultz N, et al. A cluster of cooperating tumor-suppressor gene candidates in chromosomal deletions. Proc Natl Acad Sci U S A 2012;109:8212-8217.
42. Szabo G, Bala S. MicroRNAs in liver disease. Nat Rev Gastroenterol Hepatol 2013;10:542-552.
43. Vazquez-Chantada M, Fernandez-Ramos D, Embade N, Martinez-Lopez N, Varela-Rey M, Woodhoo A, et al. HuR/methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis. Gastroenterology 2010;138:1943-1953.
44. Jones K, Timchenko L, Timchenko NA. The role of CUGBP1 in age-dependent changes of liver functions. Ageing Res Rev 2012;11:442-449.
45. Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, et al. Lin28 promotes transformation and is associated with advanced human malignancies. Nat Genet 2009;41:843-848.
46. Doyle GA, Bourdeau-Heller JM, Coulthard S, Meisner LF, Ross J. Amplification in human breast cancer of a gene encoding a c-myc mRNA-binding protein. Cancer Res 2000;60:2756-2759.
47. Christiansen J, Kolte AM, Hansen T, Nielsen FC. IGF2 mRNA-binding protein 2: biological function and putative role in type 2 diabetes. J Mol Endocrinol 2009;43:187-195.
48. Elcheva I, Tarapore RS, Bhatia N, Spiegelman VS. Overexpression of mRNA-binding protein CRD-BP in malignant melanomas. Oncogene 2008;27:5069-5074.
49. Gu L, Shigemasa K, Ohama K. Increased expression of IGF II mRNA-binding protein 1 mRNA is associated with an advanced clinical stage and poor prognosis in patients with ovarian cancer. Int J Oncol 2004; 24:671-678.
50. Kato T, Hayama S, Yamabuki T, Ishikawa N, Miyamoto M, Ito T, et al. Increased expression of insulin-like growth factor-II messenger RNA-binding protein 1 is associated with tumor progression in patients with lung cancer. Clin Cancer Res 2007;13:434-442.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Manuskript 3

Zirkel A, Lederer M, Stöhr N, Pazaitis N, Hüttelmaier S.

IGF2BP1 promotes mesenchymal cell properties and migration of tumor-derived cells by enhancing the expression of LEF1 and SNAI2 (SLUG).

Nucleic Acids Research
Juli 2013, 41:6618-36.

6618–6636 *Nucleic Acids Research, 2013, Vol. 41, No. 13* *Published online 15 May 2013*
doi:10.1093/nar/gkt410

IGF2BP1 promotes mesenchymal cell properties and migration of tumor-derived cells by enhancing the expression of LEF1 and SNAI2 (SLUG)

Anne Zirkel, Marcell Lederer, Nadine Stöhr, Nikolaos Pazaitis and Stefan Hüttelmaier*

Institute of Molecular Medicine, Department of Molecular Cell Biology, Martin-Luther-University, Heinrich-Damerow-Str.1, 06120 Halle, Germany

Received September 12, 2012; Revised and Accepted April 23, 2013

ABSTRACT

The oncofetal IGF2 mRNA-binding protein 1 (IGF2BP1) controls the migration and invasiveness of primary as well as tumor-derived cells *in vitro*. Whether the protein also modulates epithelial-mesenchymal-transition (EMT), a hallmark of tumor progression involved in tumor cell dissemination, remained elusive. In this study, we reveal that IGF2BP1 enhances mesenchymal-like cell properties in tumor-derived cells by promoting the expression of the transcriptional regulators LEF1 and SLUG (SNAI2). IGF2BP1 associates with LEF1 transcripts and prevents their degradation in a 3′-UTR-dependent manner resulting in an upregulation of LEF1 expression. LEF1 promotes transcription of the mesenchymal marker fibronectin by associating with the fibronectin 1 promoter. Moreover, LEF1 enforces the synthesis of the 'EMT-driving' transcriptional regulator SNAI2. Accordingly, IGF2BP1 knockdown causes MET-like (mesenchymal-epithelial-transition) morphological changes, enhances the formation of cell–cell contacts and reduces cell migration in various mesenchymal-like tumor-derived cells. However, in epithelial-like tumor-derived cells characterized by a lack or low abundance of IGF2BP1, the protein fails to induce EMT. These findings identify IGF2BP1 as a pro-mesenchymal post-transcriptional determinant, which sustains the synthesis of 'EMT-driving' transcriptional regulators, mesenchymal markers and enhances tumor cell motility. This supports previous reports, suggesting a role of IGF2BP1 in tumor cell dissemination.

INTRODUCTION

Epithelial-mesenchymal-transition (EMT) is essential during embryogenesis and is considered a hallmark in the progression of carcinomas [reviewed in (1,2)]. In cancer, the term EMT describes a complex network of molecular and cellular trans-differentiation phenomena by which epithelial-like tumor cells acquire mesenchymal-like properties leading to reduced inter-cellular adhesion, increased migratory capacity and elevated invasive potential. Accumulating evidence indicates that the post-transcriptional control of gene expression facilitated by microRNAs essentially modulates EMT and its reversal, mesenchymal-epithelial-transition (MET). One of the most studied post-transcriptional mechanisms promoting EMT is facilitated via the miR-200 family. This antagonizes TGF-β (TGFB)-induced EMT by interfering with the expression of ZEBs, two key transcriptional repressors of E-cadherin (CDH1) [reviewed in (3)]. Another double-negative feedback loop modulating cell plasticity essentially relies on the miR-34 family, which links p53 signaling and negative regulation of Snail (SNAI1) expression, another 'EMT-driving' transcriptional regulator (4,5). Surprisingly, little is known about the role of RNA-binding proteins (RBPs) in modulating EMT in cancer-derived cells, although at least two RBPs were proposed as essential modulators of malignant EMT/MET. The splicing regulator Sam68 was shown to control EMT through alternative splicing-activated nonsense-mediated mRNA decay of the proto-oncogene SF2/ASF (6). More recently, it was demonstrated that La enhances the IRES-mediated translation of the extracellular matrix protein laminin B1 during malignant EMT (7).

IGF2 mRNA-binding proteins (IGF2BPs) comprise a group of three proteins, two of which, IGF2BP1 and IGF2BP3, were proposed to serve essential functions during embryogenesis and in cancer [reviewed in (8–10)]. In contrast to IGF2BP2, which appears to be the main or even exclusive IGF2BP member expressed in non-neoplastic adult tissue, IGF2BP1 and IGF2BP3 were found to be severely upregulated in various cancers [reviewed in (8,11)]. However, in view of the multitude of descriptive studies indicating elevated expression of IGF2BP1/3 to

*To whom correspondence should be addressed. Tel: +49 345 5522860; Fax: +49 345 5522894; Email: Stefan.huettelmaier@medizin.uni-halle.de

correlate with tumor aggressiveness, their role in cancer cells remains poorly understood. Studies in tumor-derived as well as primary cells suggest two main functions of IGF2BPs, growth control and the regulation of cell migration. Evidence for a role in regulating cell growth and proliferation was provided by findings indicating that all IGF2BPs promote or interfere with IGF2 protein synthesis. IGF2BP1 was suggested to inhibit IGF2 mRNA translation by associating with the highly structured leader3 5′-UTR of the transcript, one of four alternative 5′-UTRs reported (12,13). In contrast, IGF2BP2 and IGF2BP3 were shown to enhance translation of the IGF2 mRNA, presumably involving the phosphorylation of IGF2BP2 by mTORC1 (14,15). Another target transcript via which IGF2BP1 was proposed to modulate cell proliferation is the oncogenic transcriptional regulator c-Myc (MYC). On associating with a rare-codon comprising coding region determinant (CRD), IGF2BP1 was shown to prevent cleavage of the MYC mRNA by endonucleases when ribosomes are slowed down on entering the rare-codon region of the CRD (16). In agreement, we observed that IGF2BP1 knockdown results in a significantly reduced half-life of the MYC mRNA in most tumor-derived cells and accordingly is associated with severely decreased cell proliferation (17,18). Finally, it was proposed that IGF2BP1 modulates β-catenin (CTNNB1) signaling (19,20). On the one hand, it was shown that IGF2BP1 transcription is enhanced in a CTNNB1/TCF-dependent manner but then negatively feeds back on CTNNB1-dependent signaling by enhancing the expression of beta-TrCP1, which among others facilitates CTNNB1 protein degradation (21). On the contrary, IGF2BP1 was demonstrated to enhance CTNNB1 expression by preventing degradation of the CTNNB1 mRNA (20). The interplay of IGF2BP1 and CTNNB1-dependent signaling was moreover suggested to negatively regulate the migration of breast cancer-derived cells *in vitro* (22). In contrast, various observations indicate that IGF2BP1 and its ortholog Vg1RBP/Vera promote the migration of primary as well as tumor-derived cells [reviewed in (23)]. In *Xenopus*, Vg1RBP/Vera was shown to enhance the migration of neural crest cells during development (24). In agreement, we reported that IGF2BP1 promotes the directed migration of tumor cells derived from osteosarcoma, ovarian carcinoma as well as glioblastoma (25). This was also demonstrated in colorectal as well as mammary carcinoma-derived cells, in which IGF2BPs enhance the formation of lamellipodia and promote directed migration, respectively (26,27). Finally, IGF2BP1 and IGF2BP3 were suggested to enhance the invasive potential of cervical carcinoma-derived HeLa cells by interfering with the degradation of the CD44 mRNA (28). This results in elevated CD44 expression and enforced formation of invadopodia *in vitro*. One common theme in the IGF2BP-facilitated regulation of cell migration, adhesion and potentially invasion is the regulation of actin dynamics [reviewed in (23)]. IGF2BP1, also termed zipcode-binding protein (ZBP) in chicken, facilitates the localization of β-actin (ACTB) encoding transcripts to the leading edge of primary fibroblasts as well as the growth cones of developing neurons (29–31). This enforcement of spatially restricted ACTB mRNA levels was proposed to provide a pool of transcripts for the rapid activation of ACTB protein synthesis and thus enhance directed cell protrusion in response to external guidance cues [reviewed in (32)]. In agreement, IGF2BP1 was observed to promote actin-driven neurite protrusion by controlling translation of the ACTB mRNA in a spatiotemporal and Src-kinase controlled manner (33). Moreover, IGF2BP1 and its ortholog Vg1RBP/Vera were revealed to modulate growth cone guidance during neuronal development (34,35). Like in primary neurons, IGF2BP1 also serves essential roles in regulating actin dynamics in tumor-derived cells. In recent studies, we proposed that the protein modulates the cellular G-/F-actin equilibrium by controlling ACTB protein synthesis and HSP27 (HSPB1)-dependent sequestering of monomeric actin (25). The latter is facilitated by IGF2BP1-directed inhibition of MAPK4 mRNA translation, which limits the activation of MK5-directed phosphorylation of HSPB1 and thereby reduces sequestering of monomeric actin by this small heat shock protein [reviewed in (23)]. Despite these various studies indicating a regulatory role of IGF2BPs, in particular IGF2BP1, in directing the migration and invasive potential of tumor-derived cells *in vitro*, it remains elusive whether IGF2BPs also regulate tumor cell dissemination *in vivo*. One key aspect that remains to be addressed in this respect is whether IGF2BPs serve a role in modulating mesenchymal versus epithelial properties of cancer-derived cells. This has been barely investigated, although one recent study suggests that IGF2BP1 promotes the formation of cell–cell contacts by enhancing the spatially restricted expression of CDH1 in proximity to cell–cell contacts (36).

MATERIALS AND METHODS

Plasmids

Full-length LEF1 (NM_016269) as well as alternative 3′-UTRs of LEF1 isoforms (A: NM_016269; NM_001130713; NM_001166119; B: NM_001130714) were generated by RT-PCR from HEK293 cells. The LEF1 coding sequence was inserted via BamHI/ EcoRI in pcDNA3.1zeo-Flag, pLVX-puro and pLVX-puro GFP plasmids, respectively. The LEF1 3′UTRs were inserted via EcoRI/ XhoI into pcDNA3.1neo-LUC (LUC: Firefly luciferase), as recently described (25). The SNAI2 3′UTR was amplified by RT-PCR from HT-144 cells and inserted into the pmirGLO (Promega) vector via BamHI/ XhoI. The fibronectin (FN1) minimal promoter (−839 to +1), the 5′UTR (+1 to +266_ATG) and the starting ATG of human FN1 (Chr.2q34) were identified by *in silico* prediction using Proscan (http://www-bimas.cit.nih.gov/molbio/proscan/). The longest FN1-promoter fragment (1105 nt; −839 to starting ATG) as well as SNAI2 promoter (37) was PCR-amplified from HEK293 genomic DNA and transferred into pGL4.21 (Promega) via XhoI/ BglII sites. ShRNA-encoding lentiviral vectors were generated by inserting annealed oligonucleotides via BamHI/EcoRI in the modified pLVX-shRNA2 or

modified pLVX-shRNA2-Crimson-puro. In the latter, the ZsGreen cassette was replaced by a PGK-promoter driven cassette encoding for E2-Crimson fused to the puromycin resistance via an EMCV IRES to allow for tracing and selection of transduced cells. All PCR-amplified products and modified vectors were validated by sequencing. The following plasmids were obtained from Addgene: SNAI2-directed shRNA lentiviral vector (ID: 10905); SNAI1 cDNA (ID: 36976), subcloned in pLVX-puro GFP; SNAI2 cDNA (ID: 36986), subcloned in pLVX-puro GFP; SNAI1 promoter (ID: 31694). For PCR primers used for cloning and plasmids, refer to Supplementary Table ST1.

Cell culture, transfection and lentiviruses

All cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. To reduce bias by cell density-dependent regulation of epithelial or mesenchymal marker expression, cells were harvested or analyzed at ~80% confluence. Cells were transfected with siRNAs by RNAiMax (72 h) or plasmids by Lipofectamine 2000 (48 h), as previously described (25). SiRNA and shRNA sequences are listed in Supplementary Tables ST1 and ST2. For knockdown-recovery studies, cells were co-transfected with indicated shRNA encoding and Flag-tagged protein-encoding plasmids for 72 h. Where indicated, cells were treated with actinomycin D (ActD; 5 μM) to block mRNA synthesis and monitor mRNA decay, as recently described (25). Lentiviruses were produced essentially as recently described (25). Transduced cell populations were subsequently cultured in the presence of puromycin (1 μg/ml). All lentiviral transfer vectors are indicated in Supplementary Table ST1.

Immunofluorescence and microscopy

Cells were grown on coverslips (48 h) and processed for immunostaining with indicated antibodies on fixation by formaldehyde, as previously described (38). Nuclei were stained by DAPI, and F-actin was labeled by phalloidin-TRITC. Representative images are shown. Images were acquired using a Leica LSM-SP5× microscope, as recently described (25). Antibodies used for immunostaining are indicated in Supplementary Table ST3. Bright field images of living cells were acquired using a Nikon TE-100 inverse microscope equipped with a Nikon CoolPix990 camera and a 40× Plan Apo objective. For wound closure analyses, cells (1×10^5/well) were cultured for 24 h in a 24-well plate and scratched before time lapse microscopy using a Leica LSM-SP5× microscope equipped with a Ludin Cube live cell chamber and a 20× Plan Fluor objective. Images were acquired every 15 min. Movies of all cell populations were analyzed simultaneously using automated cell segmentation and wound closure algorithms recently described (39).

RT-PCR and qRT-PCR

RNA isolation and reverse transcription were carried out as previously described (25). Briefly, total RNA was isolated by Trizol reagent followed by Chloroform extraction. Reverse transcription was performed using M-MLV-RT (Promega) and oligo-dT priming at 42°C for 2 h. The cDNA samples were then analyzed using SYBR® Select Master Mix (Life Technologies) and the 7900HT Fast Real-Time PCR System (Applied Biosystems) in triplicates. RNA abundance was determined using the ΔC_t or $\Delta\Delta C_t$ method, respectively. Primers used for quantitative PCR analyses are listed in Supplementary Table ST4.

Luciferase reporter analysis

Luciferase activities were determined using DualGlo reagent (Promega), as previously reported (18,25). For promoter analyses, HEK293 cells were co-transfected with indicated luciferase reporters and protein encoding plasmids for 30 h or shRNA encoding vectors for 48 h. For analyses of 3′UTR-containing reporters, cells were transfected with siRNAs 48 h before the transfection of luciferase reporters for an additional 24 h. Renilla luciferase served as an internal normalization control in all analyses.

Western blotting

For western blotting, cells were harvested by a rubber policeman to minimize degradation of trans-membrane proteins like CDH1. Total protein was extracted in RIPA-buffer [20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate , 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4] supplemented with protease inhibitor cocktail (Sigma Aldrich). Protein abundance was analyzed by western blotting with indicated antibodies using the Odyssey infrared scanner (LICOR), as previously described (18,25). Antibodies used for western blotting are indicated in Supplementary Table ST3.

Flow cytometry

The volume and number of detached cells was determined by flow cytometry measurements using a MACSQuant (Miltenyi Biotec). The relative cell size was determined by forward scattering.

Enzyme-linked immunosorbent assay

Soluble FN1 protein levels secreted by HEK293 cells were determined using a human FN1 enzyme-linked immunosorbent assay (ELISA) (Boster Biological Technology). The assay was performed according to the manufacturer's instructions. HEK293 cells were transfected with the indicated siRNAs for 72 h and starved with fetal bovine serum free Dulbecco's modified Eagle's medium for 16 h before the collection of the cell culture supernatant. Fibronectin protein amounts were normalized to cell numbers determined by flow cytometry.

RNA-immunoprecipitation

HEK293 cells were harvested and cross-linked with 0.1% formaldehyde in PBS (10^7 cells in 1 mL) for 10 min before quenching by 0.1 M Tris–HCl for 5 min. Cells were

extracted in RNA-immunoprecipitation (RIP)-buffer [10 mM HEPES (pH 7.2), 150 mM KCl, 5 mM $MgCl_2$, 0.5% NP40] supplemented with protease inhibitor cocktail (Sigma Aldrich) and RNasin (Promega). Antibodies for control-IP [immunoglobulin G (IgG) mouse] or the IGF2BP1-IP were coupled to proteinG Dynabeads (Life Technologies) in wash buffer [WB: 50 mM Tris–HCl (pH 7.4), 300 mM NaCl, 0.01% NP40, 5 mM $MgCl_2$] supplemented with yeast tRNA (20 μg/ml). After antibody coupling to beads, cell lysates were added in a 1:1 (v/v) ratio and incubated at 4°C overnight with constant agitation. The beads were washed once with WB and three times with WB containing 0.5 M urea. Protein–RNA complexes were eluted in WB supplemented with 1% SDS at 65°C for 10 min. Reversal of the cross-link was achieved by adding proteinase K (Roche) for 1 h at 65°C. RNA was purified by phenol-chloroform extraction. RNA samples were treated with RQ1-DNase before reverse transcription with M-MLV reverse transcriptase and random hexamer primers. RNA abundance was assessed by semi-quantitative and quantitative PCR using primers listed in Supplementary Table ST4.

Chromatin immunoprecipitation

The chromatin immunoprecipitation was performed using the SimpleChIP™ Enzymatic Chromatin IP Kit (Cell Signaling) essentially according to the manufacturer's instructions. For each ChIP experiment, 4×10^7 HEK293 cells were used. Co-purification of indicated genomic DNA fragments was analyzed by semi-quantitative as well as quantitative PCR using primers listed in Supplementary Table ST4.

RESULTS

IGF2BP1 promotes mesenchymal cell properties

Aiming to reveal whether IGF2BP1 modulates mesenchymal versus epithelial cell properties, we analyzed its role in transformed embryonic kidney-derived 293 A (HEK293) cells. HEK293 cells express all IGF2BPs, in particular substantial amounts of IGF2BP1, and show epithelial-like as well as mesenchymal-like cell characteristics with few CTNNB1/CDH1-positive cell–cell contacts and expression of mesenchymal markers like FN1. The transient knockdown of IGF2BP1 induced an increased size and apparent flattening of adherent HEK293 cells (Figure 1A). The observed enlargement of adhesive cells was confirmed by the quantitative assessment of cell size using LSM-microscopy and two distinct IGF2BP1-directed siRNAs (Figure 1B and Supplementary Figure S1A). Flow cytometry revealed that the overall size of detached cells, as determined by forward scattering, remained largely unaffected by IGF2BP1 knockdown (Supplementary Figure S1B). This suggested that the shift in cell size was due to altered cytoskeletal organization rather than an overall increase in cell mass. Notably, the used siRNAs were highly IGF2BP paralogue selective supporting an IGF2BP1-dependent role in controlling cell morphology (Figure 1C and Supplementary Figure S1C). Consistent with the inhibitory role of IGF2BP1 in ACTB mRNA translation (25,33), depletion of the protein resulted in an increase of ACTB protein levels in HEK293 cells (Supplementary Figure S1D). To evaluate whether IGF2BP1 depletion also enhanced epithelial-like cell characteristics, the formation of cell–cell contacts was analyzed by immunostaining for CTNNB1 and CDH1 as well as monitoring F-actin organization by phalloidin (Figure 1D, E and Supplementary Figure S1F). In contrast to the previously observed disturbance of stress-fibers in U2OS cells (25), IGF2BP1 knockdown induced an enrichment of cortical actin in HEK293 cells. Concomitantly, the recruitment of CTNNB1 as well as CDH1 to cell–cell contacts sites was markedly pronounced by IGF2BP1 knockdown using two distinct siRNAs. This morphological re-organization was associated with a modest increase in CDH1 protein and mRNA levels (Figure 1F and Supplementary Figure S1E). CTNNB1 protein amounts remained largely unaffected, although CTNNB1 mRNA abundance was decreased by IGF2BP1 knockdown, as previously described (20). On the contrary, the abundance of secreted FN1 protein as well as FN1 mRNA was significantly decreased by IGF2BP1 depletion (Figure 1G and Supplementary Figure S1E). Taken together, these observations suggested that IGF2BP1 depletion promotes epithelial-like and interferes with mesenchymal-like cell properties.

IGF2BP1 promotes the expression of LEF1

In recent studies, we identified various novel candidate target transcripts of IGF2BP1 using the selective stabilization of mRNAs by the protein during cellular stress as a screening criterion in osteosarcoma-derived U2OS cells (25). These analyses suggested LEF1 paralogue encoding mRNAs as putative target transcripts of IGF2BP1. Notably, LEF1 was identified as a regulator of FN1 and CDH1 expression in tumor-derived cells and was proposed to act in both, a CTNNB1-dependent as well as -independent manner (40,41). Hence, we hypothesized that IGF2BP1-directed regulation of pro-mesenchymal cell properties could be facilitated at least in part via LEF1.

The knockdown of IGF2BP1 with two distinct siRNAs induced a significant decrease in the levels of all three major LEF1 protein isoforms observed in HEK293 cells (Figure 2A). This was well correlated with a modest, but significant, decrease in LEF1 mRNA levels at steady state, whereas ACTB transcript abundance remained largely unaffected, as previously demonstrated (Figure 2B). Notably, the steady state levels of LEF1 mRNAs were not affected by the knockdown of IGF2BP2 or IGF2BP3 (Supplementary Figure S2A and B). This suggested that IGF2BP1 promoted LEF1 expression by stabilizing the LEF1 mRNA, as previously shown for CD44, MYC or PTEN (18,25,28). To evaluate a role of IGF2BP1 in preventing LEF1 mRNA degradation, the turnover of LEF1 transcripts was monitored on IGF2BP1 knockdown by using ActD to block mRNA synthesis. These analyses revealed a significant destabilization of LEF1 mRNAs in response to IGF2BP1 depletion, whereas RPLP0 as well as FN1 mRNA turnover remained

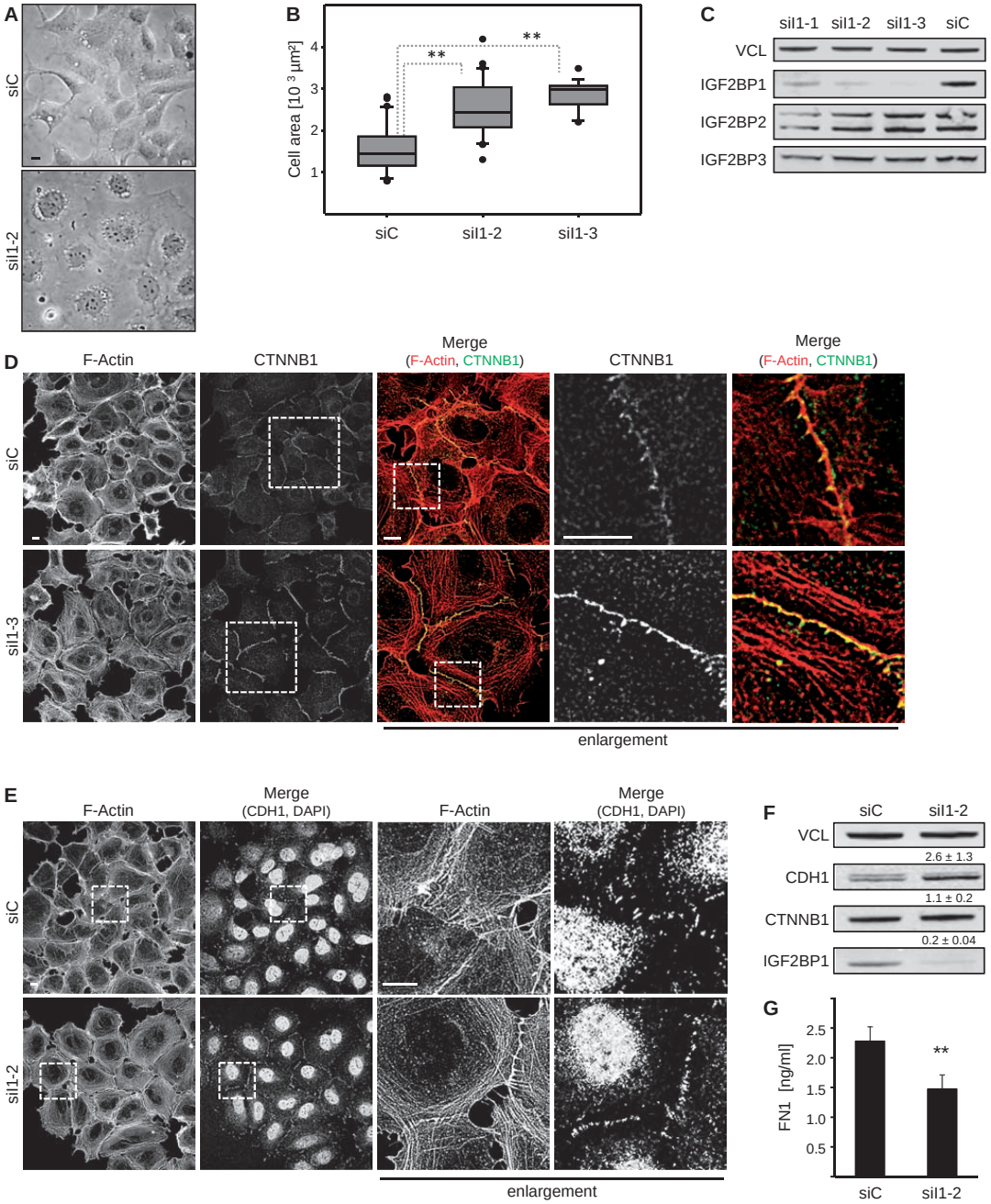


Figure 1. IGF2BP1 knockdown promotes epithelial-like cell properties in HEK293 cells. (**A** and **B**) HEK293 cells were transfected with control (siC) or IGF2BP1-directed (siI1-2 or siI1-3) siRNAs for 72 h. Cell morphology was monitored by light microscopy (A). The size of adherent cells was analyzed on immunostaining for CTNNB1 as well as F-actin labeling by phalloidin and is depicted as box plots (B). Images were acquired by LSM microscopy. Adherent cells were traced by manual labeling using CTNNB1-defined cell borders to determine the cell area (µm^2) using the Leica-SP5× software (also see Supplementary Figure S1A). (**C**) HEK293 cells were transfected with control (siC) or three distinct IGF2BP1-directed siRNAs (siI1-1, siI1-2 or siI1-3) for 72 h. IGF2BP1 paralogue-specific knockdown was analyzed by western blotting using IGF2BP1-, IGF2BP2- or IGF2BP3-directed monoclonal antibodies. VCL served as a loading control. (**D** and **E**) HEK293 cells were transfected with indicated siRNAs as in (A).

(continued)

largely unaffected (Figure 2C). This suggested that the IGF2BP1 knockdown induced decrease in FN1 resulted from an indirect impairment of FN1 transcription. In contrast, IGF2BP1 interfered with LEF1 mRNA turnover, presumably by associating with LEF1 transcripts. The latter was tested by RIP using formaldehyde-facilitated cross-linking to stabilize cytoplasmic mRNPs followed by IGF2BP1 immunopurification (Figure 2D and E). Semi- as well as quantitative RT-PCR confirmed MYC and ACTB mRNAs as direct targets of IGF2BP1, as previously demonstrated in U2OS cells (25). Selective association was also observed for LEF1 transcripts, whereas no association was determined for PPIA, vinculin (VCL) and FN1-encoding mRNAs, indicating LEF1, but not N1, as a direct target transcript of IGF2BP1. Surprisingly, we could not confirm association of IGF2BP1 with the CTNNB1-encoding mRNA providing further evidence that the protein does not regulate CTNNB1 expression in HEK293 cells (Figure 2E).

The mRNA decay as well as RIP studies indicated that IGF2BP1 interfered with LEF1 mRNA degradation by associating with LEF1-encoding transcripts. Although IGF2BP1 was suggested to prevent MYC or PTEN mRNA degradation by associating with the respective coding regions of these transcripts, the protein was proposed to prevent decay of the CD44 mRNA via the 3′-UTR. In contrast to the MYC or PTEN mRNAs, no significant enrichment of rare codons was observed in the coding region of LEF1 mRNAs (data not shown). However, recent PAR-CLIP studies identified various putative association sites for IGF2BPs in the 3′-UTR of LEF1 encoding mRNAs, which with the exception of a small 5′-region is shared by all reported LEF1 transcripts (42). This suggested that IGF2BP1 controlled the fate of LEF1 mRNAs essentially via the 3′-UTR. To test this in further detail, the activity of luciferase reporters harboring either of the two so far reported LEF1-3′UTRs was analyzed on IGF2BP1 knockdown. The activity of the reporter comprising the BGH-derived 3′-UTR was only modestly decreased by IGF2BP1 depletion. On the contrary, the activity of the two analyzed LEF1 reporters was significantly reduced by IGF2BP1 depletion (Figure 2F). Reporter activity remained largely unaffected by the knockdown of IGF2BP2 or IGF2BP3, as observed for steady levels of LEF1 mRNAs (Supplementary Figure S2C).

To exclude that IGF2BP1-facilitated regulation of LEF1 expression was exclusively observed in HEK293 cells, we analyzed how IGF2BP1 modulates mesenchymal properties and LEF1 synthesis in U2OS cells. Notably, we recently demonstrated that IGF2BP1 promotes U2OS migration and cell-matrix contact formation, two *bona fide* mesenchymal cell properties (23,25). In contrast to HEK293 cells, the depletion of IGF2BP1 had an only marginal effect on the morphology of U2OS cells, although actin fiber integrity was severely compromised as previously reported (Figure 3A). Despite only modest morphological alterations, IGF2BP1 depletion caused a decrease in LEF1 as well as FN1 mRNA and protein levels, whereas CTNNB1 protein abundance remained largely unaffected in U2OS cells (Figure 3B–E). Owing to its low abundance, altered expression of CDH1 could not be evaluated in U2OS cells (data not shown). To validate that IGF2BP1 promotes the expression of LEF1 and FN1, the chicken ortholog of human IGF2BP1, termed ZBP1, was stably expressed in U2OS cells, which compared with HEK293 cells express significantly lower levels of IGF2BP1 (8). In comparison with U2OS cells stably expressing GFP, GFP-tagged ZBP1 enhanced the expression of both, LEF1 and FN1 (Figure 3F and G). The expression of RPLP0, VCL as well as CTNNB1 remained essentially unaffected on the protein as well as mRNA level. In summary, these studies revealed that IGF2BP1 enhances the expression of LEF1 and FN1 in HEK293 and U2OS cells, suggesting largely cell context independent regulatory mechanisms. IGF2BP1 interfered with LEF1 mRNA degradation in a 3′-UTR-dependent manner. In contrast, the protein indirectly enhanced FN1 expression, potentially by stimulating LEF1-dependent FN1 transcription.

LEF1 promotes mesenchymal-like cell properties

Although IGF2BP1 could promote mesenchymal-like cell properties by enhancing the expression of LEF1 was investigated by analyzing the role of the transcriptional regulator LEF1 in HEK293 as well as U2OS cells. Similar to IGF2BP1 knockdown, the depletion of LEF1 resulted in significant morphological changes in HEK293 cells (Figure 4A). Cells appeared flattened, and the overall area covered by adherent cells was significantly increased, whereas the cell volume remained largely unaffected (Figure 4B and Supplementary Figure S2D and E). This suggested that the knockdown of LEF1 paralogues enhanced epithelial-like cell properties in HEK293 cells. In support of this, the localization of CTNNB1 and CDH1 to cell–cell contacts was obviously increased by LEF1 depletion (Figure 4C and Supplementary Figure S2G). Consistent with the assumption that LEF1 is not a potent repressor of CDH1 expression, CDH1 mRNA

Figure 1. Continued
The F-actin cytoskeleton and cell–cell contact formation was analyzed by phalloidin labeling and immunostaining for CTNNB1 (D) or CDH1 (E). Where indicated nuclei were stained by DAPI. Enlargements of boxed regions (left panels) are shown in the right panels (enlargement). Note the enrichment of CTNNB1 and CDH1 at adherens junctions and a knockdown-induced enhancement of cortical F-Actin (also see Supplementary Figure S1F). Representative images were acquired by LSM microscopy; bars, 10 µm. (**F**) HEK293 cells were transfected with indicated siRNAs as in (A). CDH1, CTNNB1 and IGF2BP1 protein abundance was analyzed by western blotting with indicated antibodies. Protein levels on IGF2BP1 knockdown were determined relative to controls (siC) by normalization to VCL, as indicated above panels. Representative western blots of three independent analyses are shown. (**G**) Soluble FN1 levels were analyzed by ELISA in HEK293 cells transfected with indicated siRNAs for 72 h. Statistical significance was validated by Student's *t*-test: $^{**}P < 0.005$. Error bars indicate standard deviation (SD) of at least three independent analyses.

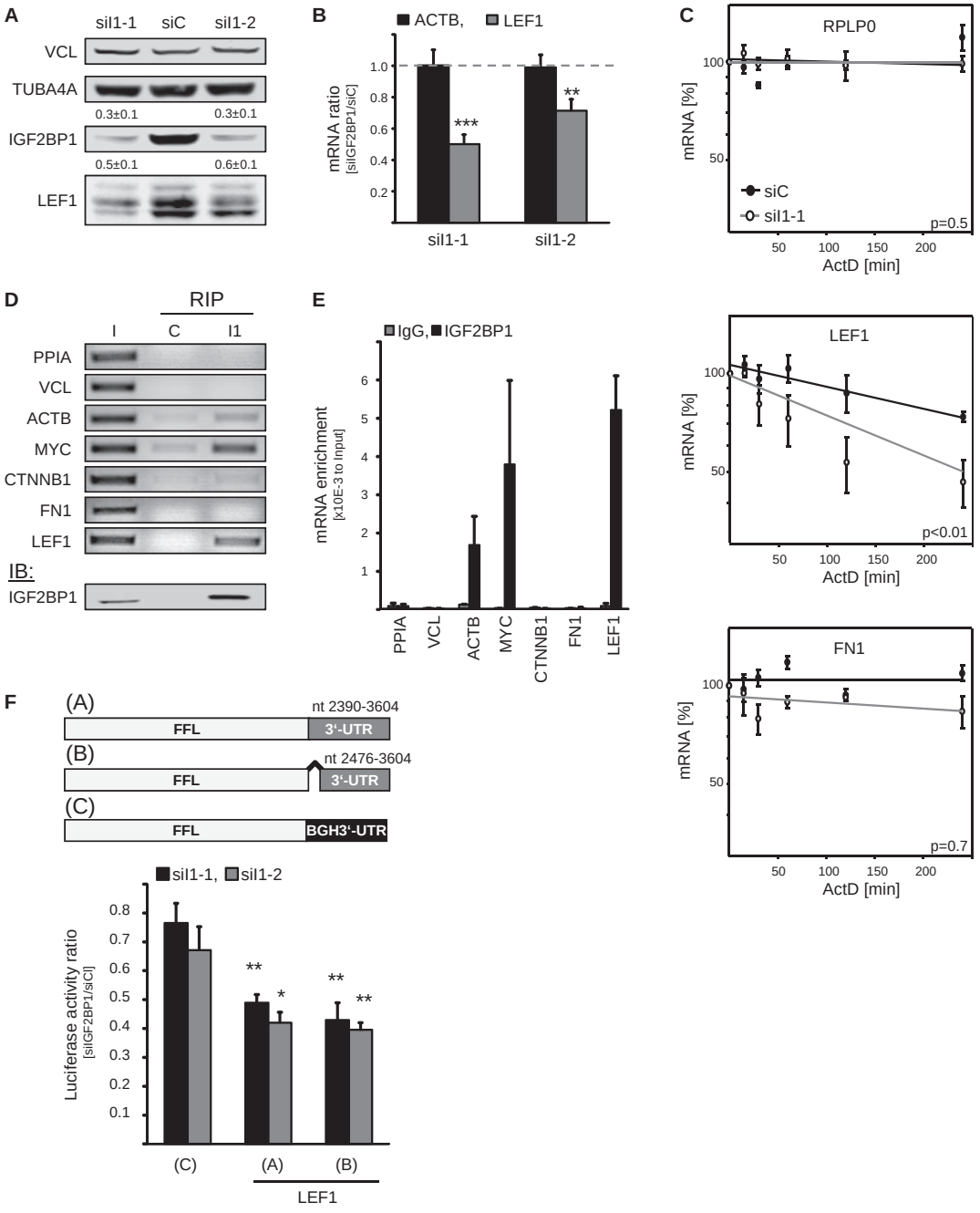


Figure 2. IGF2BP1 promotes LEF1 expression by preventing LEF1 mRNA degradation. (**A** and **B**) HEK293 cells were transfected with control (siC) or indicated IGF2BP1-directed (siI1-1, siI1-2) siRNAs for 72 h. Protein abundance on IGF2BP1 knockdown was determined relative to controls (siC) by western blotting using VCL and TUBA4A for cross-normalization, as indicated above panels. Representative western blots of three independent analyses are shown. ACTB and LEF1 mRNA levels were analyzed by qRT-PCR. Changes in RNA abundance on IGF2BP1 knockdown (siIGF2BP1) were determined relative to controls (siC) by the $\Delta\Delta C_t$-method using PPIA for normalization. (**C**) RNA decay was monitored in HEK293 cells transfected with indicated siRNAs for 72 h by blocking mRNA synthesis using ActD (5 μM) for indicated times. RNA levels were determined by qRT-PCR using normalization to PPIA by the $\Delta\Delta C_t$-method. RPLP0 served as a control. RNA decay is depicted in semi-logarithmic

(continued)

and protein levels were only modestly yet reproducibly upregulated by LEF1 knockdown (Figure 4D and E). More strikingly, LEF1 depletion resulted in a 2-fold reduction of FN1 mRNA and secreted protein levels (Figure 4E, F and Supplementary Figure S2F). As for IGF2BP1, this was further validated in U2OS cells. In these, FN1 mRNA and protein levels were decreased by LEF1 knockdown (Figure 4G, H). The opposite was observed on stable transfection of the longest LEF1 paralogue, which led to an upregulation of FN1 expression (Figure 4I and J). Hence, LEF1 promoted mesenchymal-like cell properties and enforced the expression of FN1 in HEK293 and U2OS cells.

IGF2BP1 promotes FN1 and SNAI2 (SLUG) transcription through LEF1

IGF2BP1 promoted the expression of FN1 indirectly suggesting the protein induced FN1 transcription through LEF1, which was proposed to positively regulate FN1 mRNA synthesis (40,43). In accord, transient expression of the longest LEF1 paralogue enhanced FN1 mRNA abundance and secreted FN1 protein levels in HEK293 cells (Supplementary Figure S3A–C). Moreover, FN1 expression was restored in IGF2BP1 knockdown cells by the transient expression of LEF1 (Supplementary Figure S3D). Together, this supported the view that IGF2BP1 enhances the expression of FN1 through LEF1.

In silico analyses of the genomic sequence upstream of the starting ATG of the human FN1 gene (Chr.2q34) suggested a putative minimal promoter of approximately 1 kb comprising five candidate LEF1-targeting sites (Figure 5A). To analyze whether LEF1 stimulates FN1 transcription through the FN1 promoter, the Renilla luciferase normalized activity of Firefly luciferase reporters driven by indicated fragments of the FN1 promoter were analyzed in HEK293 cells. In cells, co-transfected with red fluorescent protein (RFP), FN1 reporter activity was only observed for reporters comprising the predicted binding site four (Figure 5B, RFP). All shorter FN1 promoter fragments showed an only basal activity, which was indistinguishable from background activity determined for the control reporter lacking any promoter (Figure 5B, pGL4). Activity of reporters comprising binding site four was ~4-fold increased by the transient expression of the longest LEF1 paralogue reported in human (Figure 5B, LEF1). Together, this suggested that LEF1 promotes FN1 transcription by associating with site four in the FN1 promoter. However, activity of the longest promoter reporter (FN-839) remained essentially unaffected by the deletion of site four, which could be due to the conditioning of binding by surrounding sequences (Supplementary Figure S3E). We therefore analyzed whether LEF1 associates with the FN1 promoter using ChIP studies (Figure 5C and D). Immunopurification of LEF1 followed by semi-quantitative PCR analyses revealed robust copurification of two genomic sequences located in the human FN1 promoter, whereas binding to intergenic elements was not observed (Figure 5C, P1-P2; schematic shows position of ChIP PCR amplicons and putative binding sites). Histone H3 served as a non-promoter selective positive and IgG-agarose as a negative control. Quantitative assessment of the ChIP analyses indicated selective binding of LEF1 to the FN1 promoter and suggested association at or in proximity to the predicted binding sites three and four (Figure 5D). Finally, IGF2BP1 as well as LEF1-dependent transcriptional regulation of FN1 was evaluated by determining the activity of the longest reporter (FN-839) in response to IGF2BP1 or LEF1 knockdown (Figure 5E). The depletion of both factors reduced the reporter activity significantly supporting the view that IGF2BP1 promoted FN1 transcription in a LEF1-dependent manner. However, these analyses could not exclude whether IGF2BP1 also modulates the expression of additional factors directly or indirectly regulating the transcription of FN1.

Recent studies revealed that LEF1 enhances the expression of two transcriptional 'drivers' of EMT [reviewed in (2)], ZEB2 and SNAI2 (SLUG), in breast carcinoma-derived MDA-MB-231 cells (44). Moreover, it was postulated that LEF1 enhances SNAI2 transcription (37). Accordingly, it was tempting to speculate that IGF2BP1, by enhancing the expression of LEF1, also induces the expression of other pro-mesenchymal transcriptional regulators. To evaluate this hypothesis, SNAI2 expression in HEK293 cells was first determined in response to IGF2BP1 depletion (Figure 5F and Supplementary Figure S3F). Steady state SNAI2 protein as well as mRNA levels were reduced on IGF2BP1 knockdown. However, SNAI2 mRNA turnover remained unchanged, as determined by mRNA decay analyses in response to blocking transcription by ActD (Figure 5G). Moreover, direct association of IGF2BP1 was only observed for the LEF1, but not the SNAI2 mRNA by RIP analyses (Supplementary Figure S3H). This suggested that IGF2BP1 sustains SNAI2 expression

Figure 2. Continued
scale. Statistical significance determined over three independent analyses was analyzed by Student's *t*-test, as shown in panels (*P*-values). (**D** and **E**) The association of indicated mRNAs with IGF2BP1 in HEK293 cells was analyzed by RIP using formaldehyde fixation to stabilize mRNPs prior purification. Endogenous IGF2BP1 was immunopurified (I1) by a monoclonal antibody, as indicated by western blotting in the lower panel (IB). Co-purification of indicated mRNAs was analyzed relative to the input fraction (I, 10% of cell lysates) by semi-quantitative (D) as well as qRT-PCR (E). IgG-agarose served as a control (C) for unspecific mRNA binding. The enrichment of mRNAs by immunopurification of IGF2BP1 (I1) was determined relative to the input fraction by using the ΔC_t-method (E). (**F**) Upper panel: Scheme of used Firefly reporters comprising the two alternative LEF1 3′-UTRs (A: Acc.No., NM_016269 /001130713/ 001166119; B: Acc.No., NM_001130714) or the vector-encoded BGH-3′UTR (C). Lower panel: HEK293 cells were transfected with control or indicated IGF2BP1-directed siRNAs for 48 h before the co-transfection of Firefly luciferase reporters (A–C: see scheme in upper panel) and Renilla luciferase control reporters for 24 h. Changes in Firefly luciferase reporter activities on IGF2BP1 knockdown (siIGF2BP1) were determined relative to controls (siC) on normalization by Renilla activities. Statistical significance was validated by Student's *t*-test: $*P < 0.05$; $**P < 0.005$; $***P < 0.0005$. Error bars indicate SD of at least three independent analyses.

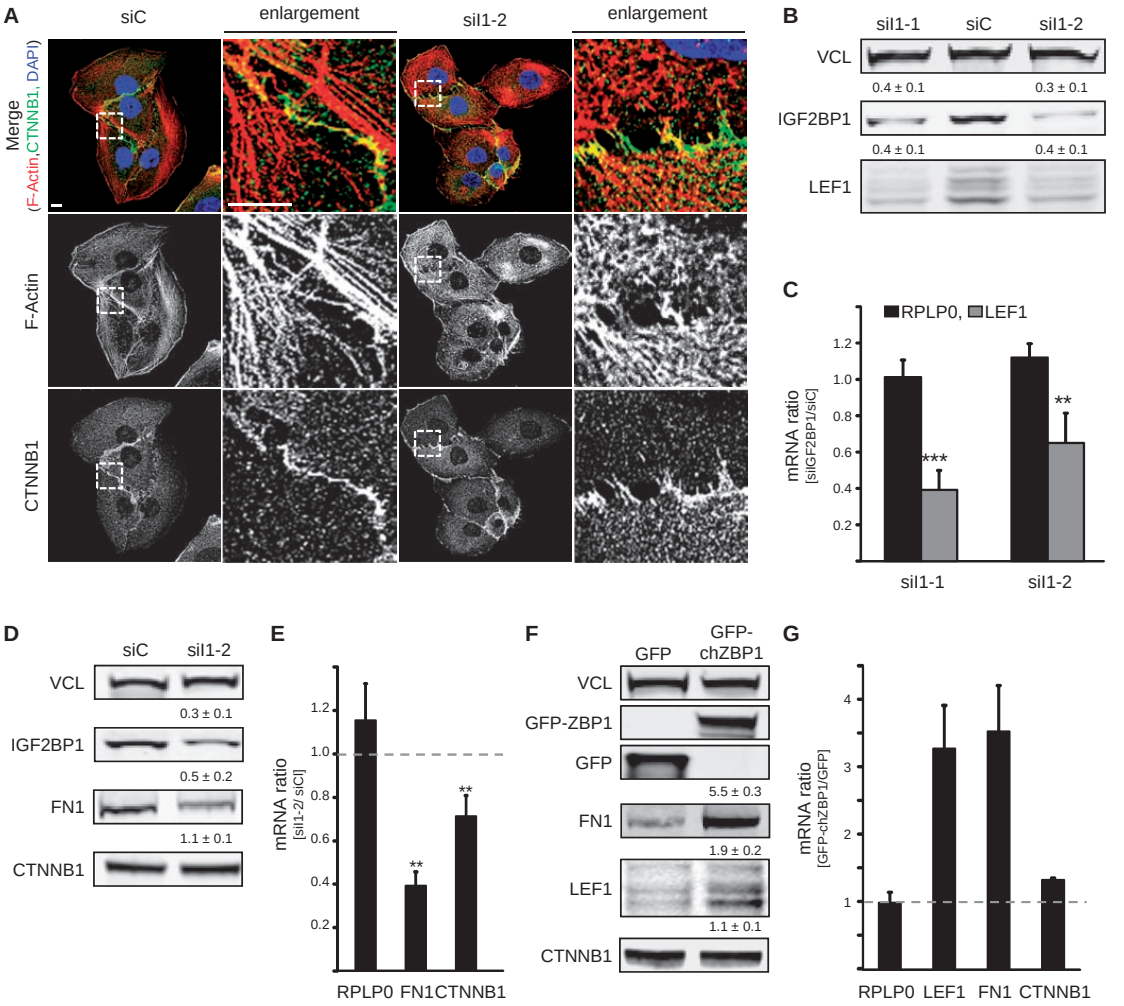


Figure 3. IGF2BP1 promotes the expression of LEF1 and FN1 in U2OS cells. (**A**) U2OS cells were transfected with IGF2BP1-directed (siI1-2) or control (siC) siRNAs for 72 h. Cell morphology and the actin cytoskeleton were analyzed by monitoring CTNNB1 localization using immunostaining or phalloidin labeling, respectively. Nuclei were labeled by DAPI. Enlargements of boxed regions (left panel) are shown in the right panels (enlargement). Bars, 10 µm. (**B** and **C**) The abundance of LEF1 protein and mRNA in response to IGF2BP1 knockdown (siI1-1 and siI1-2) was analyzed 72 h post-transfection by western blotting (B) or qRT-PCR (C), respectively. VCL served as the loading control to determine protein abundance relative to controls (siC), as is indicated above panels (B). LEF1 mRNA levels were determined relative to siC-transfected controls by the $\Delta\Delta C_t$-method using PPIA for normalization. RPLP0 mRNA served as a control. (**D** and **E**) FN1 and CTNNB1 protein (D) and mRNA (E) abundance was analyzed in U2OS cells 72 h post-transfection of indicated siRNAs as described in (B and C). CDH1 was not detectable in U2OS cells. (**F** and **G**) FN1, CTNNB1 and LEF1 protein (F) and mRNA (G) levels were investigated in U2OS cells stably transfected with GFP-ZBP1, the chicken ortholog of IGF2BP1 or GFP. Protein and mRNA abundance was essentially analyzed as described in (B and C). Statistical significance was determined by Student's *t*-test: $^{**}P < 0.005$; $^{***}P < 0.0005$. Error bars indicate SD of at least three independent analyses.

indirectly, potentially by promoting the expression of LEF1. To test this directly, SNAI2 expression was monitored on LEF1 knockdown in HEK293 cells (Figure 5H and Supplementary Figure S3G). As expected and previously reported for breast cancer-derived tumor cells, LEF1 depletion resulted in decreased steady state SNAI2 protein as well as mRNA levels. Whether LEF1 similar to FN1 controls SNAI2 expression by promoting the transcription of this 'EMT-driver' was analyzed via a previously validated luciferase reporter comprising the SNAI2-promoter [Figure 5I, (37)]. A reporter driven by the promoter of SNAI1, another 'EMT-driving' transcriptional regulator, served as control [Figure 5I, (45)]. In accord with previous studies, transiently expressed LEF1 enhanced the activity of the SNAI2, but not the SNAI1 reporter (Figure 5J). The opposite was observed on

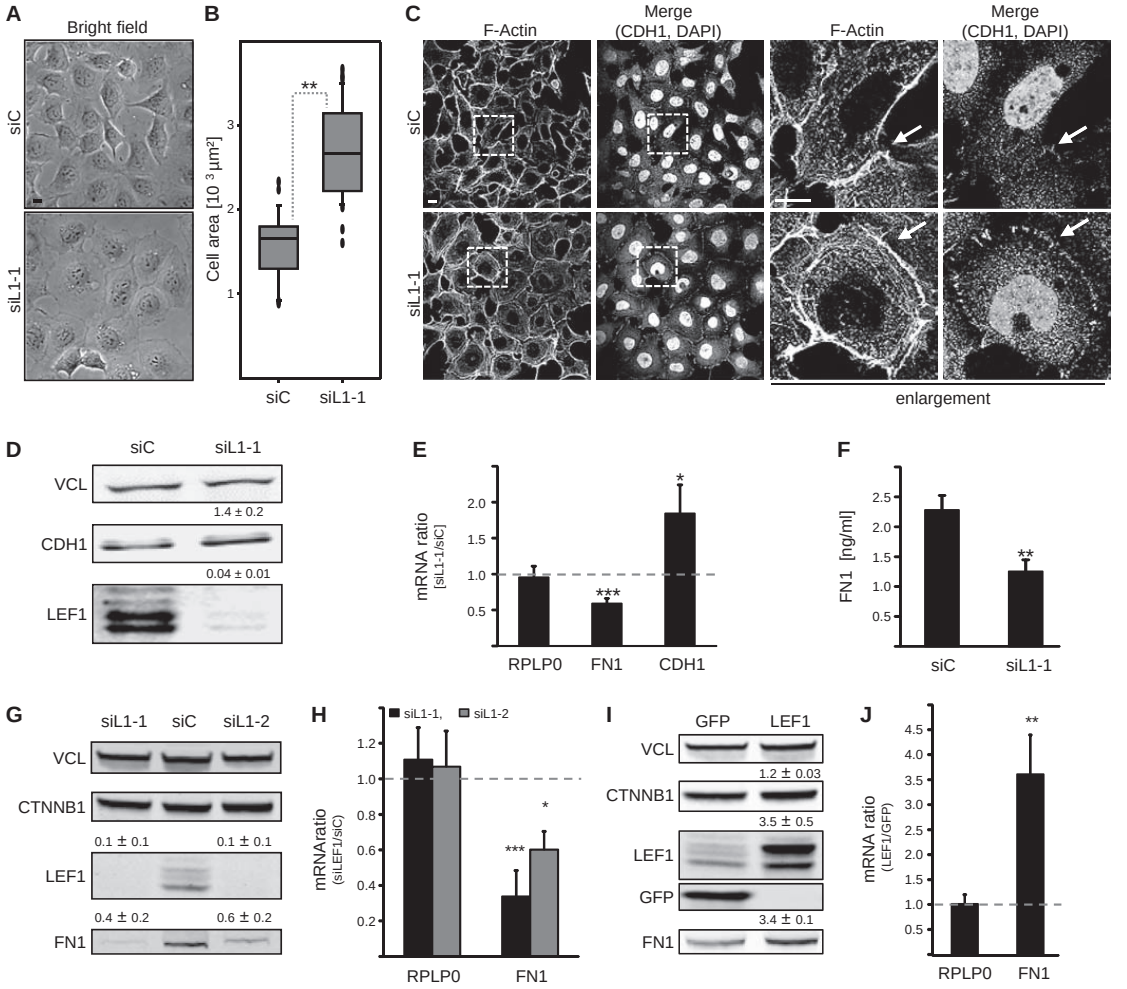


Figure 4. LEF1 promotes mesenchymal cell properties. HEK293A (**A–F**) or U2OS cells (**G** and **H**) were transfected with LEF1-directed (siL1-1 or siL1-2) or control (siC) siRNAs for 72 h. U2OS cells stably transfected with GFP or Flag-LEF1 were analyzed in (**I** and **J**). (A) Cell morphology was monitored by light microscopy; bar, 10 µm. (B) The size of adherent cells was determined by LSM microscopy, as described in Figure 1B. Please also refer to Supplementary Figure S2D. (C) The F-actin cytoskeleton and cell–cell contact formation was analyzed by phalloidin labeling and immunostaining for CDH1, where indicated nuclei were labeled by DAPI. Enlargements of boxed regions (left panels) are shown in right panels (enlargement); bars, 10 µm. The knockdown of LEF1 results in an enhanced recruitment of CDH1 to cell–cell contacts, whereas the F-actin cytoskeleton remains largely unaffected. (D) The abundance of CDH1 protein was analyzed by western blotting on LEF1 knockdown. Levels of LEF1 and CDH1 proteins were determined relative to controls (siC), as depicted above panels. VCL served as a loading control. (E) The abundance of FN1 and CDH1 mRNAs was investigated by qRT-PCR using the $\Delta\Delta C_t$-method and PPIA for normalization. RPLP0 served as a control. (F) Soluble FN1 concentrations in response to LEF1 knockdown were determined by ELISA as described in Figure 1G. (G and H) In U2OS cells transfected with indicated siRNAs, FN1 abundance was analyzed on protein (G) and mRNA (H) levels relative to controls as described in (D and E). (I and J) FN1, CTNNB1 and LEF1 protein (I) and FN1 mRNA (J) abundance was determined in U2OS cells stably transduced with Flag-LEF1 relative to GFP expressing controls. VCL served as loading control. Statistical significance was validated by Student's *t*-test: $*P < 0.05$; $**P < 0.005$; $***P < 0.0005$. Error bars indicate SD of at least three independent analyses.

IGF2BP1 as well as LEF1 knockdown. Although SNAI2 reporter activity was decreased by the depletion of IGF2BP1 or LEF1, SNAI1 reporter activity remained unaffected (Figure 5K). Notably, we also attempted to validate direct association of LEF1 with the SNAI2 promoter by ChIP, as previously demonstrated in human osteoblasts (46). However, we could not confirm association of LEF1 with the SNAI2 (46) or CDH1 (43) promoter in HEK293 cells (Supplementary Figure S3I). Recent studies reported that the expression of 'EMT-driving' transcriptional regulators like ZEBs, SNAILs and potentially LEF1 is essentially modulated by

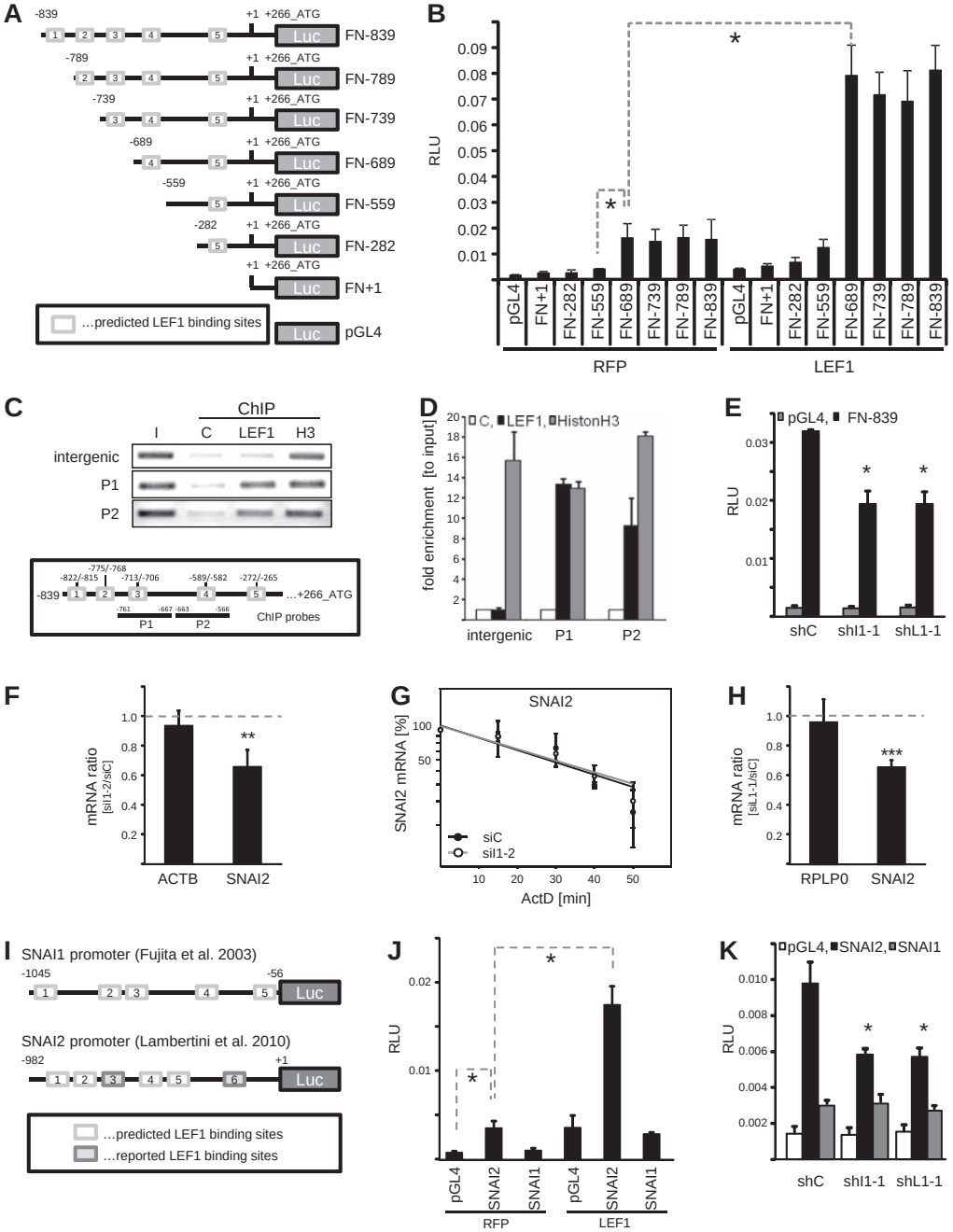


Figure 5. IGF2BP1 modulates FN1 and SNAI2 (SLUG) transcription via LEF1. (**A**) Schematic of luciferase reporters comprising the full-length *in silico* predicted (FN-839) or 5′-truncated fragments of the human FN1 promoter. The proposed transcription start is indicated by +1 with a reported 5′-UTR of 266 nt. Putative LEF1-binding sites predicted by 'PROMO' are depicted as white boxes with labels '1-5' in 5′-to-3′ direction. (**B**) The Firefly luciferase activity of indicated promoter fragments or empty pGL4 vector was monitored in HEK293 cells on transient co-transfection with RFP or LEF1 for 30 h. Firefly activities were normalized by Renilla activities [relative luciferase units (RLU)], serving as internal controls. All reporters comprising the putative LEF1-binding site four showed promoter activity and were activated by LEF1. (**C** and **D**) Binding of endogenous LEF1 protein to the human FN1 promoter in HEK293 cells was assessed by ChIP. The association of endogenous LEF1 or histone H3 to the FN1

(continued)

regulatory feedback loops facilitated via microRNAs [reviewed in (3,4)]. To evaluate whether LEF1 could regulate the expression of SNAI2 in a microRNA-dependent manner, the activity of reporters comprising the SNAI2 3′-UTR was analyzed in response to LEF1 overexpression (Supplementary Figure S3J). Activity of the SNAI2 3′-UTR comprising reporter was significantly reduced compared with the control reporter supporting inhibition of SNAI2 expression by microRNAs, for instance the miR-34 family (5). The overexpression of LEF1, however, had no significant effect on reporter activity. This suggested that LEF1 promotes SNAI2 transcription rather than modulating the post-transcriptional fate of the SNAI2 mRNA in HEK293 cells. Whether this regulation is facilitated via direct association with the SNAI2 promoter or in an indirect manner via additional yet to identify factors remained elusive, as no association of LEF1 with the SNAI2 promoter could be determined by ChIP. Taken together, these analyses indicated that IGF2BP1 promotes the expression of two 'EMT-driving' transcriptional regulators, LEF1 and SNAI2. IGF2BP1 enhanced LEF1 expression by interfering with LEF1 mRNA degradation resulting in presumably LEF1-dependent enhancement of FN1 transcription and SNAI2 expression.

IGF2BP1 sustains mesenchymal-like tumor cell properties

IGF2BP1 promotes tumor cell migration [reviewed in (23)], sustained the expression of pro-mesenchymal transcriptional regulators, and its depletion interfered with mesenchymal-like cell morphology in HEK293 as well as U2OS cells. This supported the view that IGF2BP1 serves an essential pro-mesenchymal role in tumor-derived cells, suggesting the protein as a mesenchymal marker. To evaluate this assumption, we analyzed the expression of IGF2BP1 and mesenchymal as well as epithelial markers in a panel of 10 cell lines derived from distinct tumors or metastases (Figure 6A). Except ovarian carcinoma-derived OVCAR cells, which expressed CDH1 and KRT8 but barely any of the analyzed mesenchymal markers, significant IGF2BP1 expression was only observed in mesenchymal-like tumor- or metastases-derived cells. With the exception of breast carcinoma-derived MDA-MB-231 cells, IGF2BP1 expression was well correlated with the expression of the mesenchymal marker vimentin (VIM). This provided further evidence for an essential function of IGF2BP1 in inducing and/or sustaining mesenchymal-like properties in various, although not all, mesenchymal-like tumor cells. To test this in further detail, the role of IGF2BP1 was analyzed in melanoma-derived HT-144 and 1F6 cells as well as ovarian carcinoma-derived ES-2 cells. Notably, we recently demonstrated that IGF2BP1 promotes ES-2 cell migration (25). As observed in HEK293 or U2OS cells, the transient knockdown of IGF2BP1 as well as LEF1 led to reduced FN1 as well as SNAI2 expression in all three cell lines. VIM or CDH1 protein abundance remained largely unaffected (Figure 6B and Supplementary Figure S4A and D). Despite unaffected expression of these markers, cell morphology was significantly altered, and cell–cell contact formation, as determined by CTNNB1 localization at cell borders, appeared increased on the depletion of IGF2BP1 or LEF1 (Supplementary Figures S4B, C, E, F and S5A and B). Whether the stable knockdown of IGF2BP1, LEF1 or SNAI2 promoted epithelial-like cell characteristics and marker expression in a more 'sustained' manner was analyzed in HT-144 cells transduced with shRNA-encoding lentiviral vectors. The stable knockdown of IGF2BP1 led to ~3-fold reduced IGF2BP1 abundance, which was associated with a significant downregulation of LEF1, FN1, SNAI2 and also VIM. Expression of the epithelial marker CDH1 was modestly, but reproducibly, increased (Figure 6C and D). The same was observed on the stable knockdown of LEF1 and SNAI2, although significant upregulation of CDH1 was only observed on SNAI2 depletion confirming the pivotal role of this transcriptional 'EMT-driver' in the repression of CDH1. Notably, the knockdown of LEF1 as well as SNAI2 also interfered with IGF2BP1 expression, which could indicate that IGF2BP1 expression is controlled by 'EMT-driving' transcriptional regulators like LEF1 and/or SNAI2. Consistent with the observed shift in the expression of mesenchymal versus epithelial markers, the knockdown

Figure 5. Continued
promoter was monitored by semi-quantitative (C) as well as quantitative PCR (D) using to FN1 promoter specific amplicons (P1 and P2, indicated in lower panel). An intergenic probe served as positive control. IgG-agarose was used to monitor unspecific binding (C, negative control). In (D), the enrichment of indicated genomic DNA fragments (P1 and P2) or the intergenic control (intergenic) was determined relative to the diluted input fraction (**I**) normalized by IgG-controls using the ΔC_t-method. (**E**) HEK293 cells were co-transfected with FN-839 luciferase reporter and IGF2BP1-directed (shI1-1), LEF1-directed (shL1-1) or control shRNA encoding vectors for 48 h. RLUs were determined as described in (B). (**F**) HEK293 cells were transfected with IGF2BP1-directed (siI1-2) or control siRNAs (siC) for 72 h. The abundance of SNAI2 mRNA in response to IGF2BP1 knockdown was analyzed by qRT-PCR using the $\Delta\Delta C_t$-method and PPIA for normalization. ACTB served as control. (**G**) HEK293 cells transfected as in (F) were treated with ActD (5 μM) to block transcription for indicated times. SNAI2 mRNA turnover was analyzed by qRT-PCR using the $\Delta\Delta C_t$-method and PPIA for normalization. RNA decay is depicted in semi-logarithmic scale revealing no significant difference in mRNA turnover (*P*-value not shown). (**H**) HEK293 cells were transfected with LEF1-directed (siL1-1) or control siRNAs (siC) for 72 h. The abundance of SNAI2 mRNA in response to LEF1 depletion was analyzed by qRT-PCR using the $\Delta\Delta C_t$-method and PPIA for normalization. RPLP0 served as control. (**I**) Schematic of Firefly luciferase reporters comprising the SNAI1 or SNAI2 promoter sequences, as previously reported (37,45). Indicated putative LEF1-binding sites within the SNAI1 or SNAI2 promoter were predicted [white boxes; as described in (A)] or as previously reported [gray boxes, only for SNAI2; (37)]. (**J**) The Firefly activity of SNAI1 or SNAI2 promoter fragments cloned in pGL4 as well as the activity of empty pGL4 vector was monitored in HEK293 cells on transient co-transfection with RFP or LEF1 for 30 h. RLUs were determined as described in (B). LEF1 only enhanced the activity of the SNAI2 promoter. (**K**) HEK293 cells were co-transfected with SNAI1 or SNAI2 promoter reporters and indicated shRNA-encoding vectors for 48 h. RLUs were determined as described in (B). SNAI2 promoter activity was reduced by IGF2BP1 as well as LEF1 knockdown, whereas the SNAI1 reporter activity remained largely unaffected and was barely elevated compared with the empty control reporter. Statistical significance was validated by Student's *t*-testing: $*P < 0.05$; $***P < 0.0005$. Error bars indicate SD of at least three independent analyses.

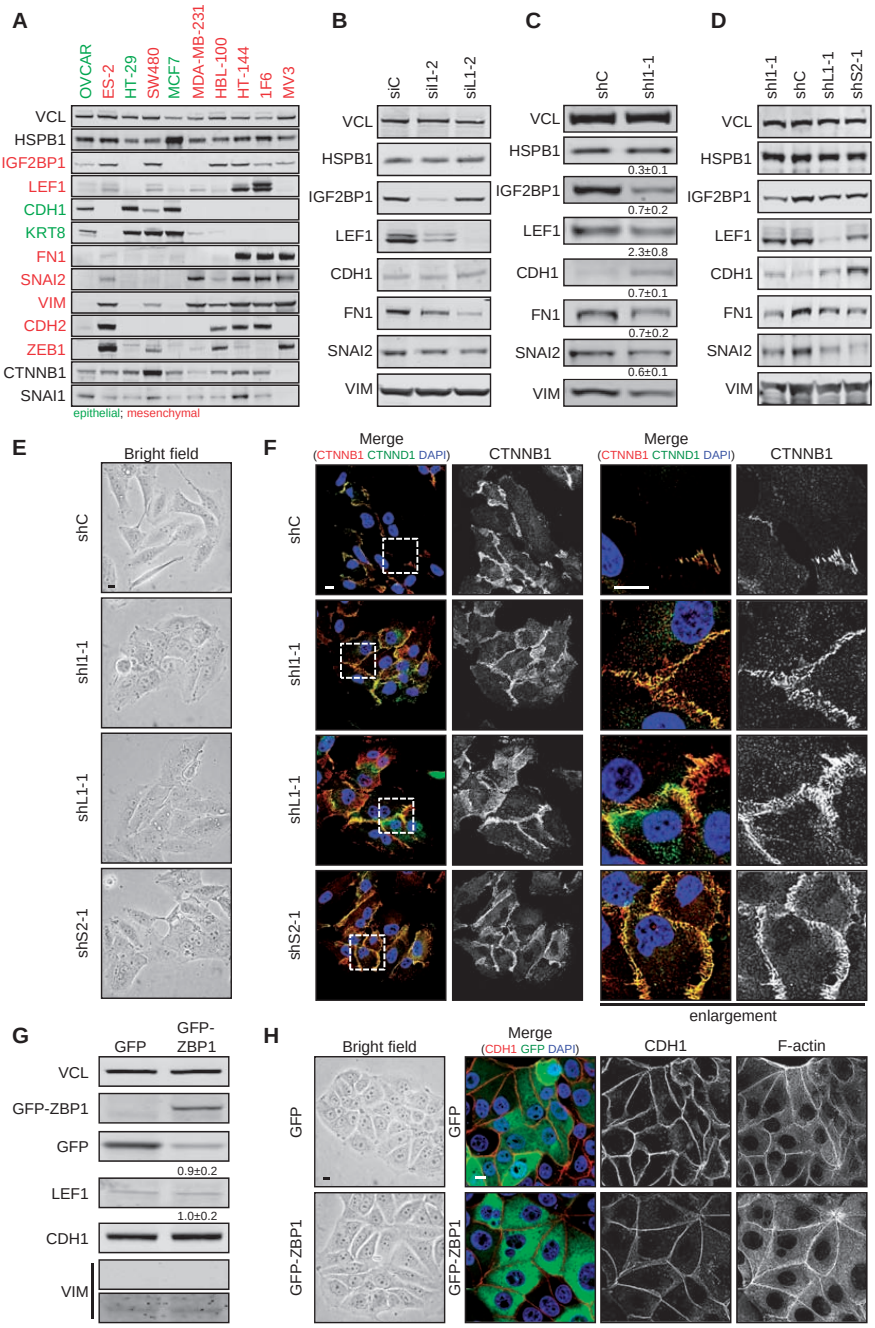


Figure 6. IGF2BP1 sustains mesenchymal-like tumor cell properties. (**A**) Indicated tumor-derived cell lines were cultured (48 h) and harvested at 80% confluence before analyzing the abundance of indicated proteins by western blotting. Epithelial-like cell lines and marker proteins are labeled in green. Mesenchymal-like cell lines and marker proteins are depicted in red. VCL and HSPB1 (HSP27) served as loading controls. IGF2BP1 is almost exclusively expressed in the following tumor-derived mesenchymal-like cells: ES-2 ovarian carcinoma (ATCC#: CRL-1978); SW480 colorectal carcinoma (ATCC#: CCL228); MDA-MB-231 (ATCC#: HTB-26) and HBL-100 (ATCC#: HTB-124) mammary carcinoma; 1F6 (no ATCC# available) and HT-144 (ATCC#: HTB-63) melanoma. In epithelial-like adenocarcinoma-derived cells [OVCAR (ATCC#: HTB-161) ovarian adenocarcinoma; MCF7 (ATCC#: HTB-22) breast adenocarcinoma; HT-29 (ATCC#: HTB-38) colorectal adenocarcinoma], expression of IGF2BP1 was only observed

(continued)

of all three factors caused severe morphological changes in HT-144 with an increase of CTNNB1 positive cell–cell contact sites (Figure 6E and F). These findings indicated that IGF2BP1 sustains the expression of mesenchymal markers and mesenchymal-like cell morphology involving the enhancement of LEF1 and SNAI2 expression. However, it remained elusive whether IGF2BP1 also induces mesenchymal-like cell properties or even EMT. To address this in further detail, we transduced breast cancer-derived MCF7 cells, which express IGF2BP1 at barely detectable levels (see Figure 6A), with lentiviral vectors encoding either GFP or GFP-fused ZBP1, the chicken ortholog of IGF2BP1. Two to three weeks after infection, the expression of low abundant LEF1 as well as highly expressed CDH1 remained essentially unaffected by GFP-ZBP1 (Figure 6G). Likewise, no significant increase was observed for the expression of VIM, which was barely detectable to begin with. In accord with the unaltered expression of mesenchymal and epithelial markers, the overall cell morphology remained essentially unchanged with no obvious defect in cell–cell contact formation, as evidenced by CDH1 immunostaining (Figure 6H). This was further analyzed in epithelial MDCK, which, despite their epithelial morphology, expresses IGF2BP1 (Supplementary Figure S7D). The stable expression of GFP-ZBP1 using lentiviral transduction significantly increased the size of adherent MDCK cells (Supplementary Figure S7A and B). However, cell–cell contact formation and the expression of the epithelial marker CDH1 or the mesenchymal marker VIM remained unaffected by ZBP1 (Supplementary Figure S7C and D). Finally, we analyzed whether the forced expression of ZBP1 increased the migratory potential of MDCK cells. Wound closure studies revealed that the motility of MDCK cells was unchanged (Supplementary Figure S7E and F). Taken together, this indicated that IGF2BP1 rather sustains than induces mesenchymal-like cell properties in tumor-derived or immortalized cells. However, its potential role in inducing mesenchymal-like cell properties or even EMT has been validated here for only two cell lines (MCF7 and MDCK). In contrast, IGF2BP1-dependent sustainment of mesenchymal cell properties could be validated for all so far analyzed mesenchymal-like tumor-derived cells expressing IGF2BP1.

IGF2BP1 promotes migration and mesenchymal cell morphology through LEF1 and SNAI2

In previous studies, we demonstrated that IGF2BP1 promotes the migratory potential as well as cell-matrix contact formation of various tumor-derived cells. These analyses indicated that IGF2BP1 modulates these *bona fide* mesenchymal-like cell properties by fine tuning actin dynamics in a MK-signaling dependent manner. However, pharmacological inhibition of MK-signaling only partially, although significantly, restored cell migration and the formation of focal adhesions (23,25). Therefore, it was tempting to speculate that the role of IGF2BP1 in directing cell migration and sustaining mesenchymal-like cell properties is also modulated through the pro-mesenchymal regulators LEF1 and/or SNAI2. Accordingly, cell migration was analyzed by wound healing analyses in HT-144 cells using automated segmentation algorithms to quantify wound closure (39). Consistent with recent findings, stable IGF2BP1 knockdown reduced wound closure by ~2-fold (Figure 7A and B). Significantly reduced cell migration was also observed on the stable knockdown of LEF1 or SNAI2, although cell migration was only moderately affected by LEF1 depletion when compared with the knockdown of IGF2BP1 or SNAI2. Whether LEF1 or SNAI2 could recover IGF2BP1 knockdown-induced impairment of tumor cell migration was determined by their stable expression in IGF2BP1-depleted cells. In comparison with IGF2BP1 knockdown cells stably expressing GFP, cell migration was restored substantially by the forced expression of either LEF1 or SNAI2. Surprisingly, however, the stable expression of LEF1 or SNAI2 had only moderate effects on FN1 or CDH1 abundance in IGF2BP1 knockdown cells (Figure 7C). Despite largely unaltered expression of these markers, mesenchymal-like cell morphology with reduced cell–cell contacts was observed on the expression of LEF1 and SNAI2 in cells stably transduced with IGF2BP1-directed shRNAs (Figure 7D and E). IGF2BP1 knockdown cells showed pronounced cell–cell contact formation with increased recruitment of CTNNB1 to cell–cell contact sites. This was correlated with an enhanced association of cells observed by bright field analyses (Figure 7D). The stable expression of LEF1 or SNAI2 induced a more mesenchymal-like appearance of cell morphology with reduced association of cells and

Figure 6. Continued
in OVCAR cells. (**B**) Melanoma-derived HT-144 cells were transiently transfected with indicated siRNAs for 72 h. The abundance of indicated proteins was analyzed by western blotting. IGF2BP1 as well as LEF1 depletion result in reduced FN1 and SNAI2 protein abundance, whereas CDH1 and VIM levels remain essentially unchanged. (**C–F**) HT-144 cells were stably transduced by lentiviral vectors encoding IGF2BP1 (shI1-1), LEF1 (shL1-1), SNAI2 (shS2-1) directed or control (shC) shRNAs. Three weeks after transduction, cells were cultured for 48 h before analyzing protein abundance by western blotting with indicated antibodies (C and D). Protein abundance on IGF2BP1 knockdown was determined relative to controls (siC) using VCL and HSPB1 for cross-normalization, as indicated above panels (C). Standard deviation of at least three independent analyses is shown. The stable knockdown of SNAI2, LEF1 and IGF2BP1 promotes the expression of the epithelial marker CDH1, whereas all mesenchymal marker proteins were reduced. Cell morphology was monitored by bright field microscopy (E). Cells were cultured on collagen coated coverslips for 48 h before immunostaining of CTNNB1 and CTNND1 (p120 Catenin) to label cell–cell contacts (F). Enlargements of boxed regions (left panels) are shown in right panels (enlargement). All three stable knockdowns promote the formation of cell–cell contacts, suggesting an enhancement of epithelial-like cell morphology. (**G** and **H**) MCF7 cells were stably transduced with GFP-ZBP1 (the chicken ortholog of IGF2BP1) or GFP. Three weeks after transduction, cells were cultured for 48 h before determining the abundance of indicated proteins by western blotting (G). Cell morphology was monitored by bright field microscopy (H, left panel) and immunostaining for CDH1 as well as labeling of F-actin by phalloidin (H, right panel). Neither CDH1 expression nor cell-cell contact formation is compromised by GFP-ZBP1, although cell size appeared modestly increased. Bars, 10 μm.

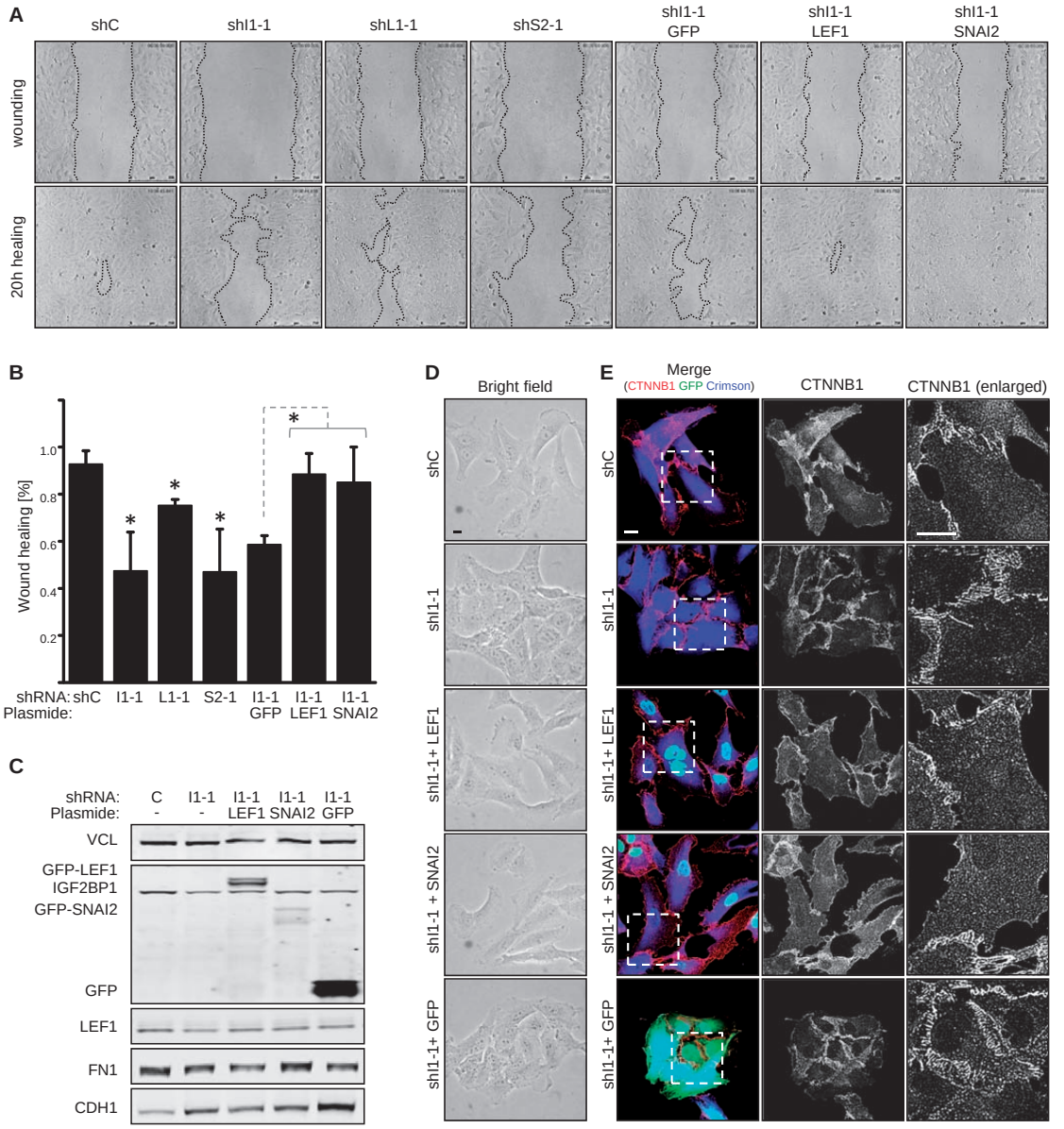


Figure 7. IGF2BP1 promotes migration and mesenchymal-like cell morphology via LEF1 and SNAI2. HT-144 cells were stably transduced by lentiviral vectors encoding IGF2BP1 (shI1-1), LEF1 (shL1-1), SNAI2 (shS2-1) directed or control (shC) shRNAs, where indicated IGF2BP1 knockdown populations were transduced with GFP, GFP-LEF1 or GFP-SNAI2 cDNA-encoding lentiviral vectors 3 weeks after the infection with shRNA-encoding vectors. (**A** and **B**) Cell migration was analyzed using wound closure analyses monitored by time lapse microscopy over 20 h (A; Bars, 250 µm). Cell migration was assessed by quantitative means relative to cells transduced with control shRNA (shC) using automated segmentation algorithms (B), as recently described (39). SD was determined over three independent analyses. Statistical significance was validated by Student's *t*-test: $*P < 0.05$. The depletion of IGF2BP1, LEF1 or SNAI2 significantly reduces cell migration. Migration of IGF2BP1 knockdown populations is restored by the expression of LEF1 or SNAI2. (**C**) The abundance of indicated epithelial or mesenchymal markers was analyzed by western blotting in indicated cell populations as described in Figure 6C. (**D** and **E**) Cell morphology of indicated cell populations was monitored by bright field microscopy (D) and immunostaining for CTNNB1 (E). Transduction with shRNA-encoding lentiviral vectors was monitored by E2-Crimson (pseudo-colored in blue), whereas the expression of GFP-LEF1, GFP-SNAI2 or GFP is depicted in green. Mesenchymal-like cell morphology was essentially restored by the stable expression of GFP-LEF1 or GFP-SNAI2 in IGF2BP1 knockdown populations. Enlargements of boxed regions are shown in the right panels (E, enlarged). Bars, 10 µm.

less striking recruitment of CTNNB1 to cell borders. CTNNB1 appeared to be enriched in the cytoplasm and was even observed, although at moderate levels, in the nucleus of some cells transduced with LEF1 or SNAI2 (Figure 7E). Hence, although FN1 and CDH1 are presumably not the key markers involved, these findings supported the view that the role of IGF2BP1 in promoting tumor cell migration and sustaining mesenchymal-like cell morphology involves the upregulation of LEF1 and SNAI2.

DISCUSSION

This study identifies a novel mechanism by which the RBP IGF2BP1 sustains mesenchymal tumor cell properties and promotes the migration of tumor-derived cells *in vitro*. This regulation is essentially facilitated by IGF2BP1-directed upregulation of the 'EMT-driving' transcriptional regulators LEF1 and SNAI2 [reviewed in (1,2)]. IGF2BP1 interferes with LEF1 mRNA degradation in a 3′-UTR dependent manner resulting in the enhancement of LEF1 expression. This in turn promotes transcription of the extracellular matrix component FN1, a *bona fide* mesenchymal marker. Moreover, IGF2BP1 indirectly promotes SNAI2 (SLUG) transcription, presumably in a LEF1-dependent manner. In agreement with this pro-mesenchymal role of IGF2BP1, the protein is predominantly observed in mesenchymal-like tumor-derived cells in which it enhances motility. In addition to controlling actin dynamics (23,25), IGF2BP1 sustains mesenchymal cell properties and modulates tumor cell migration also through the upregulation of LEF1 and SNAI2. However, the stable expression of IGF2BP1 in epithelial-like MCF7 tumor-derived cells or kidney-derived MDCK cells failed to induce EMT or a significant upregulation of mesenchymal marker expression. This indicates that IGF2BP1 sustains but does not induce pro-mesenchymal gene expression by promoting the expression of 'EMT-driving' transcriptional regulators at the post-transcriptional level. This results in elevated tumor cell migration and potentially enhances the invasive potential of tumor cells (Supplementary Figure S8).

In vitro, IGF2BP1 was identified as a pro-migratory RBP, which in various tumor-derived cells promotes both the velocity and directionality of migration. Expression profiling of primary tumors and metastases provided substantial evidence for an upregulation or even *de novo* synthesis of IGF2BP1 and/or IGF2BP3 in almost all solid cancers analyzed so far [reviewed in (8,23)]. Together, this suggests a fundamental role of IGF2BP1 and potentially IGF2BP3 in tumor cell dissemination, which presumably is observed in a broad variety of tumors. In support of this, it was reported that the transgenic expression of mouse IGF2BP1 (CRD-BP) in mammary tissue promotes the formation of primary breast carcinomas as well as metastases (47). In contrast, IGF2BP1 was proposed to interfere with the *in vitro* migration of breast cancer-derived cells and enhance the formation of cell–cell contacts (22,36). Hence, it remained contradictory whether IGF2BP1 promotes mesenchymal-like properties of tumor cells and modulates their migration in a largely cell context independent manner. Therefore, we addressed how perturbing IGF2BP1 expression in tumor-derived cells and non-tumorigenic HEK293 cells, which express exceedingly high levels of IGF2BP1, affects mesenchymal- versus epithelial-like cell properties. In HEK293 cells, depletion of the protein induced severe morphological changes, which were associated with an increase in cell–cell contact formation, moderate upregulation of the epithelial marker CDH1 and significant downregulation of the mesenchymal marker FN1. These pro-epithelial changes in cell morphology and marker expression were observed to varying extend for all analyzed tumor-derived cells expressing IGF2BP1. Accordingly, we propose that IGF2BP1 is a pro-mesenchymal marker that sustains mesenchymal-like cell properties and promotes migration of tumor-derived cells in a largely context-independent manner.

IGF2BPs control the cytoplasmic fate of specific target mRNAs by regulating their turnover, translation and/or transport [reviewed in (8,10)]. This implied that the pro-mesenchymal role of IGF2BP1 is facilitated by regulating the fate of target transcripts encoding either regulators or markers of pro-mesenchymal gene expression signatures. In recent studies, we identified various novel candidate target mRNAs of IGF2BP1 by using a loss-of-function screen in stressed U2OS cells (25). These analyses suggested mRNAs encoding the pro-mesenchymal or 'EMT-driving' transcriptional regulator LEF1 as candidate target transcripts of IGF2BP1. Analyses of how IGF2BP1 modulates LEF1 mRNA fate revealed that the protein interferes with the degradation of LEF1 mRNAs resulting in elevated expression of this TCF family member. Similar to the IGF2BP-directed control of CD44 expression (28), IGF2BP1 prevents degradation of LEF1 mRNAs via the 3′-UTR essentially shared by all four reported human LEF1 transcripts. Notably, IGF2BP1-controlled LEF1 expression could be validated by loss- as well as gain-of-function analyses and was observed in all mesenchymal-like tumor-derived cells analyzed in this study. This suggests LEF1 as a prime, although not exclusive candidate, through which IGF2BP1 promotes mesenchymal-like tumor cell properties.

The TCF/LEF family of transcriptional regulators was identified as a key mediator of both Wnt/CTNNB1- or TGFB/SMAD-dependent developmental and malignant EMT [reviewed in (2,48)]. In agreement with 'EMT-driving' functions, LEF1 depletion was associated with reduced FN1 expression in all cells analyzed in this study, whereas the opposite was observed by stable LEF1 expression in U2OS or HEK293 cells. This supports LEF1-dependent upregulation of FN1 transcription observed by promoter-reporter and ChIP studies. However, LEF1 is presumably not the only mediator of FN1 expression, as FN1 is also expressed in cells lacking LEF1, for instance melanoma-derived MV3 cells. In contrast to FN1, LEF1 depletion or overexpression barely affected the expression of CDH1 supporting the view that LEF1 is not a potent repressor of CDH1

transcription. This is surprising, as LEF1 was shown to enhance the transcription of SNAI2 (SLUG) a *bona fide* 'EMT-driving' regulator suppressing CDH1 expression (37,49). However, LEF1-driven upregulation of SNAI2 may simply be too moderate to push SNAI2 abundance to levels sufficient for CDH1 repression. Consistent with IGF2BP1-promoted expression of LEF1, IGF2BP1 and LEF1 depletion resulted in reduced SNAI2 expression, presumably owing to reduced transcription. Direct regulation of SNAI2 mRNA fate by IGF2BP1 could be excluded, as the protein neither associates with the SNAI2 mRNA nor modulates its turnover. Thus, taken together, our studies suggest that IGF2BP1 can promote the expression of mesenchymal markers and modestly interfere with the expression of epithelial markers. This regulation is likely to be facilitated via LEF1 and SNAI2 but presumably also involves additional regulators like ZEBs or TWISTs. Notably, we have substantial evidence that IGF2BP1 promotes the expression of ZEB1, another potent 'EMT-driving' transcriptional regulator, in anaplastic thyroid carcinoma-derived tumor cells (Mensch *et al*., in preparation). Importantly, in none of the analyzed tumor-derived cells, IGF2BP1 depletion was sufficient to induce upregulation of epithelial markers to a level expected for MET. Likewise, stable IGF2BP1 or ZBP1 expression in epithelial-like MCF7 or MDCK cells failed to induce EMT. This supports the view that IGF2BP1 sustains mesenchymal-like cell properties and potentially EMT-induced reprograming of gene expression at the post-transcriptional level. However, it fails to induce this reprograming, as this requires the induction of powerful upstream drivers at the transcriptional and/or epigenetic level. Along these lines, even the stable expression of *bona fide* 'EMT-driving' transcriptional regulators like SNAI2 failed to induce EMT in MCF7 cells, although CDH1 levels were significantly reduced, and cell size was markedly increased (Supplementary Figure S6A–C). This is consistent with the assumption that in some or even most tumor-derived cells, one 'EMT-driver' is insufficient to induce trans-differentiation. Moreover, this provides further support for the view that RBPs, which only fine tune gene expression at the post-transcriptional level, with few exceptions like SXL in *Drosophila*, simply lack the potency to induce a complete reprograming of gene expression signatures. This of course does not contradict a significant influence in the sustainment of altered gene expression at the post-transcriptional level.

Strikingly, we observed that IGF2BP1-facilitated modulation of tumor cell migration involves IGF2BP1-directed control of LEF1 and SNAI2 expression. This conclusion is supported by the finding that reduced migration of HT-144 cells on IGF2BP1 knockdown was completely restored by the stable expression of LEF1 or SNAI2. Notably, this is intriguingly consistent with the reported role of both factors in tumor cell invasion and metastasis, which essentially, although by far not exclusively, relies on the migratory capability of tumor cells [e.g. (50,51)]. In support of the interdependence of EMT, enhanced migratory potential and metastasis, the stable expression of LEF1 or SNAI2 also pronounced more mesenchymal-like cell morphology with reduced cell–cell contact formation on stable IGF2BP1 knockdown in HT-144 cells. Hence, although IGF2BP1 failed to induce EMT and sustained the expression of mesenchymal markers only moderately, the post-transcriptional fine tuning of gene expression facilitated by IGF2BP1 is sufficient to substantially impact cell morphology and tumor cell migration. This suggests that IGF2BP1 modulates various pro-mesenchymal regulatory networks including the control of actin dynamics (23,25) and the sustainment of pro-mesenchymal gene expression signatures driven by transcriptional regulators like LEF1 or SNAI2. In view of the proposed multitude of target mRNAs regulated by IGF2BP1 and IGF2BP3 (42), these findings suggest a fundamental role of both factors in promoting tumor cell aggressiveness and invasive potential in a largely tumor origin-independent manner. Future studies will now have to reveal whether this conclusion can be validated *in vivo* by testing to which extent both proteins promote metastasis and via which target mRNAs or signaling networks this regulation is facilitated. We expect that such analyses will indicate IGF2BP1 and IGF2BP3 as useful biomarkers for evaluating tumor aggressiveness and will reveal avenues to pursue analyzing their suitability for targeted therapy. The latter would benefit substantially from the fact that both factors are essentially *de novo* synthesized in various tumors, whereas they are barely expressed in the vast majority of adult tissues (8).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1–4 and Supplementary Figures 1–9.

ACKNOWLEDGEMENTS

The authors thank the Core Facility Imaging (CFI) of the MLU for technical support. Indicated plasmids were obtained from Addgene.

FUNDING

Funding for open access charge: Deutsche Forschungs Gemeinschaft (DFG) [HU1547/3-1, HU1547/2-2 and GRK1591 to S.H.].

Conflict of interest statement. None declared.

REFERENCES

1. Brabletz,T. (2012) To differentiate or not—routes towards metastasis. *Nat. Rev. Cancer*, **12**, 425–436.
2. Polyak,K. and Weinberg,R.A. (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat. Rev. Cancer*, **9**, 265–273.
3. Brabletz,S. and Brabletz,T. (2010) The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? *EMBO Rep.*, **11**, 670–677.
4. Brabletz,T. (2012) MiR-34 and SNAIL: another double-negative feedback loop controlling cellular plasticity/EMT governed by p53. *Cell Cycle*, **11**, 215–216.

5. Siemens,H., Jackstadt,R., Hunten,S., Kaller,M., Menssen,A., Gotz,U. and Hermeking,H. (2011) MiR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. *Cell Cycle*, **10**, 4256–4271.
6. Valacca,C., Bonomi,S., Buratti,E., Pedrotti,S., Baralle,F.E., Sette,C., Ghigna,C. and Biamonti,G. (2010) Sam68 regulates EMT through alternative splicing-activated nonsense-mediated mRNA decay of the SF2/ASF proto-oncogene. *J. Cell Biol.*, **191**, 87–99.
7. Petz,M., Them,N., Huber,H., Beug,H. and Mikulits,W. (2012) La enhances IRES-mediated translation of laminin B1 during malignant epithelial to mesenchymal transition. *Nucleic Acids Res.*, **40**, 290–302.
8. Bell,J.L., Wachter,K., Muhleck,B., Pazaitis,N., Kohn,M., Lederer,M. and Huttelmaier,S. (2012) Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell Mol. Life Sci*, doi:10.1007/s00018-012-1186-z.
9. Yaniv,K. and Yisraeli,J.K. (2002) The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. *Gene*, **287**, 49–54.
10. Yisraeli,J.K. (2005) VICKZ proteins: a multi-talented family of regulatory RNA-binding proteins. *Biol. Cell*, **97**, 87–96.
11. Findeis-Hosey,J.J. and Xu,H. (2011) The use of insulin like-growth factor II messenger RNA binding protein-3 in diagnostic pathology. *Hum. Pathol.*, **42**, 303–314.
12. Nielsen,J., Christiansen,J., Lykke-Andersen,J., Johnsen,A.H., Wewer,U.M. and Nielsen,F.C. (1999) A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol. Cell Biol.*, **19**, 1262–1270.
13. Liao,B., Patel,M., Hu,Y., Charles,S., Herrick,D.J. and Brewer,G. (2004) Targeted knockdown of the RNA-binding protein CRD-BP promotes cell proliferation via an insulin-like growth factor II-dependent pathway in human K562 leukemia cells. *J. Biol. Chem.*, **279**, 48716–48724.
14. Dai,N., Rapley,J., Angel,M., Yanik,M.F., Blower,M.D. and Avruch,J. (2011) mTOR phosphorylates IMP2 to promote IGF2 mRNA translation by internal ribosomal entry. *Genes Dev.*, **25**, 1159–1172.
15. Liao,B., Hu,Y., Herrick,D.J. and Brewer,G. (2005) The RNA-binding protein IMP-3 is a translational activator of insulin-like growth factor II leader-3 mRNA during proliferation of human K562 leukemia cells. *J. Biol. Chem.*, **280**, 18517–18524.
16. Lemm,I. and Ross,J. (2002) Regulation of c-myc mRNA decay by translational pausing in a coding region instability determinant. *Mol. Cell Biol.*, **22**, 3959–3969.
17. Kobel,M., Weidensdorfer,D., Reinke,C., Lederer,M., Schmitt,W.D., Zeng,K., Thomssen,C., Hauptmann,S. and Huttelmaier,S. (2007) Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. *Oncogene*, **26**, 7584–7589.
18. Weidensdorfer,D., Stohr,N., Baude,A., Lederer,M., Kohn,M., Schierhorn,A., Buchmeier,S., Wahle,E. and Huttelmaier,S. (2009) Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *RNA*, **15**, 104–115.
19. Noubissi,F.K., Elcheva,I., Bhatia,N., Shakoori,A., Ougolkov,A., Liu,J., Minamoto,T., Ross,J., Fuchs,S.Y. and Spiegelman,V.S. (2006) CRD-BP mediates stabilization of betaTrCP1 and c-myc mRNA in response to beta-catenin signalling. *Nature*, **441**, 898–901.
20. Gu,W., Wells,A.L., Pan,F. and Singer,R.H. (2008) Feedback regulation between zipcode binding protein 1 and beta-catenin mRNAs in breast cancer cells. *Mol. Cell. Biol.*, **28**, 4963–4974.
21. Elcheva,I., Goswami,S., Noubissi,F.K. and Spiegelman,V.S. (2009) CRD-BP protects the coding region of betaTrCP1 mRNA from miR-183-mediated degradation. *Mol. Cell*, **35**, 240–246.
22. Gu,W., Pan,F. and Singer,R.H. (2009) Blocking beta-catenin binding to the ZBP1 promoter represses ZBP1 expression, leading to increased proliferation and migration of metastatic breast-cancer cells. *J. Cell Sci.*, **122**, 1895–1905.
23. Stohr,N. and Huttelmaier,S. (2012) IGF2BP1: a post-transcriptional "driver" of tumor cell migration. *Cell Adh. Migr.*, **6**, 312–318.
24. Yaniv,K., Fainsod,A., Kalcheim,C. and Yisraeli,J.K. (2003) The RNA-binding protein Vg1 RBP is required for cell migration during early neural development. *Development*, **130**, 5649–5661.
25. Stohr,N., Kohn,M., Lederer,M., Glass,M., Reinke,C., Singer,R.H. and Huttelmaier,S. (2012) IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. *Genes Dev.*, **26**, 176–189.
26. Vainer,G., Vainer-Mosse,E., Pikarsky,A., Shenoy,S.M., Oberman,F., Yeffet,A., Singer,R.H., Pikarsky,E. and Yisraeli,J.K. (2008) A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. *J. Pathol.*, **215**, 445–456.
27. Lapidus,K., Wyckoff,J., Mouneimne,G., Lorenz,M., Soon,L., Condeelis,J.S. and Singer,R.H. (2007) ZBP1 enhances cell polarity and reduces chemotaxis. *J. Cell Sci.*, **120**, 3173–3178.
28. Vikesaa,J., Hansen,T.V., Jonson,L., Borup,R., Wewer,U.M., Christiansen,J. and Nielsen,F.C. (2006) RNA-binding IMPs promote cell adhesion and invadopodia formation. *EMBO J.*, **25**, 1456–1468.
29. Ross,A.F., Oleynikov,Y., Kislauskis,E.H., Taneja,K.L. and Singer,R.H. (1997) Characterization of a beta-actin mRNA zipcode-binding protein. *Mol. Cell Biol.*, **17**, 2158–2165.
30. Farina,K.L., Huttelmaier,S., Musunuru,K., Darnell,R. and Singer,R.H. (2003) Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. *J. Cell Biol.*, **160**, 77–87.
31. Zhang,H.L., Eom,T., Oleynikov,Y., Shenoy,S.M., Liebelt,D.A., Dictenberg,J.B., Singer,R.H. and Bassell,G.J. (2001) Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron*, **31**, 261–275.
32. Condeelis,J. and Singer,R.H. (2005) How and why does beta-actin mRNA target? *Biol. Cell*, **97**, 97–110.
33. Huttelmaier,S., Zenklusen,D., Lederer,M., Dictenberg,J., Lorenz,M., Meng,X., Bassell,G.J., Condeelis,J. and Singer,R.H. (2005) Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature*, **438**, 512–515.
34. Yao,J., Sasaki,Y., Wen,Z., Bassell,G.J. and Zheng,J.Q. (2006) An essential role for beta-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nat. Neurosci.*, **9**, 1265–1273.
35. Leung,K.M., van Horck,F.P., Lin,A.C., Allison,R., Standart,N. and Holt,C.E. (2006) Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat. Neurosci.*, **9**, 1247–1256.
36. Gu,W., Katz,Z., Wu,B., Park,H.Y., Li,D., Lin,S., Wells,A.L. and Singer,R.H. (2012) Regulation of local expression of cell adhesion and motility-related mRNAs in breast cancer cells by IMP1/ZBP1. *J. Cell Sci.*, **125**, 81–91.
37. Lambertini,E., Franceschetti,T., Torreggiani,E., Penolazzi,L., Pastore,A., Pelucchi,S., Gambari,R. and Piva,R. (2010) SLUG: a new target of lymphoid enhancer factor-1 in human osteoblasts. *BMC Mol. Biol.*, **11**, 13.
38. Stohr,N., Lederer,M., Reinke,C., Meyer,S., Hatzfeld,M., Singer,R.H. and Huttelmaier,S. (2006) ZBP1 regulates mRNA stability during cellular stress. *J. Cell Biol.*, **175**, 527–534.
39. Glaß,M., Möller,B., Zirkel,A., Wächter,K., Hüttelmaier,S. and Posch,S. (2012) Cell migration analysis: Segmenting scratch assay images with level sets and support vector machines. *Pattern Recognit.*, **45**, 3154–3165.
40. Gradl,D., Kuhl,M. and Wedlich,D. (1999) The Wnt/Wg signal transducer beta-catenin controls fibronectin expression. *Mol. Cell Biol.*, **19**, 5576–5587.
41. Nawshad,A., Medici,D., Liu,C.C. and Hay,E.D. (2007) TGFbeta3 inhibits E-cadherin gene expression in palate medial-edge epithelial cells through a Smad2-Smad4-LEF1 transcription complex. *J. Cell Sci.*, **120**, 1646–1653.
42. Hafner,M., Landthaler,M., Burger,L., Khorshid,M., Hausser,J., Berninger,P., Rothballer,A., Ascano,M. Jr, Jungkamp,A.C., Munschauer,M. *et al.* (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell*, **141**, 129–141.
43. Jesse,S., Koenig,A., Ellenrieder,V. and Menke,A. (2010) Lef-1 isoforms regulate different target genes and reduce cellular adhesion. *Int. J. Cancer*, **126**, 1109–1120.

44. Huang,F.I., Chen,Y.L., Chang,C.N., Yuan,R.H. and Jeng,Y.M. (2012) Hepatocyte growth factor activates Wnt pathway by transcriptional activation of LEF1 to facilitate tumor invasion. *Carcinogenesis*, **33**, 1142–1148.
45. Fujita,N., Jaye,D.L., Kajita,M., Geigerman,C., Moreno,C.S. and Wade,P.A. (2003) MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. *Cell*, **113**, 207–219.
46. Sakai,D., Tanaka,Y., Endo,Y., Osumi,N., Okamoto,H. and Wakamatsu,Y. (2005) Regulation of Slug transcription in embryonic ectoderm by beta-catenin-Lef/Tcf and BMP-Smad signaling. *Dev. Growth Differ.*, **47**, 471–482.
47. Tessier,C.R., Doyle,G.A., Clark,B.A., Pitot,H.C. and Ross,J. (2004) Mammary tumor induction in transgenic mice expressing an RNA-binding protein. *Cancer Res.*, **64**, 209–214.
48. Yu,W., Ruest,L.B. and Svoboda,K.K. (2009) Regulation of epithelial-mesenchymal transition in palatal fusion. *Exp. Biol. Med. (Maywood)*, **234**, 483–491.
49. Hajra,K.M., Chen,D.Y. and Fearon,E.R. (2002) The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res.*, **62**, 1613–1618.
50. Nguyen,D.X., Chiang,A.C., Zhang,X.H., Kim,J.Y., Kris,M.G., Ladanyi,M., Gerald,W.L. and Massague,J. (2009) WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. *Cell*, **138**, 51–62.
51. Shih,J.Y. and Yang,P.C. (2011) The EMT regulator slug and lung carcinogenesis. *Carcinogenesis*, **32**, 1299–1304.

Manuskript 4

Köhn M, Pazaitis N, Hüttelmaier S.

Why YRNAs? About Versatile RNAs and Their Functions.

Biomolecules
Feb 2013, 3:143-56.

Biomolecules **2013**, *3*, 143-156; doi:10.3390/biom3010143

OPEN ACCESS

biomolecules

ISSN 2218-273X

www.mdpi.com/journal/biomolecules/

Review

Why Y RNAs? About Versatile RNAs and Their Functions

Marcel Köhn, Nikolaos Pazaitis and Stefan Hüttelmaier *

Martin-Luther-University Halle-Wittenberg, Institute of Molecular Medicine, Section Molecular Cell Biology, ZAMED, Heinrich-Damerow-Str.1, D-6120 Halle, Germany;
E-Mails: marcel.koehn@medizin.uni-halle.de (M.K.) pazaitis@gmx.de (N.P.)

* Author to whom correspondence should be addressed;
E-Mail: stefan.huettelmaier@medizin.uni-halle.de; Tel.: +0049-(0)3455522860;
Fax: +0049-(0)3455522894.

Received: 31 December 2012; in revised form: 27 January 2013 / Accepted: 31 January 2013 / Published: 8 February 2013

Abstract: Y RNAs constitute a family of highly conserved small noncoding RNAs (in humans: 83-112 nt; Y1, Y3, Y4 and Y5). They are transcribed from individual genes by RNA-polymerase III and fold into conserved stem-loop-structures. Although discovered 30 years ago, insights into the cellular and physiological role of Y RNAs remains incomplete. In this review, we will discuss knowledge on the structural properties, associated proteins and discuss proposed functions of Y RNAs. We suggest Y RNAs to be an integral part of ribonucleoprotein networks within cells and could therefore have substantial influence on many different cellular processes. Putative functions of Y RNAs include small RNA quality control, DNA replication, regulation of the cellular stress response and proliferation. This suggests Y RNAs as essential regulators of cell fate and indicates future avenues of research, which will provide novel insights into the role of small noncoding RNAs in gene expression.

Keywords: Y RNA; Ro60; La; ncRNA

1. Introduction

Over recent years, our view of genomic regulation was severely revised when realizing that we have overseen a yet to be explored plethora of long and short non-coding RNAs (ncRNAs). Various reports revealed that ncRNAs are important regulators of diverse cellular processes. MicroRNAs (miRNAs), for instance, were shown to be important regulators of mRNA-fate in the cytoplasm, where they

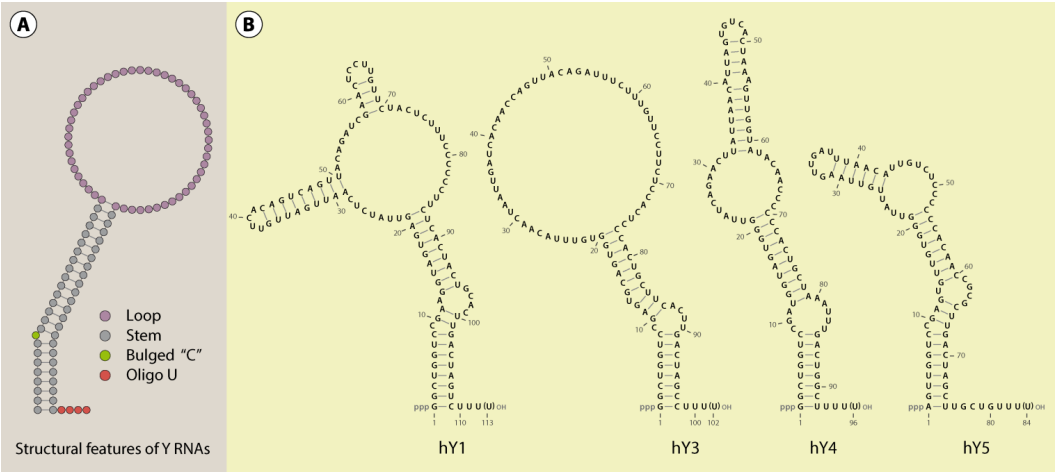
control translation and turnover of specific target transcripts (reviewed in [1]). On the other hand, long non-coding RNAs can serve as scaffolds for the assembly of subnuclear bodies (e.g., paraspeckles and NEAT1-RNA, [2]). These results reflect just two aspects of the various functions facilitated by ncRNAs, which modulate gene expression at various levels.

Y RNAs were originally identified in the 1980's by immunopurification with auto-antibodies from patients suffering from systemic lupus erythematosus [3]. The proteins Ro60 (TROVE2, SSA) and La (SSB), which are common auto-antigens in autoimmune diseases (like systemic lupus erythematosus and Sjögren's syndrome (reviewed in [4]), were identified as the major antigens facilitating the association with these small ncRNAs [5–7]. Subsequently, these cytoplasmic ncRNAs were named Y RNAs to distinguish them from nuclear U RNAs, another major class of small ncRNAs in the cell [3,8].

2. Structure and Evolution of Y RNAs

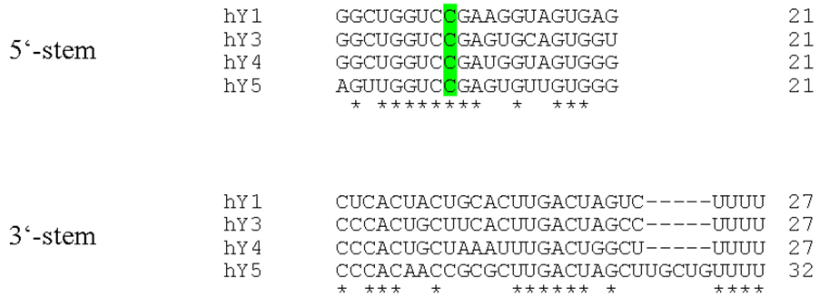
Metazoan Y RNAs are transcribed by RNA polymerase III (POLIII, [9]) from distinct promoters. Transcription is terminated at an OligoT stretch (4–6 nt), resulting in an oligo-uridinylated 3'-end of nascent Y RNA transcripts (Figures 1 and 2). This serves as the primary binding site for the La protein [10–12]. It was proposed that the OligoU stretch is removed during nuclear maturation in the nucleus to promote nuclear export of trimmed Y RNAs [13]. The trimming of POLIII-transcripts seems to occur frequently and has been observed for various other RNAs [14]. Consistent with a pivotal role of La in the 3'-end processing of Y RNAs, La-binding was shown to prohibit the transport of Y RNAs to the cytoplasm [15]. However, the molecular basis of Y RNA trimming remains poorly understood and the potentially involved nucleases are still not known. Moreover, Y RNAs were shown to possess triphosphates at their 5'-ends and not to contain large amounts of nucleotide modifications [5].

Figure 1. Y RNA structure. A schematic structure of Y RNAs illustrates the important features common to these RNAs (**A**). Furthermore, the secondary structure of the human Y RNAs (Y1, Y3, Y4 and Y5) was visualized with VARNA [17], referring to published structure probing experiments (**B**; [18,19]). According to this data, alternative structures, at least for Y3, are likely [19].



A common characteristic of all reported Y RNAs is their highly conserved stem-loop structure (Figure 1). The terminal 5'- and 3'-sequences (~20–30 nt) of Y RNAs form a double stranded region - the "stem". This region is the essential structural determinant of Y RNAs allowing there typical stem-loop fold. For some Y RNAs, these structural properties were validated by enzymatic and chemical cleavage [18,19]. The stems of Y RNAs are usually not perfect double strands. Frequently, the "upper" and "lower" parts are separated by bulged regions. One of these, a highly conserved bulged cytosine (C9 in human Y RNAs [20]), constitutes the primary binding site for the Ro60 protein (TROVE2). Deletion or mutation of this site disrupts Ro60-binding and destabilizes the entire Y RNA fold (Figure 2, [15,20,21]). Ro60-homologs and also Y RNAs have been identified from bacteria to humans, suggesting an ancient origin and co-evolution of the Ro60-Y RNA-complex [21–23]. This is supported by analyses confirming the Y RNA stem to be the most conserved part of these ncRNAs, and even the bulged cytosine base in the stem is retained in bacterial Y RNAs (Figures 1 and 2, [23,24]). Accordingly, we propose that organisms, which encode for Ro60 orthologs, presumably also express Y RNAs. Ro60 and its orthologs, as well some Y RNAs, were described or assumed in various bacterial species, lower eukaryotes, like *Chlamydomonas reinhardtii*, nematodes, like *Caenorhabditis elegans*, and vertebrates (reviewed in [21]). Putative Ro60 and Y RNA orthologs had also been suggested in arthropods, such as *Anopheles gambiae* [21,22].

Figure 2. Sequence alignments of human Y RNA stems. The terminal Y RNA stem sequences were aligned using the TCoffee web server [25,26]. Perfectly conserved nucleotides are marked with asterisks. The remarkably conserved cytosine base at position 9 within the 5'-part of the Y RNAs is highlighted in green.



RNA sequencing approaches frequently reported fragments comprising parts of Y RNAs [27,28]. In view of the conserved Y RNA structure resembling that of pre-miRNAs, it accordingly was suggested that Y RNAs could serve as miRNA precursors [28,29]. However, experimental validation of Y RNA encoded regulatory microRNAs is still lacking, and thus, the proposed Y RNA fragments could also result from degradation [30]. In support of this, it was shown that the biogenesis of some Y RNA fragments is independent of DICER1 and AGO2, providing further evidence that the identified fragments are not generated by the classical miRNA pathway [31].

The loops of Y RNAs are heterogeneous in nature and the least conserved of the ncRNAs [24,32]. The primary sequence and length of the loop distinguishes the four Y RNAs (Y1, Y3, Y4 and Y5). The longest loop is observed for Y1 (hY1: 65 nt) and the shortest for Y5 (hY5: 31 nt). The structure of the loops differs significantly among the four Y RNAs and was suggested to be largely flexible in nature [19].

The loops of Y1, Y3 and Y5 are rich in pyrimidines (human RNAs: 69 %, 65 % and 65 %, respectively); only Y1 and Y3 contain large, mostly single stranded stretches of pyrimidines. To date, in *teleostei*, only one Y RNA has been identified, which shows high homology to the human Y1/3-type of Y RNAs. Accordingly, Y3 was suggested to be the most ancestral Y RNA, at least in the vertebrate lineage [24,33]. Furthermore, additional Y RNAs possibly evolved through duplication of this ancestral RNA to fulfill novel functions within cells, which likely involves the loop region [24]. In accordance with this hypothesis, Y RNAs are able to recruit various RNA-binding proteins in a loop-dependent manner (see Table 1).

Table 1. Y RNA binding proteins.

Gene Symbol	Alternative Names	Y RNA	Binding Region	Proposed Function	Reference
SSB	La	1,3,4,5	OligoU	nuclear retention, protection of Y RNA 3'ends	[15]
TROVE2	Ro60	1,3,4,5	stem	stabilization, nuclear export, RNA quality control	[15,23,34–37]
APOBEC3G		1,3,4,5	?	?	[38−40]
NCL	nucleolin	1,3	loop	?	[41]
PTBP1	hnRNP I	1,3	loop	?	[42]
HNRNPK		1,3	loop	?	[42]
IGF2BP1	ZBP1, Imp1	(1),3	loop	nuclear Export of Ro60 and Y3	[43,44]
PUF60	RoBP1	(1,3),5	?	?	[45,46]

3. Y RNA Localization and Expression

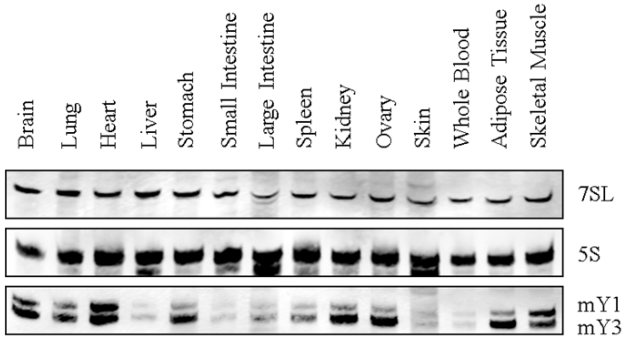
POLIII-transcripts can be transported to the cytoplasm (pre-miRNAs, tRNAs, 5S; [47,48]) or remain in the nucleus after transcription (7SK, U6; [49,50]). The localization of Y1, Y3 and Y4 was described to be mostly cytoplasmic, whereas Y5 seems to be more nuclear [51,52]. As mentioned above, the La protein is thought to interfere with nuclear export of Y RNAs by binding to their 3'-ends [15]. This export block could be released by trimming of Y RNAs, which then lack the OligoU stretch. This would trigger Ro60-dependent nuclear export of these ncRNAs [13,15]. Thus, it is tempting to speculate that Y5 differs in its association with La or nuclear trimming, allowing its nuclear retention. Nascent POLIII transcripts can accumulate in the perinucleolar compartment (PNC, [53,54]). In agreement, at least Y1, Y3 and Y5 were shown to localize to this subnuclear site by fluorescence in *in situ* hybridization (FISH, [55]). In an *in vitro* system, where labeled Y RNAs are incubated with G1 nuclei, Y RNAs were found to associate with euchromatin, and Y5 was recruited to nucleoli [56]. Notably, Y RNAs can be encapsidated into viruses, as shown for Moloney murine leukemia virus (MLV, [13]) and also for human immunodeficiency virus type 1 (HIV-1, [38]). This process is independent of Ro60-binding and seems to be initiated while Y RNAs are still in the nucleus. Whether Y RNAs modulate the lifecycle of these viruses significantly remains unknown.

The export pathways used by Y RNAs are not known in detail. It was reported that the export of Y RNAs is dependent on the small GTPase Ran, suggesting members of the karyopherin protein

family to serve as nuclear export adapters [57]. Although XPO1 and XPOT are presumably not involved, XPO5 seems to be important to direct cytoplasmic translocation of Y RNAs [15,57]. This protein usually exports minihelix containing dsRNAs, which includes VA1, some tRNAs and pre-miRNAs [58,59]. The Y RNA stem is reminiscent of a minihelix, and consistently, XPO5 was shown to associate in a complex with Y1 and RanGTP [58]. This was also supported by the crystal structure of XPO5, indicating the Y RNA stem acts as a substrate for this karyopherin [47]. Notably, there is no evidence for a re-import of Y RNAs into the nucleus. This is supported by the complete nuclear export of radiolabeled Y RNAs after injection into *Xenopus* oocytes [15,57]. Notably, the subcellular localization of Y RNAs was reported to be cell cycle-dependent and respond to cellular stress signals, like UV-irradiation [23,56,60]. Accordingly, cells accumulate both Ro60 and Y RNAs in the nucleus after UV irradiation or oxidative stress [35,44,60]. This could result from the stress-induced collapse of the Ran gradient and concomitant impairment of nuclear export [61], but may furthermore imply stress-dependent functions of the nuclear Ro60-Y RNA-complex under these conditions.

Y RNA expression has been reported in various species, including primary tissue and tumor-derived cell lines [62,63]. However, comprehensive analyses of tissue-specific Y RNA expression profiles are still lacking. Therefore, we analyzed the expression of murine Y RNAs in several adult tissues by Northern blotting (Figure 3). These studies revealed basal expression of murine Y RNAs (mY1 and mY3) in all analyzed tissues. Y RNA abundance varied significantly, with high levels observed in the brain, lung, heart, stomach, kidney, ovary, adipose tissue and skeletal muscle, in contrast to lower mY RNA abundance in the liver, gut, spleen, skin and blood. Intriguingly, the observed mY RNA expression pattern correlated to the expression signature of Ro60 [64]. This supports the view that the Y RNA-Ro60 complex co-evolved and that the protein is essential for Y RNA stabilization [34,36]. In humans, Y RNAs were shown to be significantly upregulated in a variety of tumors, for instance, bladder and kidney carcinomas. Moreover, Y RNAs promote cell proliferation, which is supported by reduced cell cycle progression upon siRNA-directed Y1 and Y3 depletion [63,65]. Little is known about the developmental expression of Y RNAs, which was analyzed exclusively in *Xenopus laevis* and *Danio rerio*, where Y RNA levels increased after the midblastula transition (MBT, [66]). Notably, the inhibition of Y RNAs by antisense morpholinos led to lethal developmental defects after MBT before gastrulation.

Figure 3. Y RNA expression in mouse tissues. A representative Northern blot for murine Y RNA tissue expression is shown. 7SL and 5S rRNA served as a loading control. Note that in mice, just Y1 and Y3 are expressed.



4. Is the Functional Role of Y RNA Determined by Associated Proteins?

4.1. Y RNP Core Proteins

All metazoan Y RNAs associate with Ro60 and La, which presumably form the core of nuclear or cytoplasmic Y RNPs (RNP: Ribonucleoprotein). As mentioned above, La binds the OligoU-stretch at the 3'-end of nascent Y RNAs [12]. On the contrary, Ro60 binds to the Y RNA stem [7]. Although La was found at the transcription site of Y RNAs, it remains to be addressed whether the protein has any influence on the transcription process itself [67]. Most likely, the protein protects nascent Y RNA transcripts from being degraded by 3'-exonucleases in the nucleus. Ro60, on the other hand, stabilizes the Y RNA structure and prevents Y RNA degradation [34,36]. In agreement, Y RNA levels are severely reduced in Ro60$^{-/-}$ cells [35]. However, the stabilizing function of Ro60 is presumed to mainly affect the cytoplasmic pool of Y RNAs, as shown for mouse fibroblasts [13]. Hence, it remains elusive whether other factors facilitate a Ro60-like role in modulating Y RNA fate in the nucleus [13,68]. Notably, the stabilization of Y RNAs by Ro60 is conserved in the bacterium *Deinococcus radiodurans*, where they act as essential facilitators of UV-resistance [23]. Additionally, the bacterial Rsr (Ro sixty related) was shown to assist in 23S rRNA maturation, and Y RNA were reported to sequester Rsr to inhibit this activity [37]. The crystal structure of Ro60-Y RNA complex revealed that Y RNAs associate with the outer surface of the HEAT-repeat-ring of Ro60, which comprises a highly conserved histidine residue (H187 in human and mouse) that is essential for direct contact formation with Y RNAs [69]. This association surface partially overlaps with the binding site for misfolded ncRNAs (like 5S), implying that Y RNAs modulate the proposed role of Ro60 in the quality surveillance of ncRNAs [60,69–71].

All four human Y RNAs associate with the antiviral cytidine deaminase APOBEC3G, which accordingly was observed in Ro60- and La-RNPs [38–40]. Mutational inactivation of the zinc-binding domain of APOBEC3G (W127A) strongly reduced its interaction with Y1 [38]. Hence, although the function of APOBEC3G-Y RNA complexes remains unknown, one could speculate that the protein facilitates C-to-U-editing to modulate Y RNA-functions and/or suppress retro-transposition of these ncRNAs [39]. Notably, retro-pseudogenes derived from Y RNAs have been described, but it is unclear if they can be re-expressed or have any other functional relevance [72]. The existence of another putative Y RNA core protein, which binds the upper Y RNA stem, is still controversial. Based on sequence requirements, this protein could be involved in nuclear export of Y RNAs and the proposed involvement of Y RNAs in DNA-replication [57,73]. *In vitro*, all four human Y RNAs were reported to associate with proteins of the pre-replication, as well as the origin recognition complex (e.g., ORC2 and CDT1, [56,66]). However, the functional composition of these Y RNPs remains to be deciphered, since the Y RNP core proteins, Ro60 and La, as well as nucleolin association, are not a prerequisite for Y RNAs to function in DNA replication [74].

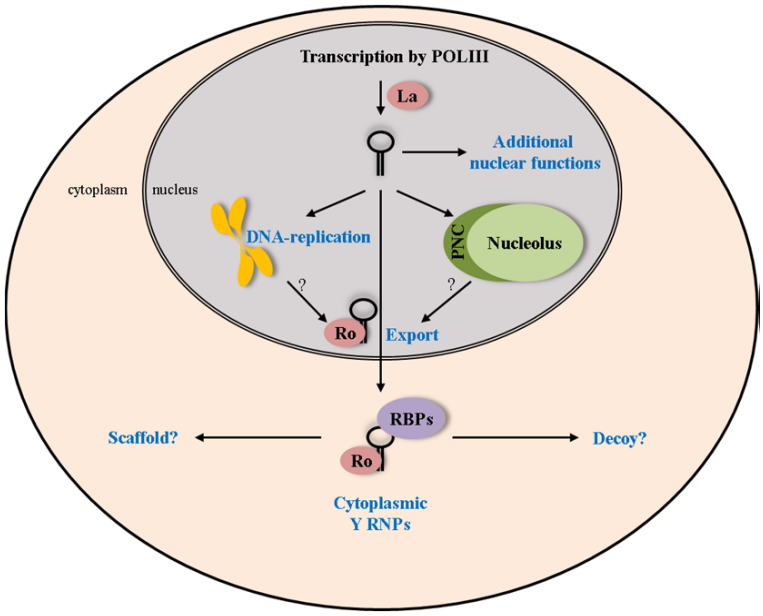
4.2. Accessory Y RNA Binding Proteins

Apart from proteins associating at the Y RNA stem, additional proteins have been shown to bind Y RNAs. Most of these associate with distinct Y RNA loops and, thus, may impose Y RNA specific functions or play a role in directing the potentially distinct lifecycle of each Y RNA (for a summary,

see Table 1 and Figure 4). Only the Y1- and Y3-RNAs contain pyrimidine-rich tracts within their loops. Accordingly, these Y RNAs associate with OligoU/C-binding proteins, including hnRNP I (PTBP1), hnRNP K (HNRNPK) and Nucleolin (NCL) [41,42]. Additionally, RoBP1 (PUF60) was shown to bind Y5 and also associates with Y1 and Y3, *in vivo* and *in vitro* [45,46]). The purification procedure used for PUF60-Y5-complexes led to the identification of RPL5 as a putative binding partner of Y5. In support of this, Y5 was shown to associate with 5S-rRNA-variants, suggesting a role of this Y RNA in the biogenesis of ribosomal RNAs [46], as demonstrated for Ro60 and La [75,76]. The identification of Ro60 in IGF2BP1 containing RNPs suggested an association of this protein with Y RNAs [77]. Recently, we and the Wolin lab could confirm this by demonstrating that mouse IGF2BP1, like its chicken ortholog ZBP1 (Zipcode binding protein), directly associates with Y3 and, to a lesser extent, with Y1 [43,44]. The depletion of IGF2BP1 resulted in the nuclear accumulation of Ro60 and Y3, suggesting an involvement of the protein in nuclear export of this Ro60-Y RNA complex [44].

Despite the various proteins described to associate with Y RNAs, the composition and properties of cellular Y RNPs remains largely elusive. Gel filtration studies indicate that Y RNPs range in size from 150–550 kDa. This suggests that one Y RNA can associate with more than one protein simultaneously. *In vitro*, we and others observed ternary complexes comprising La, Y3 and ZBP1 [43,78]. These findings suggest that Y RNPs contain at least one Y RNA, one core protein (e.g., Ro60) and one or two directly loop-associated accessory proteins (e.g., ZBP1), which could serve as binding scaffolds for additional proteins or promote oligomerization of Y RNPs (Figure 4).

Figure 4. Lifecycle of Y RNAs. The proposed cellular functions of Y RNAs rely mostly on the association with their core proteins Ro60 and La. These interactions influence various parts of the Y RNA lifecycle (e.g., nuclear export together with Ro60). Additional functions and interactions with RNA binding proteins (RBPs) have to be assumed in the nucleus, as well as the cytoplasm.



5. Future Perspectives and Conclusions

Although various Y RNA-associated proteins have been reported, the role of this highly conserved family of small ncRNAs and, in particular, the reason for their diversification remains sparse. Only two Y RNA functions have been proposed so far. During the cell cycle, they were suggested to stimulate DNA replication (Figure 4; [65,73,79]). This assumption is mainly based on *in vitro* evidence, using isolated G1-phase nuclei incubated with cellular extracts. These studies also indicated that Y RNAs are required for the establishment of new replication forks, but not for DNA elongation [79]. Although the model of Y RNAs being involved in DNA replication is intriguing, more information regarding the molecular mechanisms and *in vivo* regulation of these processes are required. However, a role of Y RNAs in DNA replication could be supported by their high conservation, as well as the devastating developmental defects and the proliferation decrease upon Y RNA inhibition [63,66]. On the other hand, Y RNAs were reported to inhibit the function of Ro60 in RNA quality control [37]. Accordingly, it was also shown that the binding site for Y RNAs in Ro60 partially overlaps with the one for misfolded RNAs, suggesting a mutually exclusive Ro60-RNP [69]. Therefore, we favor a model that Y RNAs and bound Ro60 act as cellular stress sensors. Accordingly, Ro60 can dissociate from Y RNAs under conditions, like UV-irradiation, to assist in cellular recovery by salvaging misfolded RNAs (reviewed in [21]). We propose that, in addition to Ro60, other RBPs could be sequestered by Y RNAs in a similar fashion. This is in accordance with the proposed functions of long ncRNAs, which can act as decoys and/or scaffolds to regulate gene expression [80]. A putative scaffolding role could provide both a sequestering of these regulators acting like a "molecular sink" or a chaperoning function to expedite function of the associated proteins [80]. We thus expect that Y RNAs modulate additional regulatory processes controlling the fate of mRNAs, which could include RNA processing, as well as cytoplasmic regulation of gene expression. The latter assumption is supported by the observation that Y RNAs are predominantly cytoplasmic at steady state. Surprisingly, most of the proposed roles of Y RNAs involve nuclear functions like DNA-replication or small RNA quality control. Future work will thus have to address the compartment-specific function of Y RNPs, which we expect to reveal that these small ncRNAs serve functions in modulating cytoplasmic mRNA fate. This role could essentially rely on the capability of Y RNAs to associate with various RBPs observed in mRNPs, for instance, IGF2BP1 (reviewed in [81]). By controlling the accessibility of such factors, Y RNAs could modulate the function of these regulatory RBPs in mRNA turnover, translation and, potentially, mRNA localization at regular or stress conditions. In addition to revealing the cellular role of Y RNAs, future studies also have to address the physiological significance of these small ncRNAs by the use of genetic models to address their function in development and diseases.

6. Materials and Methods: Isolation of Total RNA and Northern Blot

Tissues were isolated from athymic Nude-Foxn1^{nu}-mice (Harlan) and rapidly mixed with TRIZOL-reagent (Life Technologies). RNA-extraction was then performed using chloroform and precipitation with isopropanol. For Northern Blotting, 2.5 µg of total RNA was resolved on a 15% denaturing TBE-Urea-gel and subsequently blotted onto nylon membranes (Roche). The membranes were then

UV-crosslinked (Stratalinker 2400) and pre-hybridized with PerfectHyb Plus (Sigma-Aldrich). Northern probes (Atto680 or DY-782 label) were diluted to 100 ng/µL in PerfectHyb Plus and hybridized at 30 °C for 2 hours. Detection was conducted using the Odyssey Scanner (LI-COR). Northern Probes: 7SL: GGCATAGCGCACTACAGCCCAGAACTCCTG; Y1: ATAACTCACTACCTTCGGACCAGCC; Y3: CTGTAACTGGTTGTGATCAATTAGT; and 5S: AAGTACTAACCAGGCCCGAC.

Acknowledgments

This work was funded by the SFB 610 (DFG) to Stefan Hüttelmaier.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Krol, J.; Loedige, I.; Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* **2010**, *11*, 597–610.
2. Bond, C.S.; Fox, A.H. Paraspeckles: nuclear bodies built on long noncoding RNA. *J. Cell Biol.* **2009**, *186*, 637–644.
3. Lerner, M.R.; Boyle, J.A.; Hardin, J.A.; Steitz, J.A. Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **1981**, *211*, 400–402.
4. Franceschini, F.; Cavazzana, I. Anti-Ro/SSA and La/SSB antibodies. *Autoimmunity* **2005**, *38*, 55–63.
5. Hendrick, J.P.; Wolin, S.L.; Rinke, J.; Lerner, M.R.; Steitz, J.A. Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. *Mol. Cell. Biol.* **1981**, *1*, 1138–1149.
6. Francoeur, A.M.; Mathews, M.B. Interaction between VA RNA and the lupus antigen La: formation of a ribonucleoprotein particle in vitro. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 6772–6776.
7. Wolin, S.L.; Steitz, J.A. The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 1996–2000.
8. Will, C.L.; Lührmann, R. Splicing of a rare class of introns by the U12-dependent spliceosome. *Biol. Chem.* **2005**, *386*, 713–724.
9. Wolin, S.L.; Steitz, J.A. Genes for two small cytoplasmic Ro RNAs are adjacent and appear to be single-copy in the human genome. *Cell* **1983**, *32*, 735–744.
10. Stefano, J.E. Purified lupus antigen La recognizes an oligouridylate stretch common to the 3' termini of RNA polymerase III transcripts. *Cell* **1984**, *36*, 145–154.
11. Mathews, M.B.; Francoeur, A.M. La antigen recognizes and binds to the 3'-oligouridylate tail of a small RNA. *Mol. Cell. Biol.* **1984**, *4*, 1134–1140.
12. Pruijn, G.J.; Slobbe, R.L.; van Venrooij, W.J. Analysis of protein--RNA interactions within Ro ribonucleoprotein complexes. *Nucleic Acids Res.* **1991**, *19*, 5173–5180.

13. Garcia, E.L.; Onafuwa-Nuga, A.; Sim, S.; King, S.R.; Wolin, S.L.; Telesnitsky, A. Packaging of host mY RNAs by murine leukemia virus may occur early in Y RNA biogenesis. *J. Virol.* **2009**, *83*, 12526–12534.
14. van Hoof, A.; Lennertz, P.; Parker, R. Three conserved members of the RNase D family have unique and overlapping functions in the processing of 5S, 5.8S, U4, U5, RNase MRP and RNase P RNAs in yeast. *EMBO J.* **2000**, *19*, 1357–1365.
15. Simons, F.H.; Rutjes, S.A.; van Venrooij, W.J.; Pruijn, G.J. The interactions with Ro60 and La differentially affect nuclear export of hY1 RNA. *RNA* **1996**, *2*, 264–273.
16. Gruber, A.R.; Lorenz, R.; Bernhart, S.H.; Neuböck, R.; Hofacker, I.L. The Vienna RNA websuite. *Nucleic Acids Res.* **2008**, *36*, W70-4.
17. Darty, K.; Denise, A.; Ponty, Y. VARNA: Interactive drawing and editing of the RNA secondary structure. *Bioinformatics* **2009**, *25*, 1974–1975.
18. van Gelder, C.W.; Thijssen, J.P.; Klaassen, E.C.; Sturchler, C.; Krol, A.; van Venrooij, W.J.; Pruijn, G.J. Common structural features of the Ro RNP associated hY1 and hY5 RNAs. *Nucleic Acids Res.* **1994**, *22*, 2498–2506.
19. Teunissen, S.W.; Kruithof, M.J.; Farris, A.D.; Harley, J.B.; Venrooij, W.J.; Pruijn, G.J. Conserved features of Y RNAs: a comparison of experimentally derived secondary structures. *Nucleic Acids Res.* **2000**, *28*, 610–619.
20. Green, C.D.; Long, K.S.; Shi, H.; Wolin, S.L. Binding of the 60-kDa Ro autoantigen to Y RNAs: evidence for recognition in the major groove of a conserved helix. *RNA* **1998**, *4*, 750–765.
21. Sim, S.; Wolin, S.L. Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. *Wiley Interdiscip. Rev. RNA* **2011**, *2*, 686–699.
22. Perreault, J.; Perreault, J.-P.; Boire, G. Ro-associated Y RNAs in metazoans: evolution and diversification. *Mol. Biol. Evol.* **2007**, *24*, 1678–1689.
23. Chen, X.; Quinn, A.M.; Wolin, S.L. Ro ribonucleoproteins contribute to the resistance of Deinococcus radiodurans to ultraviolet irradiation. *Genes Dev.* **2000**, *14*, 777–782.
24. Mosig, A.; Guofeng, M.; Stadler, B.M.R.; Stadler, P.F. Evolution of the vertebrate Y RNA cluster. *Theory Biosci.* **2007**, *126*, 9–14.
25. Poirot, O.; O'Toole, E.; Notredame, C. Tcoffee@igs: A web server for computing, evaluating and combining multiple sequence alignments. *Nucleic Acids Res.* **2003**, *31*, 3503–3506.
26. Notredame, C.; Higgins, D.G.; Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **2000**, *302*, 205–217.
27. Liao, J.-Y.; Ma, L.-M.; Guo, Y.-H.; Zhang, Y.-C.; Zhou, H.; Shao, P.; Chen, Y.-Q.; Qu, L.-H. Deep sequencing of human nuclear and cytoplasmic small RNAs reveals an unexpectedly complex subcellular distribution of miRNAs and tRNA 3' trailers. *PLoS ONE* **2010**, *5*, e10563.
28. Meiri, E.; Levy, A.; Benjamin, H.; Ben-David, M.; Cohen, L.; Dov, A.; Dromi, N.; Elyakim, E.; Yerushalmi, N.; Zion, O.*; et al.* Discovery of microRNAs and other small RNAs in solid tumors. *Nucleic Acids Res.* **2010**, *38*, 6234–6246.
29. Verhagen, A.P.M.; Pruijn, G.J.M. Are the Ro RNP-associated Y RNAs concealing microRNAs? Y RNA-derived miRNAs may be involved in autoimmunity. *Bioessays* **2011**, *33*, 674–682.

30. Rutjes, S.A.; van der Heijden, A.; Utz, P.J.; van Venrooij, W.J.; Pruijn, G.J. Rapid nucleolytic degradation of the small cytoplasmic Y RNAs during apoptosis. *J. Biol. Chem.* **1999**, *274*, 24799–24807.
31. Nicolas, F.E.; Hall, A.E.; Csorba, T.; Turnbull, C.; Dalmay, T. Biogenesis of Y RNA-derived small RNAs is independent of the microRNA pathway. *FEBS Lett.* **2012**, *586*, 1226–1230.
32. Farris, A.D.; Koelsch, G.; Pruijn, G.J.; van Venrooij, W.J.; Harley, J.B. Conserved features of Y RNAs revealed by automated phylogenetic secondary structure analysis. *Nucleic Acids Res.* **1999**, *27*, 1070–1078.
33. Farris, A.D.; O'Brien, C.A.; Harley, J.B. Y3 is the most conserved small RNA component of Ro ribonucleoprotein complexes in vertebrate species. *Gene* **1995**, *154*, 193–198.
34. Xue, D.; Shi, H.; Smith, J.D.; Chen, X.; Noe, D.A.; Cedervall, T.; Yang, D.D.; Eynon, E.; Brash, D.E.; Kashgarian, M.*; et al.* A lupus-like syndrome develops in mice lacking the Ro 60-kDa protein, a major lupus autoantigen. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7503–7508.
35. Sim, S.; Weinberg, D.E.; Fuchs, G.; Choi, K.; Chung, J.; Wolin, S.L. The subcellular distribution of an RNA quality control protein, the Ro autoantigen, is regulated by noncoding Y RNA binding. *Mol. Biol. Cell* **2009**, *20*, 1555–1564.
36. Labbé, J.C.; Hekimi, S.; Rokeach, L.A. The levels of the RoRNP-associated Y RNA are dependent upon the presence of ROP-1, the Caenorhabditis elegans Ro60 protein. *Genetics* **1999**, *151*, 143–150.
37. Chen, X.; Wurtmann, E.J.; van Batavia, J.; Zybailov, B.; Washburn, M.P.; Wolin, S.L. An ortholog of the Ro autoantigen functions in 23S rRNA maturation in D. radiodurans. *Genes Dev.* **2007**, *21*, 1328–1339.
38. Wang, T.; Tian, C.; Zhang, W.; Luo, K.; Sarkis, P.T.N.; Yu, L.; Liu, B.; Yu, Y.; Yu, X.-F. 7SL RNA mediates virion packaging of the antiviral cytidine deaminase APOBEC3G. *J. Virol.* **2007**, *81*, 13112–13124.
39. Chiu, Y.-L.; Witkowska, H.E.; Hall, S.C.; Santiago, M.; Soros, V.B.; Esnault, C.; Heidmann, T.; Greene, W.C. High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15588–15593.
40. Gallois-Montbrun, S.; Holmes, R.K.; Swanson, C.M.; Fernández-Ocaña, M.; Byers, H.L.; Ward, M.A.; Malim, M.H. Comparison of cellular ribonucleoprotein complexes associated with the APOBEC3F and APOBEC3G antiviral proteins. *J. Virol.* **2008**, *82*, 5636–5642.
41. Fouraux, M.A.; Bouvet, P.; Verkaart, S.; van Venrooij, W.J.; Pruijn, G.J.M. Nucleolin associates with a subset of the human Ro ribonucleoprotein complexes. *J. Mol. Biol.* **2002**, *320*, 475–488.
42. Fabini, G.; Raijmakers, R.; Hayer, S.; Fouraux, M.A.; Pruijn, G.J.; Steiner, G. The heterogeneous nuclear ribonucleoproteins I and K interact with a subset of the ro ribonucleoprotein-associated Y RNAs in vitro and in vivo. *J. Biol. Chem.* **2001**, *276*, 20711–20718.
43. Köhn, M.; Lederer, M.; Wächter, K.; Hüttelmaier, S. Near-infrared (NIR) dye-labeled RNAs identify binding of ZBP1 to the noncoding Y3-RNA. *RNA* **2010**, *16*, 1420–1428.
44. Sim, S.; Yao, J.; Weinberg, D.E.; Niessen, S.; Yates, J.R.; Wolin, S.L. The zipcode-binding protein ZBP1 influences the subcellular location of the Ro 60-kDa autoantigen and the noncoding Y3 RNA. *RNA* **2012**, *18*, 100–110.

45. Bouffard, P.; Barbar, E.; Brière, F.; Boire, G. Interaction cloning and characterization of RoBPI, a novel protein binding to human Ro ribonucleoproteins. *RNA* **2000**, *6*, 66–78.
46. Hogg, J.R.; Collins, K. Human Y5 RNA specializes a Ro ribonucleoprotein for 5S ribosomal RNA quality control. *Genes Dev.* **2007**, *21*, 3067–3072.
47. Okada, C.; Yamashita, E.; Lee, S.J.; Shibata, S.; Katahira, J.; Nakagawa, A.; Yoneda, Y.; Tsukihara, T. A high-resolution structure of the pre-microRNA nuclear export machinery. *Science* **2009**, *326*, 1275–1279.
48. Murdoch, K.; Loop, S.; Rudt, F.; Pieler, T. Nuclear export of 5S rRNA-containing ribonucleoprotein complexes requires CRM1 and the RanGTPase cycle. *Eur. J. Cell Biol.* **2002**, *81*, 549–556.
49. Peterlin, B.M.; Brogie, J.E.; Price, D.H. 7SK snRNA: a noncoding RNA that plays a major role in regulating eukaryotic transcription. *Wiley Interdiscip. Rev. RNA* **2012**, *3*, 92–103.
50. Spiller, M.P.; Boon, K.-L.; Reijns, M.A.M.; Beggs, J.D. The Lsm2-8 complex determines nuclear localization of the spliceosomal U6 snRNA. *Nucleic Acids Res.* **2007**, *35*, 923–929.
51. Gendron, M.; Roberge, D.; Boire, G. Heterogeneity of human Ro ribonucleoproteins (RNPS): nuclear retention of Ro RNPS containing the human hY5 RNA in human and mouse cells. *Clin. Exp. Immunol.* **2001**, *125*, 162–168.
52. Farris, A.D.; Puvion-Dutilleul, F.; Puvion, E.; Harley, J.B.; Lee, L.A. The ultrastructural localization of 60-kDa Ro protein and human cytoplasmic RNAs: association with novel electron-dense bodies. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3040–3045.
53. Wang, C.; Politz, J.C.; Pederson, T.; Huang, S. RNA polymerase III transcripts and the PTB protein are essential for the integrity of the perinucleolar compartment. *Mol. Biol. Cell* **2003**, *14*, 2425–2435.
54. Pollock, C.; Huang, S. The perinucleolar compartment. *J. Cell. Biochem.* **2009**, *107*, 189–193.
55. Matera, A.G.; Frey, M.R.; Margelot, K.; Wolin, S.L. A perinucleolar compartment contains several RNA polymerase III transcripts as well as the polypyrimidine tract-binding protein, hnRNP I. *J. Cell Biol.* **1995**, *129*, 1181–1193.
56. Zhang, A.T.; Langley, A.R.; Christov, C.P.; Kheir, E.; Shafee, T.; Gardiner, T.J.; Krude, T. Dynamic interaction of Y RNAs with chromatin and initiation proteins during human DNA replication. *J. Cell. Sci.* **2011**, *124*, 2058–2069.
57. Rutjes, S.A.; Lund, E.; van der Heijden, A.; Grimm, C.; van Venrooij, W.J.; Pruijn, G.J. Identification of a novel cis-acting RNA element involved in nuclear export of hY RNAs. *RNA* **2001**, *7*, 741–752.
58. Gwizdek, C.; Ossareh-Nazari, B.; Brownawell, A.M.; Doglio, A.; Bertrand, E.; Macara, I.G.; Dargemont, C. Exportin-5 mediates nuclear export of minihelix-containing RNAs. *J. Biol. Chem.* **2003**, *278*, 5505–5508.
59. Calado, A.; Treichel, N.; Müller, E.-C.; Otto, A.; Kutay, U. Exportin-5-mediated nuclear export of eukaryotic elongation factor 1A and tRNA. *EMBO J.* **2002**, *21*, 6216–6224.
60. Chen, X.; Smith, J.D.; Shi, H.; Yang, D.D.; Flavell, R.A.; Wolin, S.L. The Ro autoantigen binds misfolded U2 small nuclear RNAs and assists mammalian cell survival after UV irradiation. *Curr. Biol.* **2003**, *13*, 2206–2211.

61. Miyamoto, Y.; Saiwaki, T.; Yamashita, J.; Yasuda, Y.; Kotera, I.; Shibata, S.; Shigeta, M.; Hiraoka, Y.; Haraguchi, T.; Yoneda, Y. Cellular stresses induce the nuclear accumulation of importin alpha and cause a conventional nuclear import block. *J. Cell Biol.* **2004**, *165*, 617–623.
62. Pruijn, G.J.; Wingens, P.A.; Peters, S.L.; Thijssen, J.P.; van Venrooij, W.J. Ro RNP associated Y RNAs are highly conserved among mammals. *Biochim. Biophys. Acta* **1993**, *1216*, 395–401.
63. Christov, C.P.; Trivier, E.; Krude, T. Noncoding human Y RNAs are overexpressed in tumours and required for cell proliferation. *Br. J. Cancer* **2008**, *98*, 981–988.
64. Wang, D.; Buyon, J.P.; Chan, E.K. Cloning and expression of mouse 60 kDa ribonucleoprotein SS-A/Ro. *Mol. Biol. Rep.* **1996**, *23*, 205–210.
65. Christov, C.P.; Gardiner, T.J.; Szüts, D.; Krude, T. Functional requirement of noncoding Y RNAs for human chromosomal DNA replication. *Mol. Cell. Biol.* **2006**, *26*, 6993–7004.
66. Collart, C.; Christov, C.P.; Smith, J.C.; Krude, T. The midblastula transition defines the onset of Y RNA-dependent DNA replication in Xenopus laevis. *Mol. Cell. Biol.* **2011**, *31*, 3857–3870.
67. Fairley, J.A.; Kantidakis, T.; Kenneth, N.S.; Intine, R.V.; Maraia, R.J.; White, R.J. Human La is found at RNA polymerase III-transcribed genes in vivo. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18350–18355.
68. Simons, F.H.; Pruijn, G.J.; van Venrooij, W.J. Analysis of the intracellular localization and assembly of Ro ribonucleoprotein particles by microinjection into Xenopus laevis oocytes. *J. Cell Biol.* **1994**, *125*, 981–988.
69. Stein, A.J.; Fuchs, G.; Fu, C.; Wolin, S.L.; Reinisch, K.M. Structural insights into RNA quality control: the Ro autoantigen binds misfolded RNAs via its central cavity. *Cell* **2005**, *121*, 529–539.
70. Hogg, J.R.; Collins, K. Structured non-coding RNAs and the RNP Renaissance. *Curr. Opin. Chem. Biol.* **2008**, *12*, 684–689.
71. Macrae, I.J.; Doudna, J.A. Ro's role in RNA reconnaissance. *Cell* **2005**, *121*, 495–496.
72. Perreault, J.; Noël, J.-F.; Brière, F.; Cousineau, B.; Lucier, J.-F.; Perreault, J.-P.; Boire, G. Retropseudogenes derived from the human Ro/SS-A autoantigen-associated hY RNAs. *Nucleic Acids Res.* **2005**, *33*, 2032–2041.
73. Gardiner, T.J.; Christov, C.P.; Langley, A.R.; Krude, T. A conserved motif of vertebrate Y RNAs essential for chromosomal DNA replication. *RNA* **2009**, *15*, 1375–1385.
74. Langley, A.R.; Chambers, H.; Christov, C.P.; Krude, T. Ribonucleoprotein particles containing non-coding Y RNAs, Ro60, La and nucleolin are not required for Y RNA function in DNA replication. *PLoS ONE* **2010**, *5*, e13673.
75. Shi, H.; O'Brien, C.A.; van Horn, D.J.; Wolin, S.L. A misfolded form of 5S rRNA is complexed with the Ro and La autoantigens. *RNA* **1996**, *2*, 769–784.
76. O'Brien, C.A.; Wolin, S.L. A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5S rRNA precursors. *Genes Dev.* **1994**, *8*, 2891–2903.
77. Jønson, L.; Vikesaa, J.; Krogh, A.; Nielsen, L.K.; Hansen, T.v.; Borup, R.; Johnsen, A.H.; Christiansen, J.; Nielsen, F.C. Molecular composition of IMP1 ribonucleoprotein granules. *Mol. Cell Proteomics* **2007**, *6*, 798–811.
78. Fabini, G.; Rutjes, S.A.; Zimmermann, C.; Pruijn, G.J.; Steiner, G. Analysis of the molecular composition of Ro ribonucleoprotein complexes. Identification of novel Y RNA-binding proteins. *Eur. J. Biochem.* **2000**, *267*, 2778–2789.

79. Krude, T.; Christov, C.P.; Hyrien, O.; Marheineke, K. Y RNA functions at the initiation step of mammalian chromosomal DNA replication. *J. Cell. Sci.* **2009**, *122*, 2836–2845.
80. Wang, K.C.; Chang, H.Y. Molecular mechanisms of long noncoding RNAs. *Mol. Cell* **2011**, *43*, 904–914.
81. Bell, J.L.; Wächter, K.; Mühleck, B.; Pazaitis, N.; Köhn, M.; Lederer, M.; Hüttelmaier, S. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell. Mol. Life Sci.* **2012**, doi.: 10.1007/s00018-012-1186-z.

Anlagen

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
GASTROINTESTINALES SYSTEM & ANHANGSDRÜSEN								
Kolorektales CA	Ross 2001 [134]	WB und/ oder sRT-PCR		81 %	(17/21)	-	-	-
	Dimitriadis 2007 [135]	IHC		50 %	(5/10)	x	x	x
		sRT-PCR		59 %	(46/78)			
	Gu 2004 [133]	sRT-PCR		100 %	(2/2)	-	-	-
	Mongroo 2011 [136]	IHC (TMA)		-	(+/228)	-	-	-
	Vainer 2008* [150]	IHC (TMA)	*primarius*	> 60 %	(+/46)	-	-	x
			nur pT3 Fälle	97 %	(58/60)			
			LK-Met.	87 %	(73/84)			
		IHC (Einzel-schnitte)	*primarius*	-	(+/25)			
Pankreas CA	Müller-Pillasch 1997 [144]	NB		33 %	(5/15)	-	-	-
	Gu 2004 [133]	sRT-PCR		100 %	(1/1)	-	-	-
Hepatozelluläres CA	Zhou 2014 [84]	IHC		61 %	(71/116)	x	x	x
	Gutschner 2014 [79]	MA		-	(+/60)	-	-	x
		qRT-PCR		-	(+/60)			
		IHC (TMA)		-	(+/101)			
	Himoto 2005a [145]	IHC		29 %	(2/7)	-	-	-
	Himoto 2005b [146]							
	Zhang 2015 [88]	qRT-PCR		-	(+/70)	x	-	-
Oesophageal. Adeno-CA	Lin 2017 [139]	RNA-Seq. (In silico)		-	(+/80)	x	-	-

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
RESPIRATORISCHES SYSTEM								
Lungen-CA	Gu 2004 [133]	sRT-PCR	-	100 %	(2/2)	-	-	-
	Vainer 2008* [150]	IHC (TMA)	-	> 60 %	(+/45)	-	-	-
	Ioannidis 2004 [137]	sRT-PCR	NSCL	27 %	(4/15)	-	-	-
			PE-CA	38 %	(3/8)			
			Adeno-CA	17 %	(1/6)			
			großzelliges CA	0 %	(0/1)			
	Kato 2007 [138]	IHC (TMA)	NSCL	52 %	(139/267)	x	-	x
			PE-CA	36 %	(57/157)			
			Adeno-CA	75 %	(70/93)			
			großzelliges CA	77 %	(10/13)			
			adenosquamöses CA	50 %	(2/4)			
	Lin 2017 [139]	RNA-Seq. (*in silico*)	Adeno-CA	-	(+/533)	x	-	-
MÄNNLICHES REPRODUKTIVES SYSTEM								
Prostata-CA	Gu 2004 [133]	sRT-PCR	-	100 %	(1/1)	-	-	-
	Vainer 2008* [150]	IHC (TMA)	-	5 %	(+/43)	-	-	-
Hodentumoren	Hammer 2015 [109]	IHC	TIN (CIS)	100 %	(9/9)	-	-	-
			Seminom	100 %	(5/5)			
			Spermatozytisches Seminom	100 %	(1/1)			
			embryonales CA	100 %	(7/7)			
			Teratom	88 %	(7/8)			
			Leydig-Zell-Tumor	50 %	(1/2)			

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
WEIBLICHES REPRODUKTIVES SYSTEM								
Ovarial-CA	Gu 2004 [133]	sRT-PCR	Adenom	-	(+/8)	x	-	x
			borderline-Tumor	-	(+/5)			
			Adenokarzinom	-	(+/46)			
	Köbel 2007 [43]	IHC	-	69 %	(73/106)	x	x	x
	Vainer 2008* [150]	IHC (TMA)	-	> 60 %	(k.A./35)	-	-	-
	Boyerinas 2012 [140]	IHC	-	-	(+/20)	x	-	-
	Busch 2016 [47]	sRT-PCR	-	-	(+/29)	-	-	x
		qRT-PCR	-	-	(+/29)			
		MA (*in silico*)	-	-	(+/285)			
		MA (*in silico*)	-	-	(+/90)			
	Müller 2018 [48]	MA (*in silico*)	seröses	-	(+/1232)	x	x	-
Mamma-CA	Ioannidis 2003 [141]	sRT-PCR	-	59 %	(69/118)	-	-	x
	Gu 2004 [133]	sRT-PCR	-	100 %	(1/1)	-	-	-
	Doyle 2000 [132]	FISH	IDC	38 %	(12/32)	-	-	x
			ILC	0 %	(0/5)			
			DCIS & medulläres CA	33 %	(1/3)			
	Fakhraldeen 2015 [142]	IHC (TMA)	-	100 %	(243/243)	-	-	-
	Vainer 2008* [150]	IHC (TMA)	-	18 %	(+/54)	-	-	-
	Gu 2009 [72]	ISH (TMA)	*primarius*	48 %	(24/50)	-	-	-
		cDNA-Array		100 %	(4/4)			
		ISH (TMA)	Metastasen selber Patientinnen	20 %	(10/50)			
		cDNA-Array		100 %	(4/4)			
Zervix-CA	Su 2016 [90]	IHC (qRT-PCR)	-	81 %	(17/21)	-	-	-

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
ZENTRALES/ PERIPHERES NERVENSYSTEM & MENINGEN								
neuroepitheliale Tumore (maligne)	Ioannidis 2004 [137]	sRT-PCR	Glioblastom	55 %	(6/11)	-	-	-
			anaplastisches Oligodendrogliom	100 %	(2/2)			
			Astrozytom	14 %	(1/7)			
			Medulloblastom	50 %	(1/2)			
			Subependymom	100 %	(2/2)			
neuroepitheliale Tumore (benigne)	Ioannidis 2004 [137]	sRT-PCR	Schwannom	0 %	(0/2)	-	-	-
			Vestibularisschwannom	100 %	(2/2)			
			zentrales Neurozytom	0 %	(0/1)			
Meningeom	Ioannidis 2004 [137]	sRT-PCR	WHO Grad I	61 %	(11/18)	-	-	-
			WHO Grad II	100 %	(3/3)			
			WHO Grad III	0 %	(0/1)			
Hirntumor	Vainer 2008* [150]	IHC (TMA)	-	> 30 %	(+/21)	-	-	-
Glioblastom	Wang 2015 [85]	WB & qRT-PCR	-	100 %	(6/6)	-	-	-
Neuroblastom	Bell 2015 [143]	MA (*in silico*)	-	77-100 %	(+/88)	x	x	x
		MA (*in silico*)	-	77-100 %	(+/476)			
		qRT-PCR (CNV)	-	84 %	(58/69)			
		MA (*in silico*)	-	23 %	(78/341)			
		WB	-	-	(+/69)			

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
HÄMATO- & LYMPHOPOIETISCHES SYSTEM								
B-Zell-Lymphome	Natkunam 2007* [112]	IHC (TMA & Einzel-schnitte)	follikuläres Lymphom	76 %	(126/165)	-	-	-
			Grad 1	71 %	(30/42)			
			Grad 2	85 %	(45/53)			
			Grad 3	73 %	(51/71)			
			diffuses großzelliges B-Zell-Lymphom	78 %	(155/200)			
			primäres mediastinales (thymisches) großzelliges B-Zell-Lymphom	90 %	(9/10)			
			BURKITT-Lymphom	100 %	(2/2)			
			extranodales Marginalzonen-Lymphom	8 %	(2/25)			
			splenisches Marginalzonen-Lymphom	20 %	(1/5)			
			nodales Marginalzonen-Lymphom	20 %	(1/5)			
			Mantelzell-Lymphom	11 %	(2/18)			
			kleinzelliges B-Zell-Lymphom/ CLL	8 %	(3/38)			
			lymphoplasmo-zytisches-Lymphom	0 %	(0/5)			
			Haarzellleukämie	70 %	(7/10)			
			B-Vorläufer-ALL	25 %	(4/13)			
T-Zell-Lymphome	Natkunam 2007* [112]	IHC (TMA & Einzel-schnitte)	T-Vorläufer-ALL	29 %	(4/14)	-	-	-
			peripheres T-Zell-Lymphom	14 %	(3/21)			
			großzelliges anaplastisches Lymphom	75 %	(6/8)			
			NK-Zell-Lymphom	2 %	(2/91)			

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
HÄMATO- & LYMPHOPOIETISCHES SYSTEM (*Fortsetzung*)								
Plasmazell-neoplasie	Natkunam 2007* [112]	IHC (TMA & Einzel-schnitte)	multiples Myelom	5 %	(7/153)	-	-	-
			Plasmezellleukämie	0 %	(0/13)			
			monoklonale Gammopathie unklarer Signifikanz	0 %	(0/8)			
HODGKIN-**Lymphome**	Natkunam 2007* [112]	IHC (TMA & Einzel-schnitte)	lymphozytenprädo-minantes HL	92 %	(12/13)	-	-	-
			klassisches HL	94 %	(101/108)			
			noduläre Sklerose	96 %	(82/85)			
			gemischtzellige Form	83 %	(19/23)			
myeloische Leukämien	Natkunam 2007* [112]	IHC (TMA & Einzel-schnitte)	akute myeloische Leukämie	100 %	(10/10)	-	-	-
			chronische myeloische Leukämie	100 %	(1/1)			
testikulärer B-Zell-Tumor	Hammer 2005 [109]	IHC	-	0 %	(0/1)	-	-	-
B-Vorläufer-ALL	Stoskus 2011 [149]	qRT-PCR	-	-	(+/96)	-	-	x
HAUT								
malignes Melanom	Elcheva 2008 [148]	IHC	-	34 %	(13/38)	-	-	-
	Vainer 2008* [150]	IHC (TMA)	-	> 40 %	(+/16)	-	-	-
Basalzell-CA	Noubissi 2014 [147]	qRT-PCR	superfizielles	96 %	(22/23)	-	-	x

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
Bewegungsapparat & Weichgewebe								
Weichgewebstumore/ Sarkome	Ioannidis 2001 [93]	sRT-PCR	Kaposi-Sarkom	0 %	(0/1)	-	-	-
			Chondrosarkom	0 %	(0/2)			
			Ewing-Sarkom	100 %	(14/14)			
			malignes fibröses Histiozytom	50 %	(1/2)			
			Liposarkom	33 %	(1/3)			
			Synovialsarkom	50 %	(1/2)			
			aneurysmatische Knochenzyste	100 %	(1/1)			
			desmoid-Tumor	100 %	(1/1)			
			osteofibröse Dysplasie	0 %	(0/1)			
			Fibrolipom	0 %	(0/1)			
			Fibrom	100 %	(1/1)			
			Osteoklastom	0 %	(0/2)			
			Lipom	33 %	(1/3)			
Osteo-sarkom	Ioannidis 2001 [93]	sRT-PCR	-	75 %	(6/8)	-	-	-
	Qu 2016 [89]	qRT-PCR (WB)	-	-	(+/40)	-	-	-

Legende mit Erläuterungen der Abkürzungen befinden sich im Tabellenfuß auf S. 121

Tabelle A1 wird auf der nachfolgenden Seite fortgesetzt

Fortsetzung Tabelle A1

Tabelle A1 | Expression von IGF2BP1 in benignen und malignen Neoplasien und präkanzerösen Läsionen

Ursprung/ Entität/ Lokalisation	Referenz	Nachweis-methode	Subentität/ Probenart[1]	Anteil positiver Fälle ‚x'		ungünstige Korrelation mit		
				in %	an allen Fällen ‚n' (n/x)	OS	EFS	KPP
BEWEGUNGSAPPARAT & WEICHGEWEBE (*Fortsetzung*)								
Rhabdomyosarkom	Ioannidis 2001 [93]	sRT-PCR	-	100 %	(1/1)	-	-	-
	Faye 2015 [52]	WB	-	75 %	(3/4)	-	-	-
		IHC	-	75 %	(6/8)			

[1] ≙ wie in Referenz bezeichnet
* ≙ Verwendung eines PanIGF2BP1-Antikörpers
\+ ≙ keine Angaben absoluter Werte (positive Fälle enthalten)
\- ≙ keine Angaben/ keine Angaben relativer Werte
ALL ≙ akute lymphatische Leukämie
CA ≙ Karzinom
CNV ≙ Kopienzahlerhöhung
DCIS ≙ duktales *Carcinoma in situ*
EFS ≙ *event-free survival*
ERBB2 ≙ *erb-b2 receptor tyrosine kinase 2*
FIGO ≙ *Fédération Internationale de Gynécologie et d'Obstétrique*
FISH ≙ Fluoreszens *in situ*-Hybridisierung
HCC ≙ hepatozelluläres Karzinom
HL ≙ HODGKIN Lymphom
IDC ≙ invasives duktales Karzinom
IHC ≙ Immunohistochemie
ILC ≙ invasives lobuläres Karzinom
ISH ≙ *in situ*-Hybridisierung
INRG ≙ *the international neuroblastoma risk group*
KPP ≙ klinisch-pathologische Parameter (wie in Referenz angegeben werden hier subsumiert T-, N- und M-Stadium [aus der TNM-Klassifikation], sowie G- [*Grading*] und V-Status[Gefäßinvasion], FIGO-Stadium, INRG-*staging*, Tumorgröße, Rezidiv, multifokale Tumorentstehung, Aszites, Residualtumor sowie Expression und Amplifikation bekannter ungünstiger Kofaktoren wie negativer-ER-Rezeptor und ERBB2-Amplifikation)
LK-Met. ≙ Lymphknotenmetastasen
MA ≙ *microarray*
MDR1 ≙ *multidrug resistance protein 1*
NB ≙ *northern blot*
NK-Zellen ≙ *natürliche Killerzelle*
NSCLC ≙ *non-small-cell lung carcinoma*
OS ≙ *overall survival*
PCR ≙ *polymerase chain reaction*
PE-CA ≙ Plattenepithelkarzinom
qRT-PCR ≙ *quantitative real-time PCR*
RNA-Seq. ≙ *RNA-Sequencing*
sRT-PCR ≙ *semi-quantitative real-time PCR*
TMA ≙ *tissue microarray*
WB ≙ *western blot*
WHO ≙ *world health organization*
x ≙ trifft zu

Tabelle A2 | Autoantikörper gegen IGF2BP1, -2 und -3 im hepatozellulären Karzinom und seinen präkanzerösen Läsionen

Läsion	IGF2BP1		IGF2BP2		IGF2BP3		Referenz		
HCC	×		10,7 %	(8/75)	17,3 %	(13/75)	Zhang	2001	[156]
							Zhang	2002	[157]
	×		16,3 %	(26/100)	×		Soo Hoo	2002	[158]
	15,4 %	(10/65)	12,3 %	(8/65)	13,8 %	(9/65)	Zhang	2003	[172]
							Tan	2008	[166]
	×		21,1 %	(20/95)	×		Zhang	1999	[171]
	3,5 %	(3/86)	1,2 %	(1/86)	4,7 %	(4/86)	Himoto	2005a	[145]
							Himoto	2005b	[146]
	16,9 %	(24/142)	14,1 %	(20/142)	9,9 %	(14/142)	Zhang	2007	[173]
	19,2 %	(14/77)	16,9 %	(13/77)	20,8 %	(16/77)	Chen	2010	[174]
Leberzirrhose	4,8 %	(1/21)	0,0 %	(0/21)	4,8 %	(1/21)	Himoto	2005a	[145]
							Himoto	2005b	[146]
	0,0 %	(0/30)	3,3 %	(1/30)	13,3 %	(4/30)	Zhang	2007	[173]
							Chen	2010	[174]
akute Hepatitis	×		0,0 %	(0/31)	×		Zhang	1999	[171]
							Soo Hoo	2002	[158]
chronische Hepatitis	×		0,0 %	(0/20)	×		Zhang	1999	[171]
							Soo Hoo	2002	[158]
	5,6 %	(1/18)	0,0 %	(0/18)	5,6 %	(1/18)	Himoto	2005a	[145]
							Himoto	2005b	[146]
	10,0 %	(3/30)	0,0 %	(0/30)	6,7 %	(2/30)	Zhang	2007	[173]
							Chen	2010	[174]
asymptomat. HBsAg Träger	×		0,0 %	(0/26)	×		Zhang	1999	[171]
							Soo Hoo	2002	[158]
Autoimmun-erkrankungen	×		4,3 %	(6/139)	3,6 %	(5/139)	Zhang	2001	[156]
							Zhang	2002	[157]
	1,9 %	(1/103)	1,0 %	(1/103)	1,0 %	(1/103)	Zhang	2003	[172]
gesunde Individuen	×		0.9 %	(1/112)	0,9 %	(1/112)	Zhang	2001	[156]
	×		0,9 %	(1/112)	×		Soo Hoo	2002	[158]
	×		0,0 %	(0/70)	×		Zhang	1999	[171]
							Zhang	2002	[157]
	2,0 %	(7/346)	2,0 %	(7/346)	1,7 %	(6/346)	Zhang	2003	[172]
	0,0 %	(0/50)	0,0 %	(0/50)	0,0 %	(0/50)	Himoto	2005a	[145]
							Himoto	2005b	[146]
	2,4 %	(2/82)	1,2 %	(1/82)	1,2 %	(1/82)	Zhang	2007	[173]
							Tan	2008	[166]
							Chen	2010	[174]

* ≙ umfasst Patienten mit den Diagnosen systemischer Lupus erythematodes, rheumatoide Arthritis und Sjögren Syndrom

HCC ≙ hepatozelluläres Karzinom

× ≙ nicht untersucht

Anhang

Selbstständigkeitserklärung

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig, ohne unzulässige Hilfe Dritter und ohne Verwendung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderer Literatur direkt oder indirekt übernommenen Daten, Konzepte, Gedanken und andere Inhalte sind unter Angabe der Quelle gekennzeichnet. Alle Regeln zur Sicherung der guten wissenschaftlichen Praxis wurden eingehalten.

Ich versichere weiterhin, dass ich für die inhaltliche Erstellung der vorliegenden Arbeit nicht entgeltliche Hilfe von Vermittlungs- und Beratungsdiensten (Promotionsberater oder andere Person) in Anspruch genommen habe. Niemand hat von mir unmittelbar oder mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Halle (Saale), den 10 Dezember 2018

Pazaitis, Nikolaos

Erklärung über frühere Promotionsversuche

Ich erkläre an Eides statt, dass ich das Zulassungsgesuch zum Promotionsverfahren erstmalig an die Medizinische Fakultät der Martin-Luther-Universität – Halle-Wittenberg stelle. Ich versichere, dass die vorliegende Arbeit weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt wurde.

Halle (Saale), den 10 Dezember 2018

Pazaitis, Nikolaos