Medizinische Fakultät der Martin-Luther-Universität
Halle-Wittenberg

Untersuchungen zum Einfluss von Glykierung und Glykosylierung auf Meningeomzellen

Dissertation zur Erlangung des akademischen Grades Doktor der Medizinischen Wissenschaften (Dr. rer. medic.) in der Fachrichtung Molekulare Medizin

von Philipp Selke geboren am 09.03.1988 in Prenzlau

Betreuer*in: Herr Prof. Dr. Rüdiger Horstkorte

Gutachter*innen: apl. Prof. C. Scheller

Prof. O. Huber, Jena

08.03.2022

13.06.2022

Referat

Posttranslationale Modifikationen wie Glykierung und Glykosylierung von Oberflächenproteinen oder Zelladhäsionsmolekülen sind sowohl an der Entstehung als auch an der Metastasierung von Tumoren beteiligt.

Meningeome sind die am häufigsten diagnostizierten nicht-malignen intrakranialen Tumore und auch die Inzidenz von nicht –malignen Meningeomen steigt mit dem Alter. Wie die meisten Tumore, erzeugen auch Meningeome bevorzugt durch anaerobe Glykolyse Energie (Warburg Effekt) und synthetisieren so vermehrt den Metaboliten Methylglyoxal (MGO). MGO ist dafür bekannt mit Aminogruppen zu reagieren und dabei *Advanced Glycation Endproducts* (AGEs) zu bilden. Diese Reaktion wird auch als Glykierung bezeichnet. AGEs sind dafür bekannt im Alter vermehrt zu akkumulieren.

Zur Untersuchung der Effekte von Glykierung anhand von Meningeomzellen wurden zum einen die benigne Zelllinie BEN-MEN-1 und zum anderen die maligne Zelllinie IOMM-Lee genutzt. Wie alle menschlichen Zellen, sind auch Meningeomzellen sialyliert. Im Golgi werden die Sialinsäuren beispielsweise an Glykanstrukturen, Ganglioside oder auch Glykoproteine mit Hilfe von Sialyltransferasen gesetzt. Während dieser Arbeit konnte gezeigt werden, dass posttranslationale Modifikationen wie Glykierung und Sialylierungen einen elementaren Einfluss auf die Malignität in Meningeomen haben.

Die Glykierung erhöht das invasive Verhalten der benignen Meningeome, wohingegen in malignen Meningeomen gegenteilige Effekte in der *real time cell analysis* (RTCA) beobachtet werden konnten. Als nächstes konnte im Immunoblot gezeigt werden, dass die Glykierung der benignen Zelllinie zu einer Erhöhung der E-Cadherin - und einer Verminderung der N-Cadherin – Expression führt. Zusätzlich konnte in der *quantitative real time polymerase chain reaction* (qPCR) beobachtet werden, dass die Glykierung zu einer Veränderung der Expression von Sialyltransferasen wie ST3GAL1/2/3/5/6, ST6GAL1/2, ST6GALNAC2/6 und ST8SIA1/2 führt, welche vor allem für einen direkten oder indirekten Einfluss auf die Tumorentwicklung bekannt sind. Die verminderte Expression von ST3GAL5 führte zu sinkenden GM3-Gangliosid- Expression in der glykierten benignen Zelllinie, welches mit Hilfe der *Porous Graphitized Carbon* (PGC) *nano-Liquid Chromatography* (LC)- *Electro Spray Ionization* (ESI)-*Mass Spectrometry* (MS)/MS /MS *analysis* nachgewiesen werden konnte.

Selke, Philipp: Untersuchungen zum Einfluss von Glykierung und Glykosylierung auf Meningeomzellen, Halle (Saale), Univ., Med.Fak., Diss. 80 Seiten, 2022

Inhaltsverzeichnis

1. Einleitung

1.1 Meningeome

Meningeome entspringen den Meningealzellen der Arachnoidea und gehören zu den am häufigsten vorkommenden nicht-malignen Tumoren. Die WHO (World Health Organization) teilt die Meningeome nach Unterschieden in Histologie und klinischem Verhalten in Grad 1, 2 und 3 ein [1–3].

Das WHO Grad 1 Meningeom ist das am häufigsten vorkommende (benignen, 80 - 90 % aller diagnostizierten Meningeome) und ist dabei das am langsamsten wachsende Meningeom und hinzukommend haben diese eine niedrige Rezidiv-Rate [1,4]. Meningeome des WHO Grades 2 (atypisch, 10 -20 % aller diagnostizierter Meningeome) werden aufgrund der erhöhten Rezidiv-Rate in WHO Grad 2 eingeteilt. Die Inzidenz für nicht maligne Meningeome steigt mit dem Alter und steigt dramatisch bei Personen über 65 Jahren [3,5]. Die 10- Jahres- Überlebensrate für nicht- maligne Meningeome liegt bei ca 87 %. Das Alter hat einen starken Einfluss auf das Überleben des Patienten nach der Diagnose (10- Jahres- Überlebensrate bei Jugendlichen und jungen Erwachsenen: 94,5 % vs. Erwachsene > 40 Jahre: 81,2 %). Auch das Areal der Meningeome hat einen Einfluss auf das Überleben des Patienten nach der Diagnose. Die relative 10- Jahres- Überlebensrate für Meningeome in zerebralen Bereichen liegt bei ca. 83,5 %, wohingegen die 10- Jahres- Überlebensrate bei Patienten mit Meningeomen in spinalen Regionen eine deutliche höhere Überlebensrate (95,5 %) haben. Des Weiteren erkranken Frauen 2,3-mal häufiger an nicht-maligne Meningeome als Männer [3,5].

Das Meningeom Grad 3 (maligne, 1 – 3 % aller diagnostizierten Meningeome) sind für ein aggressives Wachstumsverhalten und Invasivität bekannt [1,4]. Die durchschnittliche Überlebensrate für maligne Meningeome liegt bei 53 (95% KI: 48-58) Monaten. Die relative 10- Jahres-Überlebensrate von malignen Meningeomen liegt bei unter 60 %. Für das Überleben nach der Diagnose eines malignen Meningeoms spielt auch hier das Alter eine elementare Rolle [3,5].

1.2 Entstehung von Methylglyoxal/ Glyoxal im Stoffwechsel

Carbonyl-Spezies wie Methylglyoxal (MGO) oder Glyoxal (GO) werden hauptsächlich als Beiprodukt während der Glykolyse aber auch durch Autooxidation von Glukose gebildet. Durch Degradation von Glukose mittels Retro-Aldol-Kondensation entsteht MGO. Aber auch durch Deprotonierung von Glukose werden 1,2-Enol oder 2,3-Enol und dabei auch 1-Deoxyglucoson

(1-DG) oder 3-Deoxyglucoson (3-DG) gebildet. Die Fragmentierung von 3-DG kann zur Generierung von MGO führen [6]. Die Glykolyse wird als Signalweg des Zytoplasmas beschrieben und wird von allen Zellen im menschlichen Körper zur Energieerzeugung genutzt. Glukose wird mit Hilfe des Enzyms Hexokinase unter Nutzung eines Adenosintriphosphates (ATP) zu Glukose-6-Phosphat (G- 6- P). Diese irreversible Reaktion führt zu einer Hemmung der Hexokinase-Aktivität. Als nächstes wird Fruktose-6-Phosphat (F-6-P) aus G-6-P mittels G-6-P-Isomerase erzeugt und anschließend durch eine weitere Phosphorylierung durch die Phosphofruktokinase zu Fruktose-1,6-bisphosphat (F-1,6-bisP). Diese Reaktion ist nicht nur irreversibel, sondern auch der wichtigste regulatorische Schritt innerhalb der Glykolyse. Im Folgenden teilt eine Aldolase F-1,6-bisP in Glyceraldehyd-3-P (GAH-3-P) und Dihydroxyacetonphosphat (DHA-P). Letztere wird mit Hilfe der Triosephosphat-Isomerase (TIM) in GAH-3-P umgewandelt [7–9]. Durch nicht enzymatische Eliminierung der Phosphorylierungsstellen von GAH-3-P und DHA-P wird letztendlich Methylglyoxal gebildet [10].

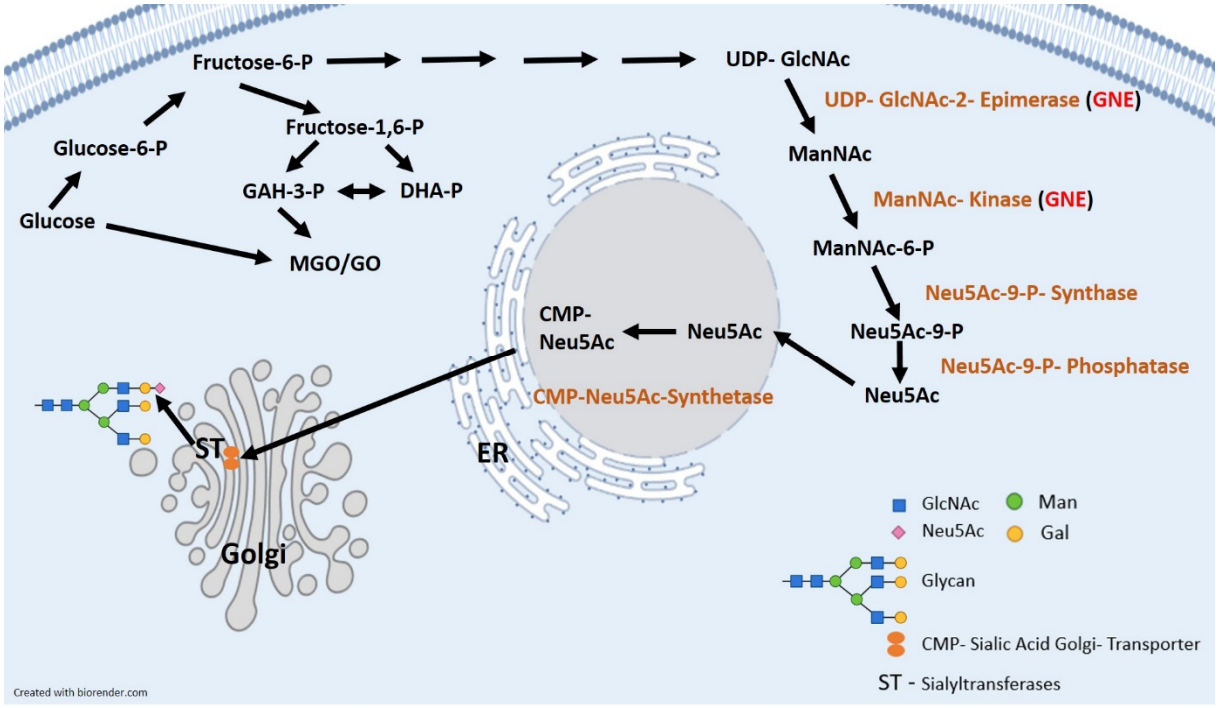

Abbildung 1: Schematische Darstellung der Sialinsäure-Biosynthese von Glucose zur Sialinsäure, Dicarbonyl-Verbindungen und der Sialylierung von Glykoproteinen (zum Beispiel N-Glykane, O-Glykane und Ganglioside) im endoplasmatischen Retikulum und Golgi. GAH-3-P = Glyceraldehyd-3-Phosphat; DHA-P = Dihydroxyaceton-Phosphat; MGO = Methylglyoxal; GO = Glyoxal; GlcNAc = N-Acetyl-Glucosamin; Man =Mannose; Gal = Galaktose; Neu5Ac = N-Acetyl-Neuraminsäure; GNE = UDP-N-Acetyl-Glukosamin-2-epimerase/N-Acetyl-Mannosamin-Kinase; ER = Endoplasmatisches Retikulum. Darstellung nach Selke et al 2021 [11].

1.3 Posttranslationale Modifikationen

Posttranslationale Modifikationen (PTMs) sind Veränderungen an Proteinen, die nach der Translation stattfinden. Häufig vorkommende PTMs sind zum Beispiel Phosphorylierung, Methylierung, Acetylierung, Glykosylierung oder auch Ubiquitinierung, aber auch Glykierung gehören dazu [12]. In dieser Arbeit wird vor allem auf die Glykierung und Sialylierung, welche eine Form der Glykosylierung darstellt, eingegangen.

1.3.1 Glykierung

Die Glykierung beschreibt eine nicht-enzymatische Reaktion bei der sich Carbonyl-Gruppen von Dicarbonylen (zum Beispiel MGO oder GO) oder von Monosacchariden (zum Beispiel Glukose oder Fruktose) mit Aminogruppen von Proteinen oder Peptiden verbinden [13]. Die Glykierung wird auch als Maillard Reaktion bezeichnet und wird in 3 Hauptstadien eingeteilt. In der frühen Phase, wobei vor allem Monosaccharide mit freien Aminogruppen von Proteinen, Nukleinsäuren und Lipiden reagieren, wird eine instabile Schiff‘sche Base und durch Neuordnung ein stabiles Ketoamin (Amadori-Produkt) gebildet [13,14]. In der fortgeschrittenen Phase werden durch Oxidation und Dehydrierung Dicarbonyle aus dem Amadori-Produkt gebildet. Diese sind stärker reaktiv als die Monosaccharide und reagieren erneut mit freien Aminogruppen. In der späten Phase reagieren diese Glykierungsagenten erneut mit Aminogruppen durch Oxidation, Dehydrierung und zyklische Reaktionen, formen schlussendlich AGEs [15].

1.3.2 Glykosylierung

Die Glykosylierung ist definiert als ein enzymatischer Prozess bei dem glykosidische Bindungen zwischen einfachen Zuckern oder Glykanen mit anderen Zuckern, Lipiden oder Proteinen gebildet werden [16]. Glykoproteine enthalten Glykan oder Glykanketten, welche in N- und O-Glykane eingeteilt werden. Die N-Glykosylierung setzt Glykane an Aminogruppen von Asparagin (Asn, N) an Glykosylierungsmuster Asn-X-Serin (Ser)/Threonin (Thr) (X steht für alle Aminosäuren außer für Prolin (Pro)). Die O-Glykosylierung setzt Glykane an Sauerstoff Atome von Hydroxylgruppen von Ser (S)- oder Thr (T)- Resten. Die N-Glykane werden in Mannose-reiche, Hybride und komplexe Glykane eingeteilt [17].

In dieser Arbeit wird speziell auf die Sialylierung eingegangen, welche eine Form der Glykosylierung darstellt. Vor allem die veränderte α 2,3- und α 2,6- verbundene Sialylierung ist mit der Tumorentwicklung assoziiert [18].

1.3.2.1 Sialylierung

Bei der Sialylierung werden Sialinsäuren auf andere Zucker, Lipide oder Proteine mit Hilfe von Sialyltransferasen (STs) transferiert. STs gehören zu den Typ II Transmembranproteinen und sind im Golgi lokalisiert. Wie viele andere Glykosyltransferasen bestehen die STs aus einer N-terminale Domäne, welche im Zytoplasma liegt, einer einzelnen Transmembran-Domäne mit 16-20 Aminosäure-Resten, einer Stammregion und einer konservierten C-terminalen katalytischen Domäne (300 ± 20 Aminosäure-Reste) im Golgi-Lumen [19]. Im Menschen sind aktuell 20 STs bekannt. Diese nutzen die CMP-aktivierte N-acetyl-5-Neuraminsäure (Neu-5-Ac; Sia) als Substrat und transferieren diese auf zum Beispiel Ganglioside, N- oder O-Glykane sowie auf Glykoproteine. Die STs werden in 4 Familien entsprechend ihrer Verbindung, die sie eingehen, aufgeteilt. Die Familie der β-Galaktosid α 2,3- Sialyltransferase (ST3Gal) besteht aus 6 Mitgliedern und transferieren über eine α 2,3 Verbindung Sialinsäuren (Sia) auf Galaktose (Gal). Die β-Galaktosid α 2,6- Sialyltransferase (ST6Gal) hat hingegen nur 2 bekannte Mitglieder. Die, dieser Gruppe angehörigen STs vermitteln den Transfer von Sia auf Galaktose in einer α 2,6 Verbindung. Die STs, die Sia auf N-acetyl galactosamin (GalNAc) übertragen werden GalNAc α 2,6- Sialyltransferase (St6GalNAc) genannt (6 Mitglieder). Der Transfer von Sia auf eine andere Sia in einer α 2,8 Verbindung wird durch die α 2,8 Sialyltransferase (ST8Sia) durchgeführt und hat auch 6 Mitglieder [20].

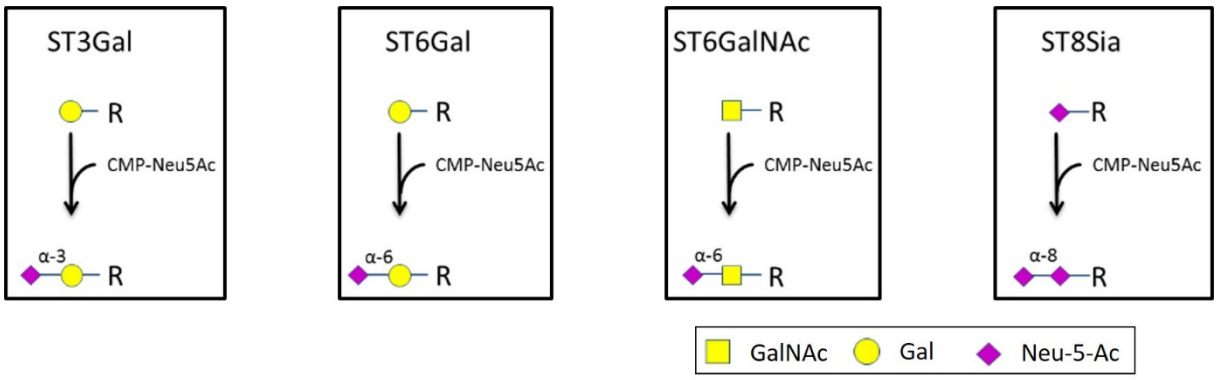

Abbildung 2: Übersicht zur Funktion der Sialyltransferase-Familien.
Es sind die 4 verschiedenen Familien der STs dargestellt. ST3Gal = β-Galaktosid α 2,3- Sialyltransferase; ST6Gal = β-Galaktosid α 2,6- Sialyltransferase; ST6GalNAc = N-Acetyl-Galaktosamin (GalNAc) α 2,6- Sialyltransferase;ST8Sia = α 2,8 Sialyltransferase; CMP-Neu-5-Ac = CMP-aktivierte Sialinsäure; Neu-5-Ac = Sialinsäure. Die Strukturen wurden nach dem Consortium of Functional Glycomics (CFG) dargestellt: gelbes Quadrat = N- Acetyl- Galaktosamin; gelber Kreis = Galaktose; pinker Diamant = N-Acetyl-Neuraminsäure. Verändert nach Rosenstock et al 2020 [21]

1.4 Zelladhäsionsmoleküle

Zelladhäsionsmoleküle (*cell adhesion molecules*; CAMs) sind Oberflächenproteine, die an Zell-Zell-Interaktion und an Zell-Matrix-Interaktion beteiligt sind. Die Adhäsionsmoleküle lassen sich in 4 Familien einteilen: Integrin- Rezeptor Familie, die Immunglobulin (Ig) Superfamilie, Selektine und Cadherine [22]. Wobei in dieser Arbeit vor allem auf die Cadherine eingegangen wird.

1.4.1 Cadherine

Cadherine sind homophile Ca^{2+} abhängige Zell-Zell-Adhäsionsmoleküle, die eine Zell-Zell-Interaktion über ihre fünf extrazellulären Domänen (*adherens junction*) vermitteln. Die klassischen Cadherine sind das epitheliale, plazentale und neurale Cadherin (E-, P- and N-Cadherin). Sie spielen im Zell-Zell-Kontakt, Gewebestrukturierung und Tumorentwicklung eine wichtige Rolle [23]. Die intrazellulär vorliegende Catenin- Bindungsdomäne (CBD) ist entscheidend für die Cadherin-Funktion und spielt eine wichtige Rolle im Erhalt der epithelialen Integrität. Sie bindet direkt an β-Catenin und p120. Diese wiederrum binden an α- Catenin und bilden somit die Verbindung zwischen Cadherin und dem Zytoskelett [24–26]. Mit Hilfe dieser Verbindung können physikalische und biochemische Signale weitergeleitet werden. β-Catenin kann aber auch die Aktivierung verschiedener Gene wie c-Myc und Cyclin-D1 unterstützen und so zu Veränderungen in der Zellmorphologie, Proliferation und Beweglichkeit führen [27].

1.5 Gangliosid Biosynthese

Ganglioside sind Glykosphingolipide welche Sia enthalten. Die Biosynthese der Ganglioside beginnt mit Ceramid (Cer) im endoplasmatischen Retikulum (ER) und wird im Golgi-Apparat fortgesetzt. Im Cis-Golgi wird Cer mittels Glucosylceramid Synthase glucosyliert und Glucosylceramid (GlcCer) gebildet [28]. Die meisten Ganglioside werden aus Laktosylceramid (LacCer) erzeugt (Gangliosid GM4 als Ausnahme wird aus Galaktosylceramid (GalCer) erzeugt), welches im trans-Golgi aus GlcCer generiert wird. Das erste Gangliosid GM3 entsteht durch den Transfer von Sia auf LacCer durch die ST3Gal5 (GM3- Synthase) [29]. Durch sequentielles Hinzufügen von Sia auf GM3 wird zuerst GD3 und durch erneute Addition GT3 gebildet. Dies wird durch die Sialyltransferase ST8Sia1 vermittelt. Die Ganglioside GM3, GD3 und GT3 dienen als Ausgangspunkt für weitere wesentlich komplexere Ganglioside, welche in a-, b- und c- Serien eingeteilt werden [30].

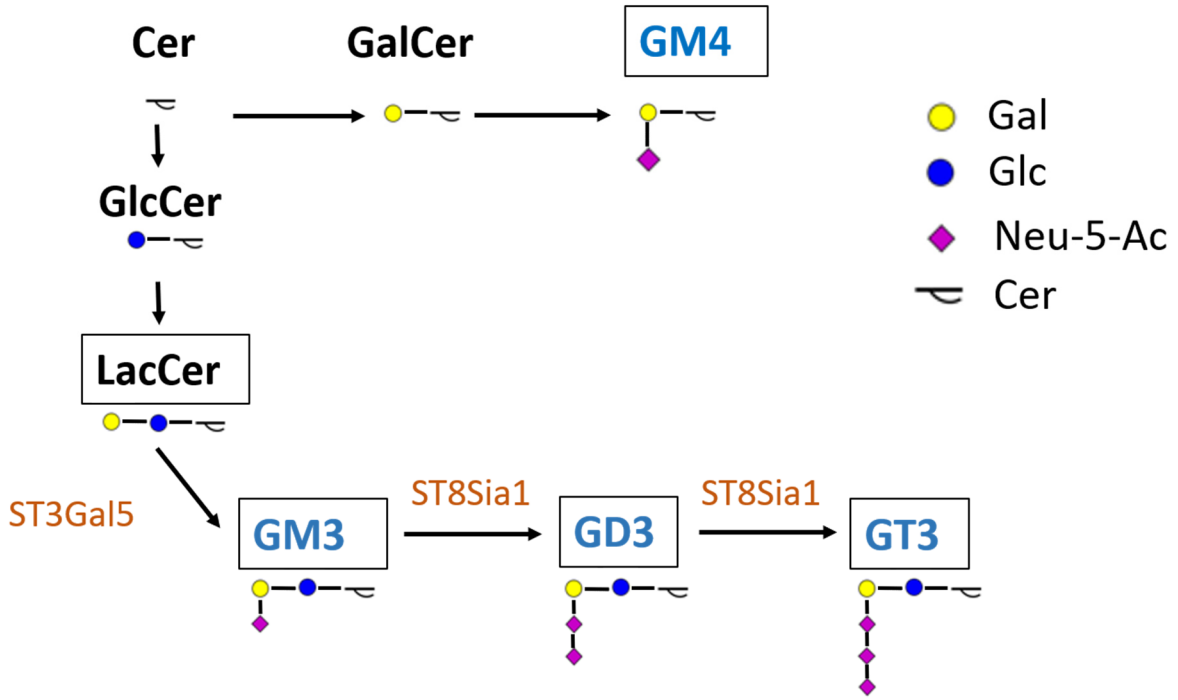

Abbildung 3: Schematische Darstellung der Gangliosid-Biosynthese.
Ceramid = Cer; Galaktosylceramid = GalCer; Monosialogangliosid 4= GM4; Glukosylceramid = GlcCer; Laktosylceramid = LacCer; β-Galaktosid α 2,3- Sialyltransferase 5= ST3Gal5; Monosialogangliosid 3 = GM3; α 2,8 Sialyltransferase 1 = ST8Sia1; Disialogangliosid 3 = GD3; Trisialogangliosid 3 = GT3. Die Strukturen wurden nach dem Consortium of Functional Glycomics (CFG) dargestellt: gelber Kreis = Galaktose; blauer Kreis = Glukose; pinker Diamant = N-Acetyl-Neuraminsäure. Verändert nach Selke et al 2021 [11].

2. Zielstellung

Ziel dieser Arbeit war es die Rolle der altersabhängigen Glykierung und Glykosylierung speziell der Sialylierung bei der Malignität von Meningeomen zu untersuchen. Diese am häufigsten vorkommende gutartige intrakraniale Tumorart umfasst drei Grade. Zum Einfluss von Glykierung auf die unterschiedlichen Grade der Meningeome ist derzeit nichts bekannt.

Dazu sollten benigne und maligne Zelllinien kultiviert und mittels verschiedener Carbonyl-haltiger Medien manipuliert und verändert werden. Hierfür sollte der Nachweis der Glykierung mittels Immunoblot erbracht, der Einfluss hinsichtlich der Zellviabilität mittels 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazoliumbromid (MTT)- Test, sowie die Rolle in Adhäsion, Migration und im invasiven Verhalten mit Hilfe des *Real-Time Cell Analyzers* (RTCA) untersucht werden. Anschließend sollte der Einfluss von Glykierung auf die Expression von Oberflächenproteinen in beiden Tumorgraden im Immunoblot analysiert werden.

Als nächstes sollte der Einfluss der altersabhängigen Glykierung auf die Sialylierung ermittelt werden. Dazu sollten zuvor Unterschiede in der Expression aller Sialyltransferasen zwischen benigner und maligner Zelllinie mit Hilfe der *Polymerase Chain Reaction* (PCR) untersucht werden. Darüber hinaus sollte der Einfluss von Glykierung auf die Sialylierung in der *quantitative real time* PCR (qPCR) auf die benigne bzw. maligne Zelllinie analysiert werden. Ziel hierbei war es herauszufinden, welche posttranslationale Modifikationen (am Beispiel der Meningeome) einen verstärkten oder verminderten Einfluss auf die Malignität haben und somit zur Tumorentwicklung beitragen.

3. Diskussion

Posttranslationale Modifikationen haben einen großen Einfluss auf die Funktionalität und Eigenschaften von Zellen. In dieser Arbeit sind nicht nur die Einflüsse von Glykierung auf verschiedene Meningeomzellen und dessen Sialylierung, sondern auch die Expression des Gangliosids GM3 dargestellt.

Zur Rolle des Glykierungsagenten MGO in Meningeomen ist vor dieser Arbeit nichts bekannt gewesen. Während dieser Arbeit konnte gezeigt werden, dass die altersabhängige Glykierung mit Hilfe von MGO verschiedener Meningeomgrade unterschiedliche Auswirkungen hat. Zum einen wird die glykierte benigne Zelllinie (BEN-MEN-1) in ihrem invasiven Verhalten verstärkt und essentielle Zelladhäsionsmoleküle wie E- und N-Cadherine in ihrer Expression verändert und zum anderen die maligne Zelllinie (IOMM-Lee) im invasiven Verhalten inhibiert. Beide Meningeomzelllinien sind unterschiedlich in der Expression ihrer Sialyltransferasen beeinflusst.

3.1 MGO nimmt als Glykierungsagens Einfluss auf die Funktionalität von Meningeomzellen

In vorrangegangen Studien konnte gezeigt werden, dass in Personen mit Diabetes und oder in Personen höheren Alters die MGO Konzentration erhöht ist [31]. Auch einen Zusammenhang zwischen Diabetes und einem erhöhten Risiko an Krebs zu erkranken konnte in Studien beobachtet werden [32,33]. Andererseits gibt es gegensätzliche Daten, die zum einen eine positive [34,35] oder auch einen gegenläufigen Zusammenhang zwischen Diabetes, Serum-Glukose-Konzentration und einem erhöhten Risiko für Meningeome aufzeigen [36].

Der Einfluss von MGO auf die Meningeomzellen hinsichtlich der Zytotoxizität zeigte, dass ≤ 0,3 mM MGO als physiologische Konzentrationen keinen Einfluss auf die Zellviabilität hat. Eine Studie von Chaplen und Kollegen hat gezeigt, dass MGO eine zelluläre Konzentration von 0,3 mM erreichen kann [37] und dass 90 - 99 % des zellulären MGO *in vivo* an Makromolekülen gebunden vorliegt [38,39]. Bei höheren nicht-physiologischen MGO-Konzentrationen konnte eine Verminderung der Zellviabilität beobachtet werden. Dies konnte auch in der mikroskopischen Inaugenscheinnahme nachgewiesen werden. Mit steigender MGO Konzentration haben sich die Zellen weniger ausgebreitet und waren apoptotisch. Zum Nachweis des programmierten Zelltodes (Apoptose) wurde im Immunoblot die Caspase 3 Aktivität nachgewiesen (keine publizierten Daten). Die gespaltene und aktivierte Caspase 3 ist einer der Schlüsselenzyme für den programmierten Zelltod. Auch andere Arbeitsgruppen konnten gleiches in humanen Glioblastom-Multiformen T98G und U87MG Zellen sowie in SH-

SY5Y Neuroblastomzellen beobachten [40,41]. Des Weiteren hat sich die Arbeitsgruppe um Lee mit einem entscheidenden Signalweg für das Zellüberleben (gp130/STAT3 Signalweg) in Schwannom RT4 Zellen der Ratte, PC12- sowie U87 Gliom-Zellen auseinandergesetzt. Sie konnten auch eine sinkende Zellviabilität, vermittelt durch den gp130/STAT3 Signalweg und damit einhergehend eine erhöhte Zytotoxizität nachweisen [42]. Das steigende Signal mit steigender MGO Konzentration im Immunoblot, zeigt einen Zuwachs glykierter Proteine und tritt daher als Schmier auf allen Banden auf. Dies wurde auch in anderen Studien beobachtet [43,44].

In vielen Studien konnte gezeigt werden, dass die Glykierung mit Hilfe von MGO einen Einfluss auf Proliferation, Adhäsion, Migration, Invasion und somit auf die Malignität haben kann [45–48].

Die altersabhängige Glykierung mit 0,3 mM MGO von Meningeomzellen konnte keinen Unterschied hinsichtlich der Adhäsion auf verschiedenen Substraten (Kollagen IV, Fibronektin) im Vergleich zur jeweiligen Kontrolle zeigen. Die Meningeome bevorzugen die Adhäsion auf Fibronektin, was auch mit vorläufigen, aber nicht publizierten Daten zur Integrin- Expression (α4, 5, 6 and β1, 3, 4) übereinstimmt. Diese Integrine sind auch dafür bekannt eine Rolle in einer Vielzahl von Funktionen wie Adhäsion, Invasion und Proliferation in Meningeomen zu spielen [49–51].

Die Behandlung mit MGO scheint gewebespezifisch unterschiedliche Auswirkungen zu erzeugen. Zum einen konnte in Neoplasien des Kolons und der Hepar eine reduzierte und zum anderen im Neuroblastom eine erhöhte Migration nach MGO-Behandlung nachgewiesen werden [45,46,48]. Für die Meningeomzellen konnte kein Einfluss auf die Chemotaxis durch die altersabhängige Glykierung mittels 0,3 mM MGO beobachtet werden.

Die benignen Meningeomzellen zeigten ein stärker invasives Verhalten nach der Behandlung mit 0,3 mM MGO, wohingegen in den malignen Meningeomzellen ein reduziertes invasives Verhalten nach der MGO-Behandlung zu beobachten war. In *anaplastic thyroid cancer* (ATC) Zellen wurde nach der Behandlung mit MGO eine Erhöhung der Invasivität erfasst [47]. Dies konnte auch in Neuroblastomzellen bestätigt werden [48]. In einer Studie mit hepatozellulären Karzinomen führte die MGO-Behandlung zu einer Verminderung der Invasivität [45]. Aber auch hier scheint die Behandlung mit MGO nicht nur gewebespezifische Auswirkungen, sondern auch die Menge, die diese erzeugt, scheint gewebespezifisch zu sein. Eine geringe Konzentration von 5 µM MGO führt in ATC Zellen zu einer erhöhten und in hepatozellulären Karzinomen zu einer Verminderung der Invasivität [45,47]. In Neuroblastomzellen führte eine Behandlung mit 0,1 mM MGO zu einem erhöhten Invasionsverhalten [48].

Utsuki und Kollegen sprechen invasiven Meningeomen eine niedrige E-Cadherin Expression zu [52]. Im Modell dieser Arbeit konnte eine niedrige Expression in den BEN-MEN-1- und eine hohe Expression von E-Cadherin in IOMM-Lee-Zellen im Immunoblot nachgewiesen werden. Nach der Glykierung mit MGO stieg die Expression in den benignen und sank in den malignen Meningeomzellen. In einzelnen Studien des Ovarialkarzinoms und des inflammatorischen Mammakarzinoms, konnte E-Cadherin gefunden werden [53,54]. Dies bedeutet, dass nicht in jeder Tumorentität der Verlust der E-Cadherin Expression für eine erhöhte Malignität steht. Zusätzlich wurde in mehreren Studien beschrieben, dass E-Cadherin zu einer erhöhten Formation zu Kolonien aber zu einer verminderten Invasivität, Apoptose-Rate und Dissemination führt und somit von den Zellen für die Metastasierung benötigt wird [55]. Andererseits ist die N-Cadherin- Expression in der glykierten benignen Zelllinie BEN-MEN-1 vermindert gegenüber den unglykierten Zellen. Eine Studie um Camand und Kollegen von 2012 ergab, dass eine reduzierte N-Cadherin- Expression in einer schnelleren und weniger gerichteten Migration von Glioblastomen mündet [56] und auch eine *in vitro* sowie *in vivo* Studie mit der C6 Gliomzelllinie stellte fest, dass eine erhöhte N-Cadherin-Expression zu einer Erniedrigung der Invasivität führt [57]. Viele Studien sehen einen Zusammenhang zwischen der N-Cadherin- und der Matrix-Metalloproteinase-9 (MMP-9) –Expression [58,59]. Vorläufige nicht publizierte Ergebnisse zur MMP-9 Expression haben eine Erhöhung nach der Behandlung mit 0,3 mM MGO in BEN-MEN-1 gezeigt. Dies müsste in Zukunft genauer untersucht werden. Man könnte vermuten, dass dies nur ein Grund für das steigende invasive Verhalten in der benignen Zelllinie ist.

3.2 MGO nimmt als Glykierungsagens Einfluss auf die Sialylierung von Meningeomzellen

Viele Arbeitsgruppen konnten einen Einfluss von Sialylierung auf die Tumorgenese beobachten [60–62]. Veränderte Expressionsmuster verschiedener Glykosyltransferasen wurden in vielen verschiedenen humanen Tumorentitäten beschrieben [63,64]. Jedoch ist bisher wenig bekannt über den Einfluss von Glykierung auf die Glykosylierung. Die Glykierung wird in verschiedenen Tumoren, aufgrund des Warburg Effekts, als erhöht beschrieben [65,66].

In Tumoren der Ovarien konnte gezeigt werden, dass eine Überexpression der Sialyltransferase ST3GAL1 zu einer *Transforming Growth Factor* (TGF)- β1- induzierten epithelialen-mesenchymalen-Transition, Migration und Invasion führt. In einem Knockdown konnte das Gegenteil beobachtet werden [67]. Die ST3GAL1-Expression in der benignen glykierten Zelllinie war auch erhöht. In der Arbeitsgruppe um Mehta et al. konnte gezeigt werden, dass in Tumoren mit einem positiven perineuralen Invasionsstatus eine erhöhte

ST3GAL2-Expression aufweisen [68]. Auch in der benignen Zelllinie konnte eine erhöhte ST3GAL2-Expression nachgewiesen werden. Die Expression von ST3GAL3, welche wichtig für die Biosynthese von Disialogangliosid (GD)1a und Trisialogangliosid (GT)1b im Gehirn ist [62], war in der glykierten BEN-MEN-1 Zelllinie erhöht und in der glykierten IOMM-Lee Zelllinie vermindert. In diesem Modell wurde in beiden glykierten Meningeom-Zelllinien eine verminderte ST3GAL5- Expression gefunden. ST3GAL5 transferiert an Lactosylceramid Sialinsäuren und bildet somit Monosialogangliosid (GM)3 und wird daher auch GM3- Synthase genannt [69]. Eine andere Studie schreibt der GM3-Synthase eine Hemmung der Phosphorylierung des *epidermal Growth Factor Receptor* (EGFR) zu, was wiederum zu einer Verminderung der Zellproliferation führt [70]. Als nächstes wird die Widerstandsfähigkeit gegenüber oxidativen Stress und Strahlentherapie, aufgrund der Hochregulierung des *Extracellular Signal-regulated Kinases* (ERK) vermittelt über die veränderte GM3-Synthase-Expression modifiziert [29]. Die Absolutmenge von GM3 in der glykierten benignen Meningeomzelllinie war reduziert. Dies ist kohärent mit der Expression von ST3GAL5 in den glykierten BEN-MEN-1-Zellen. In einer Studie von Yamashita und Kollegen konnte in einer GM3-Synthase-Knockout-Maus nicht nur eine Liganden-induzierte Insulinrezeptor-Phosphorylierung, sondern auch eine erhöhte Glukose-Sensitivität und Insulintoleranz, gezeigt werden [71]. Eine verminderte GM3-Expression führt zu einer gehemmten Zellbeweglichkeit und Adhäsion vermittelt durch eine ERK-Phosphorylierung, einer erhöhten Ras-Expression, was wiederum die Migration durch *Mitogen-Activated Protein Kinase* (MAPK) reguliert [29,72–74]. Ein anderes Glykierungsagens (Glyoxal, GO) hat in der benignen Meningeomzelllinie BEN-MEN-1 ebenfalls eine Herunterregulierung von ST3GAL5 zur Folge. In der malignen Meningeomzelllinie IOMM-Lee konnte keine Veränderung der ST3GAL5 Expression beobachtet werden. Dies könnte eine, für viele verschiedene Tumorentitäten im Zusammenhang mit Glykierung beschriebene, erhöhte Glyoxalase 1 Aktivität, welche Dicarbonyle degradiert, erklären [75–77]. Auch die ST3GAL6-Expression, welche eine elementare Rolle in der Synthese von funktionellen Sialyl-Lewis X haben [78], war in beiden glykierten Meningeom-Zelllinien reduziert. In mehreren Studien konnte durch eine reduzierte ST3GAL6-Expression eine reduzierte Migration und Invasion in Zelllinien des Harnblasenkarzinoms (5637 und J82 *Urinary Bladder Carcinom* (UBC) Zellen) [79] und auch zu einer verminderten Adhäsion und Migration in Multiplen Myelomzellen dargestellt werden [80].

In der Gruppe der Sialyltransferasen, die Sialinsäuren in einer α 2,6 Verbindung auf Galaktose transferieren, konnte eine erhöhte ST6GAL1-Expression in den glykierten BEN-MEN-1 Zellen nachgewiesen werden. Dies konnte auch in anderen Tumorentitäten wie Prostata, Glioblastom, Pulmo oder in Geweben der Mamma nachgewiesen werden [81–84]. In einer anderen Studie in A549 und H1299 Zellen (Zelllinien der Bronchialkarzinome) führte eine

verminderte ST6GAL1-Expression zu verminderten MMP-Expressionen und einem gehemmten invasiven Verhalten [82]. Andererseits hatte ST6GAL1 eine Tumor-suppressive Wirkung im Urothelkarzinom [85]. In vielen Tumorentitäten konnte eine erhöhte ST6GAL2 Expression nachgewiesen werden und vor allem in Mammakarzinomen war dies mit einer erhöhten Expression von *intracellular adhesion molecule* (ICAM)-1, *vascular adhesion molecule* (VCAM)-1, MMP2 oder auch MMP9 verbunden [86,87].

In der glykierten benignen Meningeomzelllinie BEN-MEN-1 wurde eine sinkende ST6GALNAC2-Expression beobachtet. Die verminderte Expression von ST6GALNAC2 ist mit einer schlechteren Prognose für den Patienten mit Mammakarzinom aber auch im Kolorektalen Karzinom assoziiert [88,89]. Im Mammakarzinom konnte die Metastasen-supprimierende Wirkung der ST6GALNAC2-Expression nachgewiesen werden [90]. Andererseits konnte eine andere Arbeitsgruppe um Schneider et al 2001 zeigen, dass eine hohe ST6GALNAC2 Expression mit Metastasierungen ins Lymphsystem korrelieren [91]. In der glykierten BEN-MEN-1 Zelllinie war die ST6GALNAC4 Expression erhöht. Eine erhöhte Expression dieser Sialyltransferase führte zu einer verminderten O-Glykan-Kettenverlängerung [92]. In Folikulären Thyroid Karzinom (*Follicular Thyroid Carcinoma*, FTC) -238 Zellen konnte *in vitro* und *in vivo* eine Inhibition des invasiven Verhalten durch eine verminderte Expression des ST6GALNAC4- Gens erzeugt werden [93]. In diesem Modell könnte eine geringere ST6GALNAC5 Expression in beiden glykierten Meningeomzelllinien ein Zeichen für eine Transformation sein, denn die Expression dieser Sialyltransferase ist auf das Hirngewebe beschränkt und synthetisiert GD1α [94]. Die glykierten BEN-MEN-1 Zellen zeigten eine erhöhte Expression von ST6GALNAC6. In mehreren Studien konnte eine erhöhte ST6GALNAC6-Expression, welche für die Synthese von Sialyl-Lewis (a) bekannt ist, im Tumor des Kolons beobachtet werden. Die erhöhte Expression steht im Zusammenhang mit Mechanismen, die die Tumorentwicklung vorantreiben [95,96].

In zerebralen Metastasen des malignen Melanoms ist eine erhöhte ST8SIA1 Expression (GD3-Synthase) auch für eine erhöhte GD3-Synthese verantwortlich. Dies führt zu einer schlechteren Prognose für den Patienten [97]. Auch im Glioblastom konnte eine erhöhte Malignität durch eine erhöhte Expression von GD3 sowie GD2 beobachtet werden und ST8SIA1 wird im Glioblastom sogar als elementarer Treiber für die Malignität beschrieben [98,99]. In diesem Modell hat die Glykierung zu einer erhöhten Expression in der benignen Zelllinie und zu einer Erniedrigung der ST8SIA1 Expression in der malignen Zelllinie geführt. In einer Studie zu Meningeomen und GD3 und GD2 Expression, konnte die Arbeitsgruppe um Mennel et al zeigen, dass die Expressionsmuster abhängig vom Ursprung des Tumors sind [100]. Im nicht kleinzelligen Bronchialkarzinom wurde eine erhöhte ST8SIA2 Expression mit einem erhöhten Rezidiv-Risiko in Verbindung gebracht [101]. Dies könnte auch eine Rolle in

glykierten BEN-MEN-1 Zellen im Zusammenhang mit dem erhöhten invasiven Verhalten spielen. ST8SIA5 ist bekannt für die Synthese von GD1c/GT1a Tetrasialogangliotetraosyl-Ceramid (GQ)1b aus GM1b/GD1a/GT1b. In der glykierten benignen Zelllinie ist diese Sialyltransferase erhöht [102]. Zusammenfassend kann gesagt werden, dass die Behandlung der Meningeomzellen mit einem hochreaktiven Dicarbonyl wie MGO, direkt bzw. indirekt über Veränderungen der E- und N-Cadherin Expression und über die vorwiegende Steigerung der Expression der Sialyltransferasen in benignen Meningeomzellen zu einer verstärkten Invasivität und somit zu einer erhöhte Malignität führt. Die maligne Zelllinie zeigte eine vorwiegende Verminderung der Expression der Sialyltransferasen mit der altersabhängigen Glykierung und eine Senkung des invasiven Verhaltens.

4. Literaturverzeichnis

1. Maier, A.D.; Bartek, J.; Eriksson, F.; Ugleholdt, H.; Juhler, M.; Broholm, H.; Mathiesen, T.I. Clinical and histopathological predictors of outcome in malignant meningioma. *Neurosurg. Rev.* **2020**, *43*, 643–653, doi:10.1007/s10143-019-01093-5.
2. Claus, E.B.; Bondy, M.L.; Schildkraut, J.M.; Wiemels, J.L.; Wrensch, M.; Black, P.M. Epidemiology of intracranial meningioma. *Neurosurgery* **2005**, *57*, 1088-95; discussion 1088-95, doi:10.1227/01.neu.0000188281.91351.b9.
3. Ostrom, Q.T.; Patil, N.; Cioffi, G.; Waite, K.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2013-2017. *Neuro Oncol.* **2020**, *22*, iv1-iv96, doi:10.1093/neuonc/noaa200.
4. Fathi, A.-R.; Roelcke, U. Meningioma. *Curr. Neurol. Neurosci. Rep.* **2013**, *13*, 337, doi:10.1007/s11910-013-0337-4.
5. Ostrom, Q.T.; Cioffi, G.; Gittleman, H.; Patil, N.; Waite, K.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012-2016. *Neuro Oncol.* **2019**, *21*, v1-v100, doi:10.1093/neuonc/noz150.
6. Wang, Y.; Ho, C.-T. Flavour chemistry of methylglyoxal and glyoxal. *Chem. Soc. Rev.* **2012**, *41*, 4140–4149, doi:10.1039/c2cs35025d.
7. Akram, M. Mini-review on glycolysis and cancer. *J. Cancer Educ.* **2013**, *28*, 454–457, doi:10.1007/s13187-013-0486-9.
8. Allaman, I.; Bélanger, M.; Magistretti, P.J. Methylglyoxal, the dark side of glycolysis. *Front. Neurosci.* **2015**, *9*, 23, doi:10.3389/fnins.2015.00023.
9. Kumari, A. Glycolysis. *Sweet Biochemistry*; Elsevier, 2018; pp 1–5, ISBN 9780128144534.
10. Schalkwijk, C.G.; Stehouwer, C.D.A. Methylglyoxal, a Highly Reactive Dicarbonyl Compound, in Diabetes, Its Vascular Complications, and Other Age-Related Diseases. *Physiol. Rev.* **2020**, *100*, 407–461, doi:10.1152/physrev.00001.2019.
11. Selke, P.; Bork, K.; Zhang, T.; Wuhrer, M.; Strauss, C.; Horstkorte, R.; Scheer, M. Glycation Interferes with the Expression of Sialyltransferases in Meningiomas. *Cells* **2021**, *10*, doi:10.3390/cells10123298.
12. Gill, V.; Kumar, V.; Singh, K.; Kumar, A.; Kim, J.-J. Advanced Glycation End Products (AGEs) May Be a Striking Link Between Modern Diet and Health. *Biomolecules* **2019**, *9*, doi:10.3390/biom9120888.
13. Rabbani, N.; Thornalley, P.J. The dicarbonyl proteome: proteins susceptible to dicarbonyl glycation at functional sites in health, aging, and disease. *Ann. N. Y. Acad. Sci.* **2008**, *1126*, 124–127, doi:10.1196/annals.1433.043.

14. Ahmed, N. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res. Clin. Pract.* **2005**, *67*, 3–21, doi:10.1016/j.diabres.2004.09.004.
15. Lapolla, A.; Traldi, P.; Fedele, D. Importance of measuring products of non-enzymatic glycation of proteins. *Clin. Biochem.* **2005**, *38*, 103–115, doi:10.1016/j.clinbiochem.2004.09.007.
16. Pinho, S.S.; Reis, C.A. Glycosylation in cancer: mechanisms and clinical implications. *Nat. Rev. Cancer* **2015**, *15*, 540–555, doi:10.1038/nrc3982.
17. Reily, C.; Stewart, T.J.; Renfrow, M.B.; Novak, J. Glycosylation in health and disease. *Nat. Rev. Nephrol.* **2019**, *15*, 346–366, doi:10.1038/s41581-019-0129-4.
18. Kim, Y.J.; Varki, A. Perspectives on the significance of altered glycosylation of glycoproteins in cancer. *Glycoconj. J.* **1997**, *14*, 569–576, doi:10.1023/a:1018580324971.
19. Chen, X.; Varki, A. Advances in the biology and chemistry of sialic acids. *ACS Chem. Biol.* **2010**, *5*, 163–176, doi:10.1021/cb900266r.
20. Grewal, R.K.; Shaikh, A.R.; Gorle, S.; Kaur, M.; Videira, P.A.; Cavallo, L.; Chawla, M. Structural Insights in Mammalian Sialyltransferases and Fucosyltransferases: We Have Come a Long Way, but It Is Still a Long Way Down. *Molecules* **2021**, *26*, doi:10.3390/molecules26175203.
21. Rosenstock, P.; Bork, K.; Massa, C.; Selke, P.; Seliger, B.; Horstkorte, R. Sialylation of Human Natural Killer (NK) Cells is Regulated by IL-2. *J. Clin. Med.* **2020**, *9*, doi:10.3390/jcm9061816.
22. Chooi, W.H.; Chew, S.Y. Modulation of cell-cell interactions for neural tissue engineering: Potential therapeutic applications of cell adhesion molecules in nerve regeneration. *Biomaterials* **2019**, *197*, 327–344, doi:10.1016/j.biomaterials.2019.01.030.
23. Kaszak, I.; Witkowska-Piłaszewicz, O.; Niewiadomska, Z.; Dworecka-Kaszak, B.; Ngosa Toka, F.; Jurka, P. Role of Cadherins in Cancer-A Review. *Int. J. Mol. Sci.* **2020**, *21*, doi:10.3390/ijms21207624.
24. van Roy, F.; Berx, G. The cell-cell adhesion molecule E-cadherin. *Cell. Mol. Life Sci.* **2008**, *65*, 3756–3788, doi:10.1007/s00018-008-8281-1.
25. Shapiro, L.; Weis, W.I. Structure and biochemistry of cadherins and catenins. *Cold Spring Harb. Perspect. Biol.* **2009**, *1*, a003053, doi:10.1101/cshperspect.a003053.
26. Serrano-Gomez, S.J.; Maziveyi, M.; Alahari, S.K. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Mol. Cancer* **2016**, *15*, 18, doi:10.1186/s12943-016-0502-x.
27. Rowlands, T.M.; Pechenkina, I.V.; Hatsell, S.J.; Pestell, R.G.; Cowin, P. Dissecting the roles of beta-catenin and cyclin D1 during mammary development and neoplasia. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11400–11405, doi:10.1073/pnas.1534601100.

28. Maccioni, H.J.F. Glycosylation of glycolipids in the Golgi complex. *J. Neurochem.* **2007**, *103 Suppl 1*, 81–90, doi:10.1111/j.1471-4159.2007.04717.x.
29. Shimizu, T.; Nagane, M.; Suzuki, M.; Yamauchi, A.; Kato, K.; Kawashima, N.; Nemoto, Y.; Maruo, T.; Kawakami, Y.; Yamashita, T. Tumor hypoxia regulates ganglioside GM3 synthase, which contributes to oxidative stress resistance in malignant melanoma. *Biochim. Biophys. Acta Gen. Subj.* **2020**, *1864*, 129723, doi:10.1016/j.bbagen.2020.129723.
30. Yu, R.K.; Tsai, Y.-T.; Ariga, T.; Yanagisawa, M. Structures, biosynthesis, and functions of gangliosides--an overview. *J. Oleo Sci.* **2011**, *60*, 537–544, doi:10.5650/jos.60.537.
31. Rabbani, N.; Thornalley, P.J. Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. *Biochem. Biophys. Res. Commun.* **2015**, *458*, 221–226, doi:10.1016/j.bbrc.2015.01.140.
32. Chowdhury, T.A. Diabetes and cancer. *QJM* **2010**, *103*, 905–915, doi:10.1093/qjmed/hcq149.
33. Giovannucci, E.; Harlan, D.M.; Archer, M.C.; Bergenstal, R.M.; Gapstur, S.M.; Habel, L.A.; Pollak, M.; Regensteiner, J.G.; Yee, D. Diabetes and cancer: a consensus report. *CA Cancer J. Clin.* **2010**, *60*, 207–221, doi:10.3322/caac.20078.
34. Schneider, B.; Pülhorn, H.; Röhrig, B.; Rainov, N.G. Predisposing conditions and risk factors for development of symptomatic meningioma in adults. *Cancer Detect. Prev.* **2005**, *29*, 440–447, doi:10.1016/j.cdp.2005.07.002.
35. Schwartzbaum, J.; Jonsson, F.; Ahlbom, A.; Preston-Martin, S.; Malmer, B.; Lönn, S.; Söderberg, K.; Feychting, M. Prior hospitalization for epilepsy, diabetes, and stroke and subsequent glioma and meningioma risk. *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14*, 643–650, doi:10.1158/1055-9965.EPI-04-0119.
36. Bernardo, B.M.; Orellana, R.C.; Weisband, Y.L.; Hammar, N.; Walldius, G.; Malmstrom, H.; Ahlbom, A.; Feychting, M.; Schwartzbaum, J. Association between prediagnostic glucose, triglycerides, cholesterol and meningioma, and reverse causality. *Br. J. Cancer* **2016**, *115*, 108–114, doi:10.1038/bjc.2016.157.
37. Chaplen, F.W.; Fahl, W.E.; Cameron, D.C. Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 5533–5538, doi:10.1073/pnas.95.10.5533.
38. Chaplen, F.W. Incidence and potential implications of the toxic metabolite methylglyoxal in cell culture: A review. *Cytotechnology* **1998**, *26*, 173–183, doi:10.1023/A:1007953628840.
39. Thornalley, P.J. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification-A role in pathogenesis and antiproliferative

chemotherapy. *General Pharmacology: The Vascular System* **1996**, *27*, 565–573, doi:10.1016/0306-3623(95)02054-3.

40. Yin, Q.-Q.; Dong, C.-F.; Dong, S.-Q.; Dong, X.-L.; Hong, Y.; Hou, X.-Y.; Luo, D.-Z.; Pei, J.-J.; Liu, X.-P. AGEs induce cell death via oxidative and endoplasmic reticulum stresses in both human SH-SY5Y neuroblastoma cells and rat cortical neurons. *Cell. Mol. Neurobiol.* **2012**, *32*, 1299–1309, doi:10.1007/s10571-012-9856-9.
41. Paul-Samojedny, M.; Łasut, B.; Pudełko, A.; Fila-Daniłow, A.; Kowalczyk, M.; Suchanek-Raif, R.; Zieliński, M.; Borkowska, P.; Kowalski, J. Methylglyoxal (MGO) inhibits proliferation and induces cell death of human glioblastoma multiforme T98G and U87MG cells. *Biomed. Pharmacother.* **2016**, *80*, 236–243, doi:10.1016/j.biopha.2016.03.021.
42. Lee, H.K.; Seo, I.A.; Suh, D.J.; Lee, H.J.; Park, H.T. A novel mechanism of methylglyoxal cytotoxicity in neuroglial cells. *J. Neurochem.* **2009**, *108*, 273–284, doi:10.1111/j.1471-4159.2008.05764.x.
43. Rosenstock, P.; Bezold, V.; Bork, K.; Scheffler, J.; Horstkorte, R. Glycation interferes with natural killer cell function. *Mech. Ageing Dev.* **2019**, *178*, 64–71, doi:10.1016/j.mad.2019.01.006.
44. Bezold, V.; Rosenstock, P.; Scheffler, J.; Geyer, H.; Horstkorte, R.; Bork, K. Glycation of macrophages induces expression of pro-inflammatory cytokines and reduces phagocytic efficiency. *Aging (Albany NY)* **2019**, *11*, 5258–5275, doi:10.18632/aging.102123.
45. Loarca, L.; Sassi-Gaha, S.; Artlett, C.M. Two α-dicarbonyls downregulate migration, invasion, and adhesion of liver cancer cells in a p53-dependent manner. *Dig. Liver Dis.* **2013**, *45*, 938–946, doi:10.1016/j.dld.2013.05.005.
46. He, T.; Zhou, H.; Li, C.; Chen, Y.; Chen, X.; Li, C.; Mao, J.; Lyu, J.; Meng, Q.H. Methylglyoxal suppresses human colon cancer cell lines and tumor growth in a mouse model by impairing glycolytic metabolism of cancer cells associated with down-regulation of c-Myc expression. *Cancer Biol. Ther.* **2016**, *17*, 955–965, doi:10.1080/15384047.2016.1210736.
47. Antognelli, C.; Moretti, S.; Frosini, R.; Puxeddu, E.; Sidoni, A.; Talesa, V.N. Methylglyoxal Acts as a Tumor-Promoting Factor in Anaplastic Thyroid Cancer. *Cells* **2019**, *8*, doi:10.3390/cells8060547.
48. Scheer, M.; Bork, K.; Simon, F.; Nagasundaram, M.; Horstkorte, R.; Gnanapragassam, V.S. Glycation Leads to Increased Polysialylation and Promotes the Metastatic Potential of Neuroblastoma Cells. *Cells* **2020**, *9*, doi:10.3390/cells9040868.
49. Chen, J.; Xu, X.; Wang, H. Expression of integrin-alpha(3) mRNA in meningiomas and its correlation with proliferation and invasion. *J. Huazhong Univ. Sci. Technolog. Med. Sci.* **2009**, *29*, 94–96, doi:10.1007/s11596-009-0120-6.

50. Nigim, F.; Kiyokawa, J.; Gurtner, A.; Kawamura, Y.; Hua, L.; Kasper, E.M.; Brastianos, P.K.; Cahill, D.P.; Rabkin, S.D.; Martuza, R.L.; et al. A Monoclonal Antibody Against β1 Integrin Inhibits Proliferation and Increases Survival in an Orthotopic Model of High-Grade Meningioma. *Target. Oncol.* **2019**, *14*, 479–489, doi:10.1007/s11523-019-00654-4.
51. Bello, L.; Zhang, J.; Nikas, D.C.; Strasser, J.F.; Villani, R.M.; Cheresh, D.A.; Carroll, R.S.; Black, P.M. Alpha(v)beta3 and alpha(v)beta5 integrin expression in meningiomas. *Neurosurgery* **2000**, *47*, 1185–1195, doi:10.1097/00006123-200011000-00035.
52. Utsuki, S.; Oka, H.; Sato, Y.; Kawano, N.; Tsuchiya, B.; Kobayashi, I.; Fujii, K. Invasive meningioma is associated with a low expression of E-cadherin and beta-catenin. *Clin. Neuropathol.* **2005**, *24*, 8–12.
53. Sundfeldt, K. Cell–cell adhesion in the normal ovary and ovarian tumors of epithelial origin; an exception to the rule. *Molecular and Cellular Endocrinology* **2003**, *202*, 89–96, doi:10.1016/s0303-7207(03)00068-6.
54. Kleer, C.G.; van Golen, K.L.; Braun, T.; Merajver, S.D. Persistent E-cadherin expression in inflammatory breast cancer. *Mod. Pathol.* **2001**, *14*, 458–464, doi:10.1038/modpathol.3880334.
55. Padmanaban, V.; Krol, I.; Suhail, Y.; Szczerba, B.M.; Aceto, N.; Bader, J.S.; Ewald, A.J. E-cadherin is required for metastasis in multiple models of breast cancer. *Nature* **2019**, *573*, 439–444, doi:10.1038/s41586-019-1526-3.
56. Camand, E.; Peglion, F.; Osmani, N.; Sanson, M.; Etienne-Manneville, S. N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. *J. Cell Sci.* **2012**, *125*, 844–857, doi:10.1242/jcs.087668.
57. Asano, K.; Duntsch, C.D.; Zhou, Q.; Weimar, J.D.; Bordelon, D.; Robertson, J.H.; Pourmotabbed, T. Correlation of N-cadherin expression in high grade gliomas with tissue invasion. *J. Neurooncol.* **2004**, *70*, 3–15, doi:10.1023/b:neon.0000040811.14908.f2.
58. Hsu, C.-C.; Huang, S.-F.; Wang, J.-S.; Chu, W.-K.; Nien, J.-E.; Chen, W.-S.; Chow, S.-E. Interplay of N-Cadherin and matrix metalloproteinase 9 enhances human nasopharyngeal carcinoma cell invasion. *BMC Cancer* **2016**, *16*, 800, doi:10.1186/s12885-016-2846-4.
59. Walker, A.; Frei, R.; Lawson, K.R. The cytoplasmic domain of N-cadherin modulates MMP-9 induction in oral squamous carcinoma cells. *Int. J. Oncol.* **2014**, *45*, 1699–1706, doi:10.3892/ijo.2014.2549.

60. Pihikova, D.; Kasak, P.; Kubanikova, P.; Sokol, R.; Tkac, J. Aberrant sialylation of a prostate-specific antigen: Electrochemical label-free glycoprofiling in prostate cancer serum samples. *Anal. Chim. Acta* **2016**, *934*, 72–79, doi:10.1016/j.aca.2016.06.043.
61. Fleming, S.C.; Smith, S.; Knowles, D.; Skillen, A.; Self, C.H. Increased sialylation of oligosaccharides on IgG paraproteins--a potential new tumour marker in multiple myeloma. *J. Clin. Pathol.* **1998**, *51*, 825–830, doi:10.1136/jcp.51.11.825.
62. Cui, H.-X.; Wang, H.; Wang, Y.; Song, J.; Tian, H.; Xia, C.; Shen, Y. ST3Gal III modulates breast cancer cell adhesion and invasion by altering the expression of invasion-related molecules. *Oncol. Rep.* **2016**, *36*, 3317–3324, doi:10.3892/or.2016.5180.
63. Saldova, R.; Wormald, M.R.; Dwek, R.A.; Rudd, P.M. Glycosylation changes on serum glycoproteins in ovarian cancer may contribute to disease pathogenesis. *Dis. Markers* **2008**, *25*, 219–232, doi:10.1155/2008/601583.
64. Suzuki, O.; Abe, M.; Hashimoto, Y. Sialylation by β-galactoside α-2,6-sialyltransferase and N-glycans regulate cell adhesion and invasion in human anaplastic large cell lymphoma. *Int. J. Oncol.* **2015**, *46*, 973–980, doi:10.3892/ijo.2015.2818.
65. Ahmad, S.; Khan, H.; Siddiqui, Z.; Khan, M.Y.; Rehman, S.; Shahab, U.; Godovikova, T.; Silnikov, V.; Moinuddin. AGEs, RAGEs and s-RAGE; friend or foe for cancer. *Semin. Cancer Biol.* **2018**, *49*, 44–55, doi:10.1016/j.semcancer.2017.07.001.
66. Bellier, J.; Nokin, M.-J.; Lardé, E.; Karoyan, P.; Peulen, O.; Castronovo, V.; Bellahcène, A. Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. *Diabetes Res. Clin. Pract.* **2019**, *148*, 200–211, doi:10.1016/j.diabres.2019.01.002.
67. Wu, X.; Zhao, J.; Ruan, Y.; Sun, L.; Xu, C.; Jiang, H. Sialyltransferase ST3GAL1 promotes cell migration, invasion, and TGF-β1-induced EMT and confers paclitaxel resistance in ovarian cancer. *Cell Death Dis.* **2018**, *9*, 1102, doi:10.1038/s41419-018-1101-0.
68. Mehta, K.A.; Patel, K.A.; Pandya, S.J.; Patel, P.S. "Aberrant sialylation plays a significant role in oral squamous cell carcinoma progression". *J. Oral Pathol. Med.* **2020**, *49*, 253–259, doi:10.1111/jop.12976.
69. Trinchera, M.; Parini, R.; Indellicato, R.; Domenighini, R.; dall'Olio, F. Diseases of ganglioside biosynthesis: An expanding group of congenital disorders of glycosylation. *Mol. Genet. Metab.* **2018**, *124*, 230–237, doi:10.1016/j.ymgme.2018.06.014.
70. Kawashima, N.; Nishimiya, Y.; Takahata, S.; Nakayama, K.-I. Induction of Glycosphingolipid GM3 Expression by Valproic Acid Suppresses Cancer Cell Growth. *J. Biol. Chem.* **2016**, *291*, 21424–21433, doi:10.1074/jbc.M116.751503.
71. Yamashita, T.; Hashiramoto, A.; Haluzik, M.; Mizukami, H.; Beck, S.; Norton, A.; Kono, M.; Tsuji, S.; Daniotti, J.L.; Werth, N.; et al. Enhanced insulin sensitivity in mice lacking

ganglioside GM3. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3445–3449, doi:10.1073/pnas.0635898100.

72. Kojima, N.; Hakomori, S. Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid-glycolipid interaction. *J. Biol. Chem.* **1991**, *266*, 17552–17558.
73. Hakomori, S.-I.; Handa, K. GM3 and cancer. *Glycoconj. J.* **2015**, *32*, 1–8, doi:10.1007/s10719-014-9572-4.
74. Hashiramoto, A.; Mizukami, H.; Yamashita, T. Ganglioside GM3 promotes cell migration by regulating MAPK and c-Fos/AP-1. *Oncogene* **2006**, *25*, 3948–3955, doi:10.1038/sj.onc.1209416.
75. Hu, X.; Yang, X.; He, Q.; Chen, Q.; Yu, L. Glyoxalase 1 is up-regulated in hepatocellular carcinoma and is essential for HCC cell proliferation. *Biotechnol. Lett.* **2014**, *36*, 257–263, doi:10.1007/s10529-013-1372-6.
76. Burdelski, C.; Shihada, R.; Hinsch, A.; Angerer, A.; Göbel, C.; Friedrich, E.; Hube-Magg, C.; Burdak-Rothkamm, S.; Kluth, M.; Simon, R.; et al. High-Level Glyoxalase 1 (GLO1) expression is linked to poor prognosis in prostate cancer. *Prostate* **2017**, *77*, 1528–1538, doi:10.1002/pros.23431.
77. Peng, H.-T.; Chen, J.; Liu, T.-Y.; Wu, Y.-Q.; Lin, X.-H.; Lai, Y.-H.; Huang, Y.-F. Up-regulation of the tumor promoter Glyoxalase-1 indicates poor prognosis in breast cancer. *Int. J. Clin. Exp. Pathol.* **2017**, *10*, 10852–10862.
78. Chachadi, V.B.; Bhat, G.; Cheng, P.-W. Glycosyltransferases involved in the synthesis of MUC-associated metastasis-promoting selectin ligands. *Glycobiology* **2015**, *25*, 963–975, doi:10.1093/glycob/cwv030.
79. Dalangood, S.; Zhu, Z.; Ma, Z.; Li, J.; Zeng, Q.; Yan, Y.; Shen, B.; Yan, J.; Huang, R. Identification of glycogene-type and validation of ST3GAL6 as a biomarker predicts clinical outcome and cancer cell invasion in urinary bladder cancer. *Theranostics* **2020**, *10*, 10078–10091, doi:10.7150/thno.48711.
80. Glavey, S.V.; Manier, S.; Natoni, A.; Sacco, A.; Moschetta, M.; Reagan, M.R.; Murillo, L.S.; Sahin, I.; Wu, P.; Mishima, Y.; et al. The sialyltransferase ST3GAL6 influences homing and survival in multiple myeloma. *Blood* **2014**, *124*, 1765–1776, doi:10.1182/blood-2014-03-560862.
81. Yamamoto, H.; Oviedo, A.; Sweeley, C.; Saito, T.; Moskal, J.R. Alpha2,6-sialylation of cell-surface N-glycans inhibits glioma formation in vivo. *Cancer Res.* **2001**, *61*, 6822–6829.
82. Yuan, Q.; Chen, X.; Han, Y.; Lei, T.; Wu, Q.; Yu, X.; Wang, L.; Fan, Z.; Wang, S. Modification of α2,6-sialylation mediates the invasiveness and tumorigenicity of non-small cell lung cancer cells in vitro and in vivo via Notch1/Hes1/MMPs pathway. *Int. J. Cancer* **2018**, *143*, 2319–2330, doi:10.1002/ijc.31737.

83. Wei, A.; Fan, B.; Zhao, Y.; Zhang, H.; Wang, L.; Yu, X.; Yuan, Q.; Yang, D.; Wang, S. ST6Gal-I overexpression facilitates prostate cancer progression via the PI3K/Akt/GSK-3β/β-catenin signaling pathway. *Oncotarget* **2016**, *7*, 65374–65388, doi:10.18632/oncotarget.11699.
84. Lin, S.; Kemmner, W.; Grigull, S.; Schlag, P.M. Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. *Exp. Cell Res.* **2002**, *276*, 101–110, doi:10.1006/excr.2002.5521.
85. Antony, P.; Rose, M.; Heidenreich, A.; Knüchel, R.; Gaisa, N.T.; Dahl, E. Epigenetic inactivation of ST6GAL1 in human bladder cancer. *BMC Cancer* **2014**, *14*, 901, doi:10.1186/1471-2407-14-901.
86. Cheng, J.; Wang, R.; Zhong, G.; Chen, X.; Cheng, Y.; Li, W.; Yang, Y. ST6GAL2 Downregulation Inhibits Cell Adhesion and Invasion and is Associated with Improved Patient Survival in Breast Cancer. *Onco. Targets. Ther.* **2020**, *13*, 903–914, doi:10.2147/OTT.S230847.
87. Xu, G.; Chen, J.; Wang, G.; Xiao, J.; Zhang, N.; Chen, Y.; Yu, H.; Wang, G.; Zhao, Y. Resveratrol Inhibits the Tumorigenesis of Follicular Thyroid Cancer via ST6GAL2-Regulated Activation of the Hippo Signaling Pathway. *Mol. Ther. Oncolytics* **2020**, *16*, 124–133, doi:10.1016/j.omto.2019.12.010.
88. Murugaesu, N.; Iravani, M.; van Weverwijk, A.; Ivetic, A.; Johnson, D.A.; Antonopoulos, A.; Fearns, A.; Jamal-Hanjani, M.; Sims, D.; Fenwick, K.; et al. An in vivo functional screen identifies ST6GalNAc2 sialyltransferase as a breast cancer metastasis suppressor. *Cancer Discov.* **2014**, *4*, 304–317, doi:10.1158/2159-8290.CD-13-0287.
89. Venkitachalam, S.; Revoredo, L.; Varadan, V.; Fecteau, R.E.; Ravi, L.; Lutterbaugh, J.; Markowitz, S.D.; Willis, J.E.; Gerken, T.A.; Guda, K. Biochemical and functional characterization of glycosylation-associated mutational landscapes in colon cancer. *Sci. Rep.* **2016**, *6*, 23642, doi:10.1038/srep23642.
90. Ferrer, C.M.; Reginato, M.J. Sticking to sugars at the metastatic site: sialyltransferase ST6GalNAc2 acts as a breast cancer metastasis suppressor. *Cancer Discov.* **2014**, *4*, 275–277, doi:10.1158/2159-8290.CD-14-0075.
91. Schneider, F.; Kemmner, W.; Haensch, W.; Franke, G.; Gretschel, S.; Karsten, U.; Schlag, P.M. Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. *Cancer Res.* **2001**, *61*, 4605–4611.
92. Reticker-Flynn, N.E.; Bhatia, S.N. Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. *Cancer Discov.* **2015**, *5*, 168–181, doi:10.1158/2159-8290.CD-13-0760.

93. Miao, X.; Jia, L.; Zhou, H.; Song, X.; Zhou, M.; Xu, J.; Zhao, L.; Feng, X.; Zhao, Y. miR-4299 mediates the invasive properties and tumorigenicity of human follicular thyroid carcinoma by targeting ST6GALNAC4. *IUBMB Life* **2016**, *68*, 136–144, doi:10.1002/iub.1467.
94. Bos, P.D.; Zhang, X.H.-F.; Nadal, C.; Shu, W.; Gomis, R.R.; Nguyen, D.X.; Minn, A.J.; van de Vijver, M.J.; Gerald, W.L.; Foekens, J.A.; et al. Genes that mediate breast cancer metastasis to the brain. *Nature* **2009**, *459*, 1005–1009, doi:10.1038/nature08021.
95. Tsuchida, A.; Okajima, T.; Furukawa, K.; Ando, T.; Ishida, H.; Yoshida, A.; Nakamura, Y.; Kannagi, R.; Kiso, M.; Furukawa, K. Synthesis of disialyl Lewis a (Le(a)) structure in colon cancer cell lines by a sialyltransferase, ST6GalNAc VI, responsible for the synthesis of alpha-series gangliosides. *J. Biol. Chem.* **2003**, *278*, 22787–22794, doi:10.1074/jbc.M211034200.
96. Kannagi, R. Carbohydrate antigen sialyl Lewis a--its pathophysiological significance and induction mechanism in cancer progression. *Chang Gung Med. J.* **2007**, *30*, 189–209.
97. Ramos, R.I.; Bustos, M.A.; Wu, J.; Jones, P.; Chang, S.C.; Kiyohara, E.; Tran, K.; Zhang, X.; Stern, S.L.; Izraely, S.; et al. Upregulation of cell surface GD3 ganglioside phenotype is associated with human melanoma brain metastasis. *Mol. Oncol.* **2020**, *14*, 1760–1778, doi:10.1002/1878-0261.12702.
98. Iwasawa, T.; Zhang, P.; Ohkawa, Y.; Momota, H.; Wakabayashi, T.; Ohmi, Y.; Bhuiyan, R.H.; Furukawa, K.; Furukawa, K. Enhancement of malignant properties of human glioma cells by ganglioside GD3/GD2. *Int. J. Oncol.* **2018**, *52*, 1255–1266, doi:10.3892/ijo.2018.4266.
99. Yeh, S.-C.; Wang, P.-Y.; Lou, Y.-W.; Khoo, K.-H.; Hsiao, M.; Hsu, T.-L.; Wong, C.-H. Glycolipid GD3 and GD3 synthase are key drivers for glioblastoma stem cells and tumorigenicity. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 5592–5597, doi:10.1073/pnas.1604721113.
100. Mennel, H.D.; Bosslet, K.; Geissel, H.; Bauer, B.L. Immunohistochemically visualized localisation of gangliosides Glac2 (GD3) and Gtri2 (GD2) in cells of human intracranial tumors. *Experimental and Toxicologic Pathology* **2000**, *52*, 277–285, doi:10.1016/S0940-2993(00)80046-9.
101. Hao, J.; Zeltz, C.; Pintilie, M.; Li, Q.; Sakashita, S.; Wang, T.; Cabanero, M.; Martins-Filho, S.N.; Wang, D.Y.; Pasko, E.; et al. Characterization of Distinct Populations of Carcinoma-Associated Fibroblasts from Non-Small Cell Lung Carcinoma Reveals a Role for ST8SIA2 in Cancer Cell Invasion. *Neoplasia* **2019**, *21*, 482–493, doi:10.1016/j.neo.2019.03.009.

102. Kim, Y.J.; Kim, K.S.; Do, S.; Kim, C.H.; Kim, S.K.; Lee, Y.C. Molecular cloning and expression of human alpha2,8-sialyltransferase (hST8Sia V). *Biochem. Biophys. Res. Commun.* **1997**, *235*, 327–330, doi:10.1006/bbrc.1997.6725.

5. Thesen

1. Methylglyoxal als Glykierungsagens wirkt konzentrationsabhängig zytotoxisch auf die Meningeomzellen.
2. Methylglyoxal glykiert die Meningeomzellen konzentrationsabhängig.
3. Die Adhäsion ist durch die altersabhängige Glykierung in Meningeomzellen nicht beeinflusst.
4. Die Chemotaxis in Meningeomzellen ist durch die altersabhängige Glykierung nicht verändert.
5. Ein Zusammenhang zwischen altersabhängiger Glykierung und einem erhöhten invasiven Verhalten ist in benignen Meningeomzellen ersichtlich.
6. Die altersabhängige Glykierung verändert die Expressionsmuster der Sialyltransferasen in benignen Meningeomzellen, sodass sie indirekt oder direkt eine Rolle in der Tumorentwicklung spielen könnten.

6. Publikationsteil

6.1 Glycation of benign meningioma cells leads to increased invasion

Philipp Selke [1], Philip Rosenstock [1], Kaya Bork [1], Christian Strauss [2], Rüdiger Horstkorte [1,*] and Maximilian Scheer [1,2]

1 Institute for Physiological Chemistry, Medical Faculty, Martin-Luther-University Halle-Wittenberg, 06114 Halle (Saale), Germany; philipp.selke@uk-halle.de (P.S.); philip.rosenstock@uk-halle.de (P.R.); kaya.bork@medizin.uni-halle.de (K.B.); maximilian.scheer@uk-halle.de (M.S.); Ruediger.horstkorte@medizin.uni-halle.de (R.H.)

2 Department for Neurosurgery, University Hospital Halle, 06120 Halle (Saale), Germany; Christian.strauss@uk-halle.de (C.S.)

*Correspondence: ruediger.horstkorte@medizin.uni-halle.de; Tel.: +49-0-345-557-3873

Citation: Selke P, Rosenstock P, Bork K, Strauss C, Horstkorte R, Scheer M. Glycation of benign meningioma cells leads to increased invasion. Biol Chem. 2021 Mar 17;402(7):849-859. doi: 10.1515/hsz-2020-0376. PMID: 33725749.

Abstract: Meningiomas are the most common non-malignant intracranial tumors. Like most tumors, meningiomas prefer anaerobic glycolysis for energy production (Warburg effect). This leads to an increased synthesis of the metabolite methylglyoxal (MGO). This metabolite is known to react with amino groups of proteins. This reaction is called glycation, thereby building advanced glycation endproducts (AGEs). In this study, we investigated the influence of glycation on two meningioma cell lines, representing the WHO grade I (BEN-MEN-1) and the WHO grade III (IOMM-Lee). Increasing MGO concentrations led to the formation of AGEs and decreased growth in both cell lines. When analyzing the influence of glycation on adhesion, chemotaxis and invasion, we could show that the glycation of meningioma cells resulted in increased invasive potential of the benign meningioma cell line, whereas the invasive potential of the malignant cell line was reduced. In addition, glycation increased the E-cadherin- and decreased the N-cadherin-expression in BEN-MEN-1 cells, but did not affected the cadherin-expression in IOMM-Lee cells.

Keywords: Meningioma; intracranial tumor; glycation; methylglyoxal; MGO; AGE; advanced glycation endproducts; invasion; chemotaxis; adhesion

Introduction

Meningioma represents the most common non-malignant intracranial tumor (Goldbrunner et al., 2016; Ostrom et al., 2018; Holleczek et al., 2019; Ostrom et al., 2019).

Like many tumors, meningiomas need large amounts of glucose as primary energy source, because they mainly metabolize glucose to lactate during anaerobic glycolysis (Warburg effect) (Bharadwaj et al., 2015), which generates only low amounts of adenosine triphosphate (ATP). This changed (anaerobic) energy metabolism is one of the “hallmarks of cancer” (Gill et al., 2016). In line with this, there is a correlation between serum glucose levels and meningioma risk (Michaud et al., 2011; Edlinger et al., 2012; Wiedmann et al., 2013; Niedermaier et al., 2015). However, there are many inconsistent data suggesting a positive (Schneider et al., 2005; Schwartzbaum et al., 2005) or inverse (Bernardo et al., 2016) relationship between diabetes and serum glucose levels and the risk of meningioma. Patients with type 2 diabetes have a decreased survival after surgical resection of a WHO grade I meningioma (Nayeri et al., 2016).

Methylglyoxal (MGO) has been discussed as a possible linker between diabetes and serum glucose levels and cancer (Bellier et al., 2019), since diabetic patients and aged individuals have elevated MGO concentrations (Rabbani and Thornalley, 2015). Some authors even propose MGO as a tumor promoting agent (Bellahcène et al., 2018; Antognelli et al., 2019). Approximately 0.1 – 0.4 % of the glucose is transformed to MGO during glycolysis as a regular side product from dihydroxyacetone phosphate or glyceraldehyde-3-phosphate (Allaman et al., 2015). Importantly, MGO is 20,000 times more reactive than glucose and reacts mainly with proteins (through arginine, lysine, and cysteine residues) or to a lower degree also with DNA or lipids, thereby forming advanced glycation endproducts (AGEs) (Kalapos, 2008; Falone et al., 2012; Schalkwijk, 2015). This non-enzymatic reaction between the carbonyl groups of dicarbonyls (like MGO or glyoxal) or sugars (like glucose or fructose) and the amino groups of proteins is called glycation (Ahmed, 2005; Rabbani and Thornalley, 2008). Electrophilic carbonyl groups of glucose or other reactive sugars react with free amino groups of amino acids and forming a non-stable Schiff base. This reaction is called classical Maillard reaction. Further rearrangement leads to formation of a more stable ketosamine (Amadori product). The formation of Schiff bases and Amadori products are reversible reactions. In later reactions, they form irreversible adducts or protein crosslinks (Paul and Bailey, 1996; Ahmed, 2005). The process of producing AGEs affects all proteins including cell adhesion molecules or receptors and proteins of the extracellular matrix (Pedchenko et al., 2005; Rabbani and Thornalley, 2012).

It was previously suggested that application of MGO lead to altered adhesion and migration (Loarca et al., 2013; Antognelli et al., 2019; Nokin et al., 2019).

Cadherins represent cell adhesion molecules that mediate Ca^{2+}-dependent homophilic interaction with cadherin molecules on the surface of neighboring cells. The cytoplasmic domain binds downstream to members of the catenin protein family and regulates functions like cell-cell interactions (Harrison et al., 2011; Mège and Ishiyama, 2017). Cadherins also play an important role in the epithelial – mesenchymal-transition (EMT). The EMT describes the change of epithelial markers like E-cadherin to mesenchymal markers like N-cadherin or Vimentin and can promote migration of transformed cells (Gloushankova et al., 2017; Mendonsa et al., 2018). Decreasing E-cadherin expression is mostly associated with weakening of cell-cell adhesion in tumor progression (Rodriguez et al., 2012). Expression of N-cadherin is closely related to tumor invasion and metastasis (Cao et al., 2019).

In the present study, we analyzed differences between benign and malignant meningioma cell lines before and after glycation using MGO. We could show that physiological MGO concentrations had no effect on cell morphology, metabolic activity, chemotaxis and adhesion. However, the invasiveness of the benign meningioma cells was increased whereas the invasiveness of the malignant meningioma cells was reduced in the presence of MGO, which means that glycation resulted in a switch of benign tumor to a more aggressive tumor cell. Furthermore, glycation led to increased E-cadherin and reduced N-cadherin-expression in the benign meningioma cells.

Results

MGO-treatment of meningioma cells interferes with their morphology

First of all, we analyzed whether treatment with MGO affects meningioma cells by comparing their morphology. Therefore, we cultured both cell lines in absence or presence of MGO for 24 h. Representative micrographs for the benign BEN-MEN-1 cells are shown in figure 1 A and for the malignant IOMM-Lee cells in figure 1 B. When cells were cultured in the presence of 0.1 or 0.3 mM MGO, we could not observe any changes in the morphology compared with untreated control cells. BEN-MEN-1 cells had a typical meningothelial shape and grew in monolayers. IOMM-Lee cells were more epithelial-like and grew as multilayers. After treatment with 0.6 mM MGO, we observed less cells and morphological differences compared with the controls. When culturing cells in presence of 1.0 mM MGO, cells became round and we observed less cells and less spreading in both cultures.

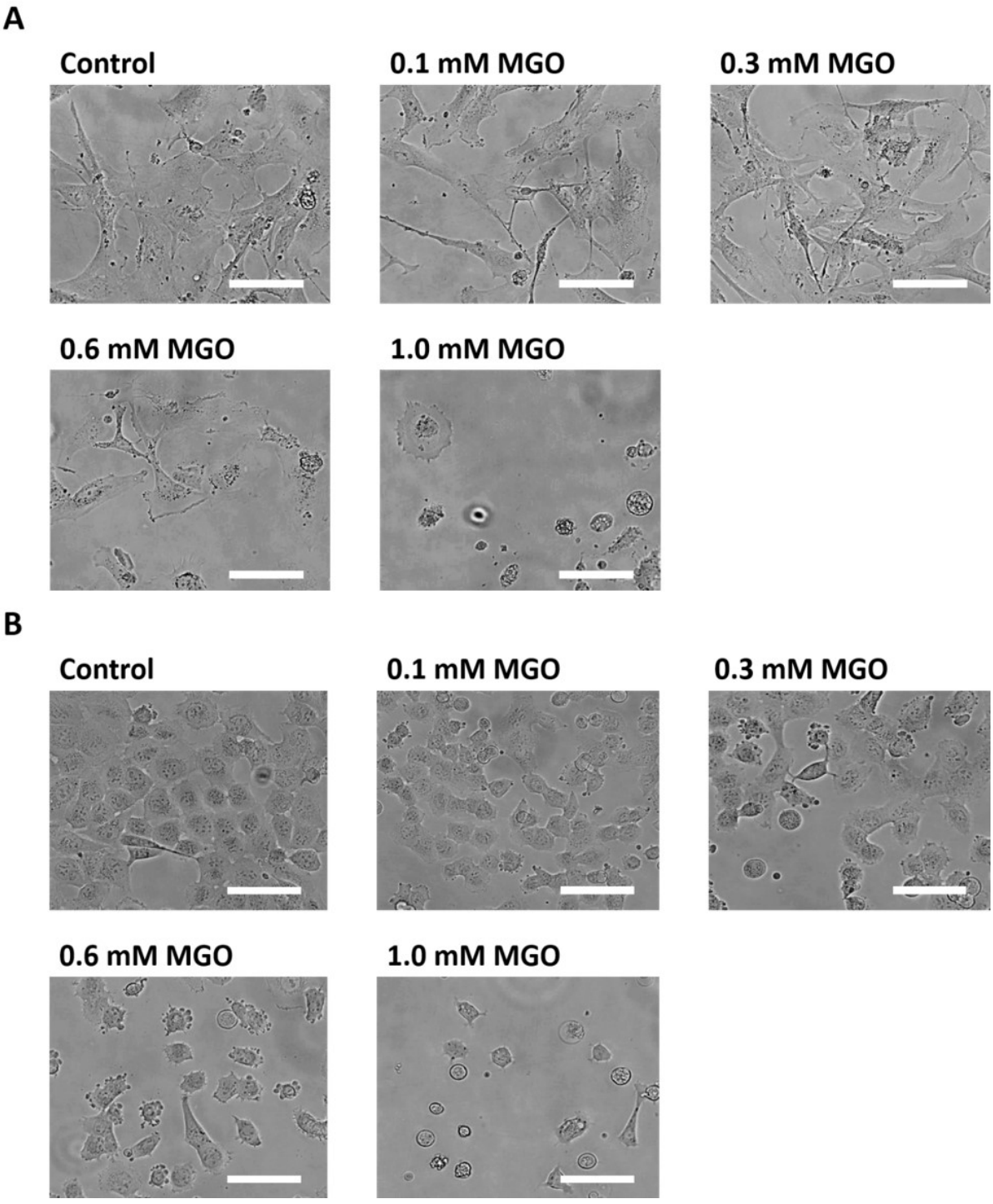

Figure 1. Micrographs of meningioma cells after 24 h MGO-treatment. Cells (**A** = BEN-MEN-1 and **B =** IOMM-Lee) were cultured in the absence (control) or presence of different concentrations (0.1 mM, 0.3 mM, 0.6 mM and 1.0 mM) of MGO. Scale bar in white: 50 µm.

High concentrations of MGO interfere with the cell viability of meningioma cells

Since morphology was changed in presence of 0.6 mM MGO or more, we examined the cell viability of both meningioma cell lines, after culturing in absence or presence of different concentrations of MGO (figure 2). We measured decreased cell viability after incubation with high concentrations of MGO. For the benign cell line, we could not show significant differences at MGO concentrations up to 0.6 mM. Only at 1.0 mM MGO, cell viability was significantly reduced compared with untreated controls (BEN-MEN-1; figure 2 A). For the malignant cell line (IOMM-Lee; figure 2 B), we observed similar effects. Only at 1.0 mM MGO, there was a significant difference compared with control, which confirmed our microscopic observations presented in figure 1 A and B. Furthermore, we analyzed caspase 3 expression by Western blot analysis. Caspase 3 was only expressed in cultures treated with 1.0 mM MGO and not in cultures treated with 0.1, 0.3 or 0.6 mM MGO (data not shown), indicating that only very high concentrations of MGO induce apoptosis in our experiments.

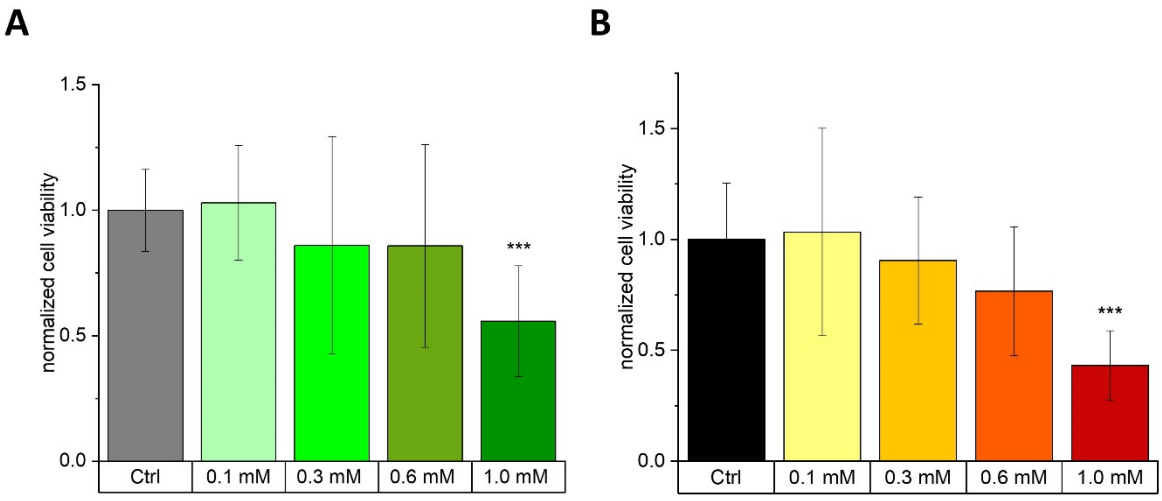

Figure 2. Cell viability of meningioma cell lines after 24 h MGO-treatment. Cell viability was analyzed using an MTT-assay for BEN-MEN-1 (**A**) and IOMM–Lee cells (**B**). Cells were seeded in absence (Ctrl) or presence of MGO (0.1 mM, 0.3 mM, 0.6 mM and 1.0 mM). Both cell lines showed a decreasing metabolic activity with increasing concentration of MGO. Statistical analysis was performed using t-test and error bars represent SD (n= 7; $p < 0.005$ = ***; (BEN-MEN-1: $p = 0.0027$; IOMM-Lee: $p = 0.00045$)).

Increased glycation with increasing concentrations of MGO

Next, we wanted to examine whether treatment of meningioma cells with MGO led to glycation. For this, we cultured both meningioma cell lines in the presence (0.1 mM, 0.3 mM, 0.6 mM and 1.0 mM) or absence of MGO for 24 h. To verify the effect of the treatment, we performed immunoblotting using anti-AGE antibodies. We could show that increasing MGO concentrations led to increasing AGE-signals, which is shown in figure 3 A and C for BEN-MEN-1 and in figure 3 B and D for IOMM-Lee cells. Glycation is quantified in figure 3 B and D. We found significant increase of glycation already at concentrations of 0.3 mM MGO (figure 3 E).

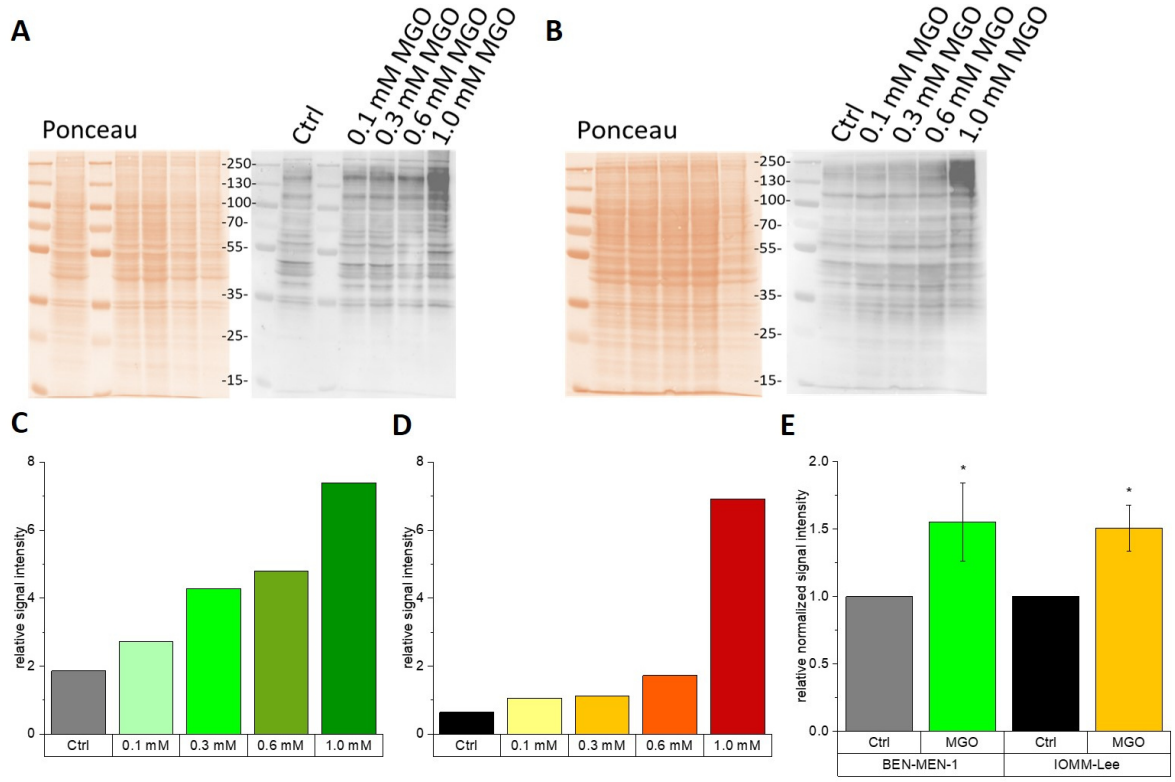

Figure 3. Glycation of meningioma cells. **A** Ponceau S staining of the total proteins (left side) and immunoblot (right side) of BEN-MEN-1 with different MGO concentrations. Controls (Ctrl) were cells without MGO treatment. We used an anti-AGE antibody to verify cellular glycation. **B**: Ponceau S staining of the total proteins (left side) and immunoblot (right side) of IOMM-Lee cells with different MGO concentrations. Controls (Ctrl) were cells without MGO treatment. We used an anti-AGE antibody to verify cellular glycation **C**: Representative quantification of the blot of BEN-MEN-1 cells shown in A. **D**: Representative quantification of the blot of IOMM-Lee cells shown in B. **E.** Quantification of glycation of BEN-MEN-1 and IOMM-Lee after treatment with 0.3 mM MGO. Non-glycated cells were used as controls (Ctrl). Statistical analysis was performed using t-test and error bars represent SD (n= 4; (BEN-MEN-1: p = 0.0452; IOMM-Lee: p = 0.0143)).

MGO has no effect on the adhesion of meningioma cells to ECM components

We then analyzed the effect of glycation on adhesion of the two meningioma cell lines. Since 0.3 mM MGO had no effect on the cell viability of both meningioma cell lines and led to significant formation of AGEs, we decided to use 0.3 mM MGO for all further experiments. To quantify adhesion, we cultured both meningioma cell lines in E-plates. Cells were seeded in absence or presence of MGO on two different matrices (collagen IV or fibronectin) which were in addition preincubated with or without MGO. **Fehler! Verweisquelle konnte nicht gefunden werden.**Figure 4 A and B show the adhesion of both meningioma cell lines on the two different matrices. No significant difference in adhesion could be detected in both cell lines after MGO treatment. Please note, that we observed that BEN-MEN-1 cells adhere much better on both substrates compared to IOMM-Lee cells and that both cancer cells prefer fibronectin to collagen IV as substrate.

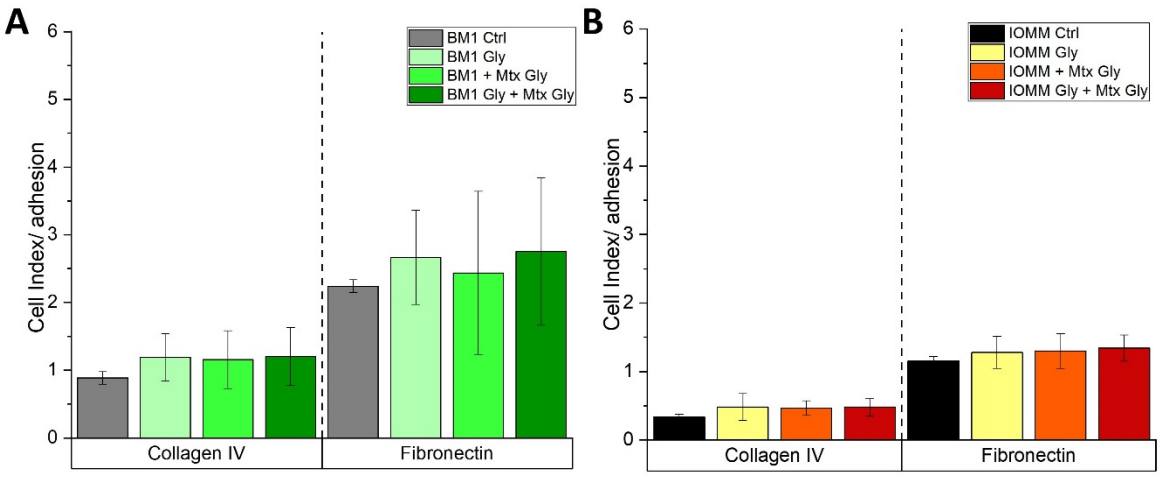

Figure 4. Adhesion of meningioma cells with MGO-treatment. BEN-MEN-1 (BM1) (**A**) and IOMM-Lee (IOMM) (**B**) cells were seeded in absence (Ctrl) or presence of 0.3 mM MGO on collagen IV (left) or fibronectin (right). Graphs display adhesion on collagen IV and fibronectin with or without treatment with untreated or treated cells after 2 h. Grey and black bars in **A** and **B** represent the untreated matrix with untreated cells as control. Untreated matrix and treated BEN-MEN-1 cells (BM1 Gly; light green bar) or treated IOMM cells (IOMM Gly; yellow bar). The green (BM1 + Mtx Gly) and orange (IOMM + Mtx Gly) bars show cell adhesion of treated matrix with untreated BEN-MEN-1 and IOMM-Lee cells. Dark green (BM1 Gly + Mtx Gly) and red (IOMM Gly + Mtx Gly) bars represent treated matrix with treated BEN-MEN-1 and IOMM-Lee cells Error bars represent SD (n = 4), (Mtx = Matrix; Gly = MGO treatment).

MGO has no effect on chemotaxis of meningioma cells

Since MGO leads to glycation of almost all cellular proteins, we next wanted to analyze whether cell surface receptors are in general inactivated after glycation. We therefore investigated the impact of glycation on the chemotaxis, which is mediated by cell surface receptors. We cultured both meningioma cell lines in CIM-plates for 24 h. Cells were cultured again in the absence or presence of 0.3 mM MGO. Figure 5 A and B show the chemotactic cell migration after 12 h or 24 h. Treatment with MGO had no effect, neither on BEN-MEN-1 (Figure 5 A) nor on IOMM-Lee cells (Figure 5 B). Please note that chemotaxis of malignant IOMM-Lee cells is higher in contrast to benign BEN-MEN-1 cells.

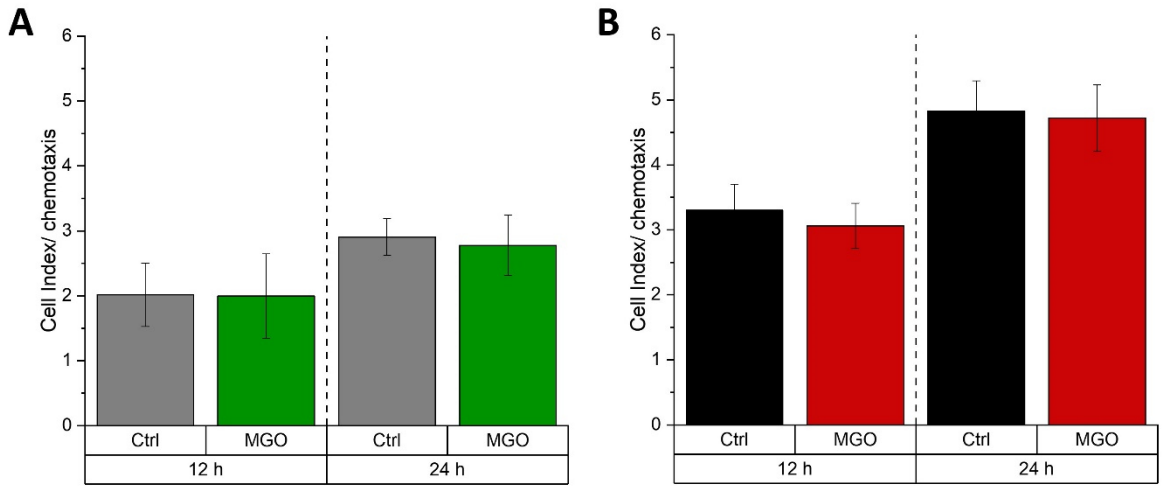

Figure 5. Chemotaxis of meningioma cells. BEN-MEN-1 (**A**) and IOMM-Lee (**B**) were cultured for 24 h in the absence (Ctrl) or presence of 0.3 mM MGO. Graphs display relative chemotaxis (presented as cell indices) with treatment (MGO) or without treatment (Ctrl) for 12 h and 24 h. Error bars represent SD (n = 4).

Glycation increases invasiveness of the benign cell line and decreases the invasiveness of malignant cell line

Since invasiveness is the most important parameter in most tumors, we finally wanted to examine whether there are changes in invasiveness due to glycation. From our chemotaxis experiments, we knew already that glycation does not interfere with the function of cellular receptors in general. We therefore cultured meningioma cell lines in CIM-plates for 48 h on matrigel in absence or presence of 0.3 mM MGO. Figure 6 A displays the invasion over 48 h for both cell lines. Untreated IOMM-Lee cells are more invasive compared with BEN-MEN-1

cells. BEN-MEN-1 (figure 6 B) cells had a significantly increased invasion after MGO-treatment after 24 h, 36 h and 48 h compared with the untreated controls. This effect could be also observed in another benign meningioma cell line (HBL-52) (data not shown). Interestingly, the malignant cell line (IOMM-Lee, figure 6 C) showed a significant reduction in their invasiveness over 48 h.

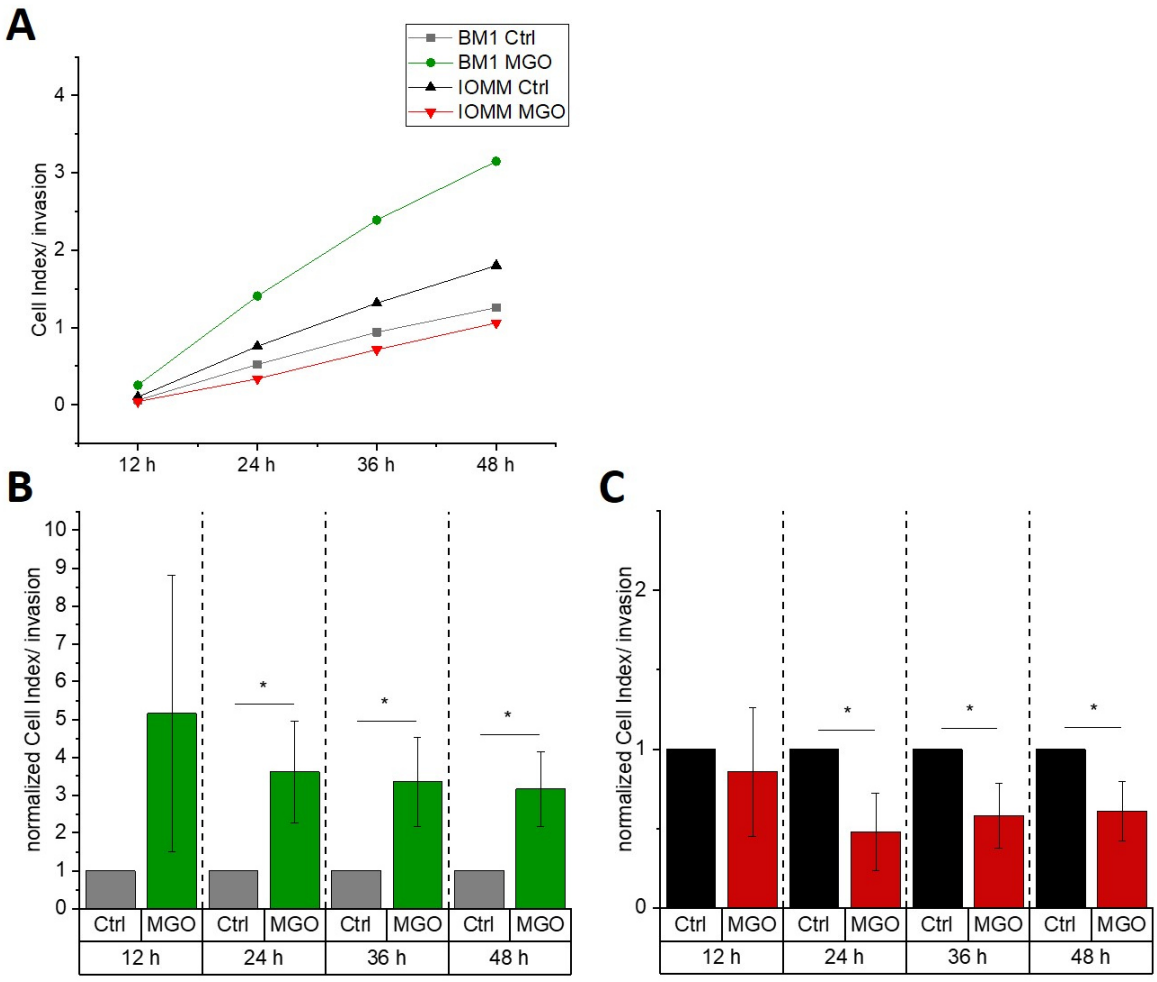

Figure 6. Invasion of meningioma cells. BEN-MEN-1 (**A, B**) and IOMM-Lee (**A**, **C**) were cultivated for 48 h in absence (Ctrl) or presence of 0.3 mM MGO. Graphs display invasion (presented as cell indices) with treatment (MGO) or without treatment (Ctrl) for 12 h, 24 h (BEN-MEN-1: $p = 0.012$; IOMM-Lee: $p = 0.0347$), 36 h (BEN-MEN-1: $p = 0.0112$; IOMM-Lee: $p = 0.0377$) and 48 h (BEN-MEN-1: $p = 0.0324$; IOMM-Lee: $p = 0.0374$). In the graphs **B** and **C** normalized cell indices were shown for both cell lines relative to the controls. Statistical analysis was performed using t-test. Error bars represent SD ($n= 4$; $p<0.05$= *).

Glycation influences the expression of E- and N-Cadherin in benign meningioma cells

Finally, we wanted to investigate whether MGO treatment influences the expression of members of the cadherin family, which are involved in epithelial-mesenchymal or mesenchymal-epithelial-transition. Therefore, we cultured meningioma cells in absence or presence of 0.3 mM MGO for 24 h and performed immunoblotting using anti-E- and N-cadherin antibodies (figure 7). The expression of E- and N-cadherin in IOMM-Lee was not changed after glycation (figure 7 A-D). However, glycation of BEN-MEN-1 cell line resulted in increasing expression of E-cadherin (figure 7 A and B) and decreasing expression of N-cadherin (figure 7 C and D).

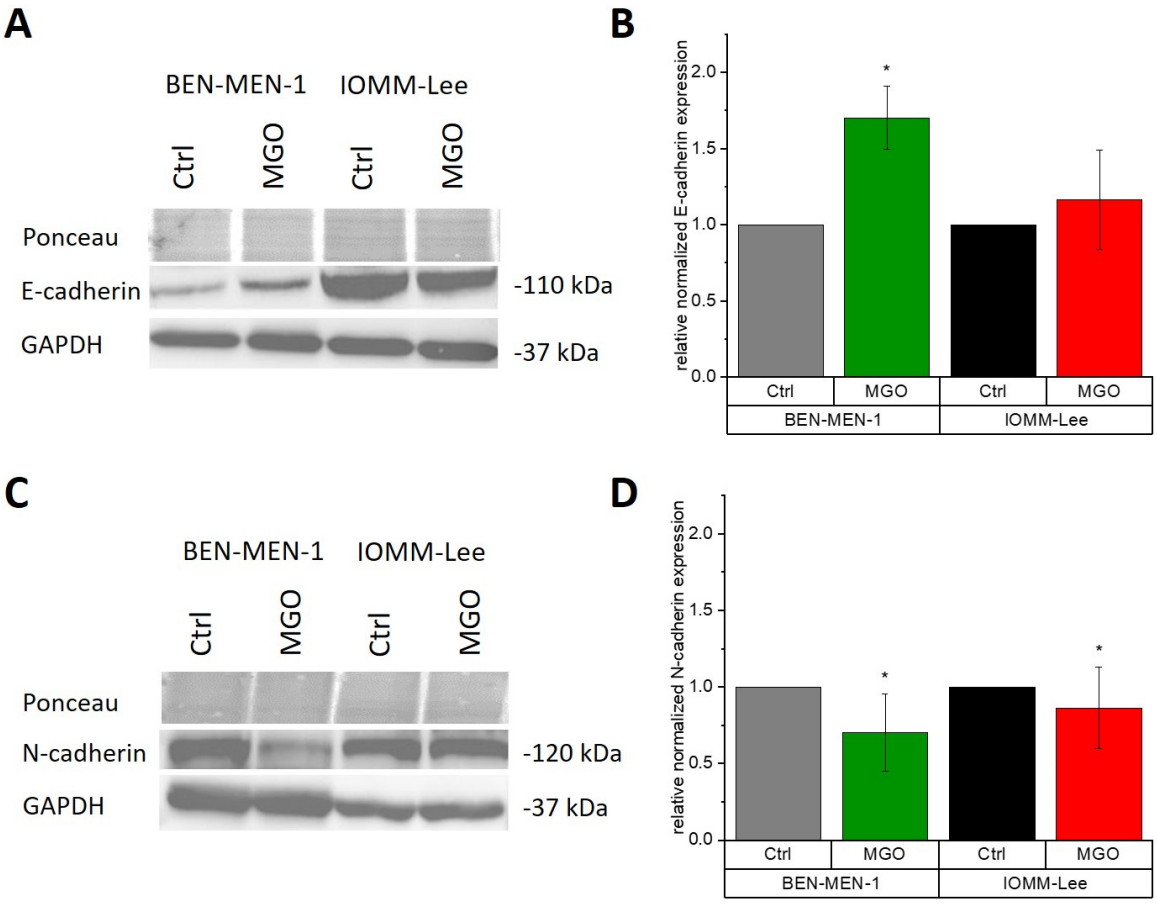

Figure 7. Cadherin expression of meningioma cells with and without MGO-treatment. **A.** BEN-MEN-1 and IOMM-Lee were cultured in the absence or presence of 0.3 mM MGO and E-Cadherin was analyzed by Westernblot. **B.** Quantification of E-Cadherin expression in BEN-MEN-1 ($p = 0.0406$) and IOMM-Lee cells before (Ctrl) and after treatment with 0.3 mM MGO (n=3). **C.** BEN-MEN-1 and IOMM-Lee were cultured in the absence or presence of 0.3 mM MGO and N-Cadherin was analyzed by Westernblot. **D.** Quantification of N-Cadherin expression in BEN-MEN-1 ($p = 0.0276$) and IOMM-Lee cells before (Ctrl) and after treatment with 0.3 mM MGO (n=7). All statistical analysis (B;D) were performed using t-test. Error bars represent SD ($p<0.05$= *).

Discussion

Little is known about the role of MGO and glycation on meningioma cells. In this study, we analyzed the effect of MGO-treatment and glycation on two meningioma cell lines, representing the WHO grade I (BEN-MEN1) and the WHO grade III (IOMM-Lee). We could show that treatment with MGO led to glycation and modulated the invasiveness of both meningioma cell lines. However, glycation did not affect cell adhesion and chemotaxis of these cell lines.

Up to 90 – 99 % of cellular MGO is bound in vivo to macromolecules; however, cellular concentrations up to 0.3 mM have been reported (Thornalley, 1996; Chaplen, 1998; Chaplen et al., 1998). Our data on cell viability using the MTT assay showed that meningioma cells have no significantly reduced metabolism at physiological concentration of 0.3 mM MGO, but are influenced by MGO at high non-physiological concentrations. This could be confirmed via microscopy, where fewer cells and less cell spreading were observed and in immunoblotting in high concentrations, we could detect the cleaved- caspase-3 activity, which is one of the key player of programmed cell death (i. e. apoptosis) (data not shown). Similar observations have been shown in human glioblastoma multiforme T98G, U87MG cells and SH-SY5Y neuroblastoma cells, where MGO interfered with proliferation (Yin et al., 2012; Paul-Samojedny et al., 2016). Another study showed that MGO affects rat schwannoma RT4 cells, PC12 cells and U87 glioma cells in cell viability by decreasing of the key signaling pathway for cell survival (gp130/STAT3 signaling) and as a consequence promotes cytotoxicity (Lee et al., 2009)

MGO led in our hands to detectable glycation of meningioma cell proteins. The influence of glycation on adhesion, migration, invasion and apoptosis of cancer cells could be demonstrated in several recent studies (Loarca et al., 2013; He et al., 2016; Scheer et al., 2020) . Our data show that cell adhesion of meningioma cells is not altered at physiological relevant MGO concentrations. Meningioma cells prefer fibronectin as substrate, what is in line with their integrin expression (α4, 5, 6 and β1, 3, 4) (preliminary data not shown), which are also known to be involved in proliferation, adhesion, migration and invasion in meningiomas (Bello et al., 2000; Chen et al., 2009; Gogineni et al., 2011; Nigim et al., 2019).

Our data on chemotaxis indicate that glycation of meningioma cells does not lead to a general loss of function. MGO-treatment seems to have cell-specific effects on behavior, since migration was reduced in liver and colon cancer cells and increased in neuroblastoma cells (Loarca et al., 2013; He et al., 2016; Scheer et al., 2020).

However, our results from the invasion of the meningioma cells indicate that MGO-treatment leads to a higher degree of invasiveness in benign meningioma cells, consequently to increased aggressiveness of meningioma cells. Antognelli et al. have shown that MGO treatment increases the invasion in anaplastic thyroid cancer (ATC) cells (Antognelli et al., 2019). This observation was also confirmed in neuroblastoma cells (Scheer et al., 2020). In liver cancer, it has been shown that invasion was inhibited by MGO treatment (Loarca et al., 2013). However, one has to keep in mind that the concentrations of MGO treatments were different in most studies. Low concentration of 5 µM MGO resulted in an increased invasion in ATC cells and a decreased invasion in liver cancer cells (Loarca et al., 2013; Antognelli et al., 2019). In neuroblastoma cells, increased invasiveness occurred after treatment with 0.1 mM MGO (Scheer et al., 2020).

Carnel et al. proposed an E-cadherin–integrin crosstalk during cancer invasion and metastasis (Canel et al., 2013). Although Utsuki and colleagues have shown that low expression of E-cadherin is associated with invasive meningioma (Utsuki et al., 2005), we found high expression of E-cadherin in the malignant grade III IOMM-Lee cells and low expression in the benign grade I BEN-MEN-1 cells (see blots in figure 7). Glycation led to increased expression of E-cadherin in BEN-MEN-1 cells, which fits nicely to the increased invasiveness after glycation. On the other hand, we found decreased expression of N-cadherin in BEN-MEN-1 cells. Asano and colleagues reported for gliomas, that an increased expression of N-cadherin correlated with a decreased invasiveness (Asano et al., 2004). Another study has shown that a decreased expression of N-cadherin resulted in a faster and less-directed migration of tumor cells (Camand et al., 2012). In our model, it appears that increased expression of E-cadherin and a decreased expression of N-cadherin are associated with a more invasive behaviour. Many studies suggest a correlation between N-cadherin expression and matrix metalloproteinase-9 (MMP-9) (Suyama et al., 2002; Walker et al., 2014; Hsu et al., 2016). Preliminary data suggest an up-regulation of MMP-9 in BEN-MEN-1 cells after treatment with 0.3 mM MGO (data not shown). Although this has to be validated, one could speculate this as one reason for the increased invasion of this cell line.

Further studies should include expression analysis of the receptor of advanced glycation endproducts (RAGE), since Dai and colleagues showed that the AGE-induced RAGE-signalling pathways promotes development and progression of meningiomas (Dai et al., 2018) and several other studies suggested also an involvement of RAGE in tumor progression (Abe and Yamagishi, 2008; Takino et al., 2010; Ahmad et al., 2018). In our hands, RAGE expression was increased in both cell lines after glycation (data not shown).

In summary, we propose that glycation has a specific effect on different cancer cells. Glycation promotes invasiveness of a WHO grade I meningioma cell line in vitro, but decreases invasive

behavior in a WHO grade III meningioma cell line. Further studies are necessary, which could include strategies for “de-glycation”, which may be important for future cancer treatment.

Materials and Methods

Cell culture

The human benign meningioma cell line BEN-MEN-1 was obtained from Leibniz-Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and the human malignant meningioma cell line IOMM-Lee (ATCC® CRL-3370™) was obtained from American Type Culture Collection (ATCC, Manassas, USA). Both cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 100 µg/mL of streptomycin, 100 U/mL of penicillin, 4 mM glutamine and 10 % fetal bovine serum (FBS, Sigma Aldrich, St. Louis, MO, USA) at 37 °C in a 5 % CO2 incubator. The cell lines were split every 2 - 3 days with 0.1 % Trypsin-EDTA (Ethylenediaminetetraacetic acid) solution for 2 min.

Cell viability and cell morphology assays

The cell viability of glycated BEN-MEN-1 and IOMM-Lee cells was measured using an MTT (Methylthiazolyldiphenyl-tetrazolium bromide, Sigma Aldrich) assay. Both cell lines were seeded into 96-well plates at a density of 7.8 x 10^4/ cm^2 cells per well in DMEM with 1 % FBS. After 2 h of attachment, cells were treated with different concentrations of MGO (Sigma Aldrich, 40% aqueous solution; diluted in 1xPBS; 0.1 mM, 0.3 mM, 0.6 mM and 1.0 mM). Controls (Ctrl) were cells (BEN-MEN-1, IOMM-Lee) without MGO treatment. Cell lines were cultivated for 24 h. Morphology of the cells was acquired with bright field microscopy (Axiovert 100, Carl Zeiss AG, Oberkochen, Germany). MTT was diluted to a final concentration of 0.5 mg/mL in normal growth medium and cells were incubated for 2 h with 100 µL MTT solution per well. After elimination of MTT-containing medium, residual formazan crystals were dissolved in 150 µL Dimethylsulfoxid (DMSO). The absorption values were measured (Plate-Reader, Clariostar, BMG Labtech GmbH, Ortenberg, Germany) at a wavelength of 570 nm (background 630 nm). The untreated control cells were set to 1 of cell viability. The changes in cell viability of the treated cells were calculated in relation to the untreated control.

Glycation and Immunoblotting

Cells were seeded in 12-well plates at a density of 3.95 x 10^4/ cm^2 in DMEM with 1 % FBS. After 2 h of attachment, the cells were treated with different concentrations of MGO (0.1 mM, 0.3 mM, 0.6 mM and 1.0 mM). Controls (Ctrl) were cells (BEN-MEN-1, IOMM-Lee) without

MGO treatment. The cell lines were cultivated for 24 h. Cells were directly lysed in hot SDS-sample buffer (2.5 % sodium dodecyl sulfate, 0.06 M TRIS (tris(hydroxymethyl)aminomethane) pH 6.8, 10 % glycerin, 0.01 %brome phenol blue, 10 mM dithiothreitol in TBS-T (TRIS buffered saline- 0.1 % tween)) to isolate the total protein. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10 %) and transferred to a nitrocellulose membrane using western blot techniques. The monoclonal anti-AGE antibody Carboxymethyllysine (CML)-26 (0.05 µg/mL, Abcam, Cambridge, UK) together with the secondary peroxidase-coupled antibody (ImmunoResearch Inc., Eagan, USA) was used to detect the glycation. Detection of E- and N-cadherin expression were done with monoclonal anti-E-cadherin antibody (0.05 µg/mL, Abcam, Cambridge, UK) and with monoclonal anti-N-cadherin antibody (0.0483 µg/mL, Abcam, Cambridge, UK) and a secondary peroxidase-coupled antibody (ImmunoResearch Inc., Eagan, USA). Images were taken using Chemidoc MP imaging system (Bio-Rad Laboratories, Hercules, USA). Ponceau S staining (0.1 % Ponceau S, 3 % trichloroacetic acid and 3 % sulfosalicylic acid) of total loaded protein and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) (0.04 µg/ml, Santa Cruz Biotechnology Inc., Dallas, USA) were used as loading controls. Band intensity of proteins of interest were transformed into numeric values using Image lab software (Bio-Rad Laboratories, Hercules) and normalized to the corresponding ponceau S staining to quantify the results.

Examination of adhesion with Real Time Cell Analysis

Fibronectin and collagen IV at a concentration of 10 µg/mL were used for each experiment and were added to the wells of the 96X E-plates® (ACEA Biosciences, San Diego, USA) and incubated for 1 h at 37 °C. E-plates® have gold microelectrode biosensors in each well of ACEA's electronic microtiter plates. After a washing step with PBS, the wells were incubated with 0.5 % BSA solution in PBS for 20 min. Before the cells and media were added, the wells were washed with PBS. The cells were trypsinized and detached. The reaction was stopped and the cells were resuspended in media with 1 % FBS. 50 µl serum-free media was added to every well in order to measure the background signal. Cells were added in density of 1.5625 x 10^4/ cm^2. The adhesion of cell lines was measured as changes in impedance with the Real Time cell electronic sensing (RT-CES®) system (ACEA Biosciences) and monitored every 15 min for a period of 4 h. The measurement was done with the Real Time Cell Analyzer dual purpose (RTCA DP) Analyzer (ACEA Biosciences,) and displayed with the Real Time Cell Analyzer (RTCA) program 2.0 (ACEA Biosciences,) as Cell Index (CI). The index is calculated as follows: CI = (impedance at time point n – impedance in the absence of cells) / nominal impedance value.

Examination of chemotaxis with Real Time Cell Analysis

Chemotaxis was analyzed in 96X Cellular invasion and migration (CIM)-plates (ACEA Biosciences). The CIM-plates are composed of an upper and a lower chamber. The bottom surface of the upper chamber consists of a microporous membrane where cells can migrate through. Underneath the membrane, a gold electrode detects the presence of adherent cells. 160 µl DMEM with 20 % FBS were added in the lower chamber. 50 µl DMEM with 1 % FBS were added in the upper chamber. CIM-plates were incubated for 1 h at 37 °C, followed by background measurement. Cells were trypsinized, detached, the reaction was stopped and the cells were resuspended in media with 1 % FBS. Cells were added to the upper chamber in density of 7.8 x 10^4/ cm^2. The chemotaxis on every label was measured as changes in impedance with the RT-CES® system and monitored every 15 min for a period of 24 h. The measurement was conducted with the RTCA DP Analyzer (ACEA Biosciences) and displayed with the RTCA program 2.0 (ACEA Biosciences).

Examination of invasion with Real Time Cell Analysis

Invasion was analyzed in 96X CIM-plates (ACEA Biosciences). The CIM-plates are composed of an upper and a lower chamber. The bottom surface of the upper chamber consists of a microporous membrane where cells can migrate through. On the underside of this membrane a gold electrode detects the presence of adherent cells. To investigate the invasion, 800 µg/ml Basement Membrane Matrix, lactose dehydrogenase-elevating virus (LDEV)-free Matrigel® (Corning, Minneapolis, MN, USA) were added in the upper chamber. After an incubation for 4 h at 37 °C, 160 µl DMEM with 20 % FBS were added in the lower chamber and 50 µl DMEM with 1 % FBS were added in the upper chamber. The CIM-plates were incubated for 1 h at 37 °C. Afterwards, the background signal was measured. Cells were trypsinized and detached. The reaction was stopped by adding media with 1 % FBS and the cells were resuspended. Cells were added to the upper chamber in density of 1.1 x 10^5/ cm^2. Invasion was measured as changes in impedance with the RT-CES® system and monitored every 15 min for a period of 48 h. The measurement was carried out with the RTCA DP Analyzer (ACEA Biosciences) and displayed with the RTCA program 2.0 (ACEA Biosciences).

Statistical analysis

All analyses and visualizations were performed using OriginPro 2019 software (OriginLab Corporation, Northampton, USA). Paired student t-test against the control group, both cell lines or a theoretical value of 1 (due to data normalization) were executed. Figures show the average mean with standard deviation (SD) and levels of significance are represented within the figures.

Acknowledgment

Special thanks to Dr. Heidi Olzscha for critical reading of the manuscript. This research was funded by Wilhelm Roux-program, FKZ 31/21 and Deutsche Forschungsgemeinschaft (DFG, ProMoAge RTG 2155).

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

Abe, R. and Yamagishi, S.-i. (2008). AGE-RAGE system and carcinogenesis. Current pharmaceutical design 14: 940–945.

Ahmad, S., Khan, H., Siddiqui, Z., Khan, M.Y., Rehman, S., Shahab, U., Godovikova, T., Silnikov, V., and Moinuddin (2018). AGEs, RAGEs and s-RAGE; friend or foe for cancer. Seminars in cancer biology 49: 44–55.

Ahmed, N. (2005). Advanced glycation endproducts--role in pathology of diabetic complications. Diabetes research and clinical practice 67: 3–21.

Allaman, I., Bélanger, M., and Magistretti, P.J. (2015). Methylglyoxal, the dark side of glycolysis. Frontiers in neuroscience 9: 23.

Antognelli, C., Moretti, S., Frosini, R., Puxeddu, E., Sidoni, A., and Talesa, V.N. (2019). Methylglyoxal Acts as a Tumor-Promoting Factor in Anaplastic Thyroid Cancer. Cells 8.

Asano, K., Duntsch, C.D., Zhou, Q., Weimar, J.D., Bordelon, D., Robertson, J.H., and Pourmotabbed, T. (2004). Correlation of N-cadherin expression in high grade gliomas with tissue invasion. Journal of neuro-oncology 70: 3–15.

Bellahcène, A., Nokin, M.-J., Castronovo, V., and Schalkwijk, C. (2018). Methylglyoxal-derived stress: An emerging biological factor involved in the onset and progression of cancer. Seminars in cancer biology 49: 64–74.

Bellier, J., Nokin, M.-J., Lardé, E., Karoyan, P., Peulen, O., Castronovo, V., and Bellahcène, A. (2019). Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. Diabetes research and clinical practice 148: 200–211.

Bello, L., Zhang, J., Nikas, D.C., Strasser, J.F., Villani, R.M., Cheresh, D.A., Carroll, R.S., and Black, P.M. (2000). Alpha(v)beta3 and alpha(v)beta5 integrin expression in meningiomas. Neurosurgery 47: 1185–1195.

Bernardo, B.M., Orellana, R.C., Weisband, Y.L., Hammar, N., Walldius, G., Malmstrom, H., Ahlbom, A., Feychting, M., and Schwartzbaum, J. (2016). Association between prediagnostic glucose, triglycerides, cholesterol and meningioma, and reverse causality. British journal of cancer 115: 108–114.

Bharadwaj, S., Venkatraghavan, L., Mariappan, R., Ebinu, J., Meng, Y., Khan, O., Tung, T., Reyhani, S., Bernstein, M., and Zadeh, G. (2015). Serum lactate as a potential biomarker of non-glial brain tumors. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia 22: 1625–1627.

Camand, E., Peglion, F., Osmani, N., Sanson, M., and Etienne-Manneville, S. (2012). N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. Journal of cell science 125: 844–857.

Canel, M., Serrels, A., Frame, M.C., and Brunton, V.G. (2013). E-cadherin-integrin crosstalk in cancer invasion and metastasis. Journal of cell science 126: 393–401.

Cao, Z.-Q., Wang, Z., and Leng, P. (2019). Aberrant N-cadherin expression in cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 118: 109320.

Chaplen, F.W. (1998). Incidence and potential implications of the toxic metabolite methylglyoxal in cell culture: A review. Cytotechnology 26: 173–183.

Chaplen, F.W., Fahl, W.E., and Cameron, D.C. (1998). Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. Proceedings of the National Academy of Sciences of the United States of America 95: 5533–5538.

Chen, J., Xu, X., and Wang, H. (2009). Expression of integrin-alpha(3) mRNA in meningiomas and its correlation with proliferation and invasion. Journal of Huazhong University of Science and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban 29: 94–96.

Dai, J., Ma, Y., Chu, S., Le, N., Cao, J., and Wang, Y. (2018). Identification of key genes and pathways in meningioma by bioinformatics analysis. Oncology letters 15: 8245–8252.

Edlinger, M., Strohmaier, S., Jonsson, H., Bjørge, T., Manjer, J., Borena, W.T., Häggström, C., Engeland, A., Tretli, S., and Concin, H., et al. (2012). Blood pressure and other metabolic syndrome factors and risk of brain tumour in the large population-based Me-Can cohort study. Journal of hypertension 30: 290–296.

Falone, S., D'Alessandro, A., Mirabilio, A., Petruccelli, G., Cacchio, M., Di Ilio, C., Di Loreto, S., and Amicarelli, F. (2012). Long term running biphasically improves methylglyoxal-related metabolism, redox homeostasis and neurotrophic support within adult mouse brain cortex. PloS one 7: e31401.

Gill, K.S., Fernandes, P., O'Donovan, T.R., McKenna, S.L., Doddakula, K.K., Power, D.G., Soden, D.M., and Forde, P.F. (2016). Glycolysis inhibition as a cancer treatment and its role in an anti-tumour immune response. Biochimica et biophysica acta 1866: 87–105.

Gloushankova, N.A., Rubtsova, S.N., and Zhitnyak, I.Y. (2017). Cadherin-mediated cell-cell interactions in normal and cancer cells. Tissue barriers 5: e1356900.

Gogineni, V.R., Nalla, A.K., Gupta, R., Gujrati, M., Klopfenstein, J.D., Mohanam, S., and Rao, J.S. (2011). α3β1 integrin promotes radiation-induced migration of meningioma cells. International journal of oncology 38: 1615–1624.

Goldbrunner, R., Minniti, G., Preusser, M., Jenkinson, M.D., Sallabanda, K., Houdart, E., Deimling, A. von, Stavrinou, P., Lefranc, F., and Lund-Johansen, M., et al. (2016). EANO guidelines for the diagnosis and treatment of meningiomas. The Lancet Oncology 17: e383-e391.

Harrison, O.J., Jin, X., Hong, S., Bahna, F., Ahlsen, G., Brasch, J., Wu, Y., Vendome, J., Felsovalyi, K., and Hampton, C.M., et al. (2011). The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. Structure (London, England : 1993) 19: 244–256.

He, T., Zhou, H., Li, C., Chen, Y., Chen, X., Li, C., Mao, J., Lyu, J., and Meng, Q.H. (2016). Methylglyoxal suppresses human colon cancer cell lines and tumor growth in a mouse model by impairing glycolytic metabolism of cancer cells associated with down-regulation of c-Myc expression. Cancer biology & therapy 17: 955–965.

Holleczek, B., Zampella, D., Urbschat, S., Sahm, F., Deimling, A. von, Oertel, J., and Ketter, R. (2019). Incidence, mortality and outcome of meningiomas: A population-based study from Germany. Cancer epidemiology 62: 101562.

Hsu, C.-C., Huang, S.-F., Wang, J.-S., Chu, W.-K., Nien, J.-E., Chen, W.-S., and Chow, S.-E. (2016). Interplay of N-Cadherin and matrix metalloproteinase 9 enhances human nasopharyngeal carcinoma cell invasion. BMC cancer 16: 800.

Kalapos, M.P. (2008). Methylglyoxal and glucose metabolism: a historical perspective and future avenues for research. Drug metabolism and drug interactions 23: 69–91.

Lee, H.K., Seo, I.A., Suh, D.J., Lee, H.J., and Park, H.T. (2009). A novel mechanism of methylglyoxal cytotoxicity in neuroglial cells. Journal of neurochemistry 108: 273–284.

Loarca, L., Sassi-Gaha, S., and Artlett, C.M. (2013). Two α-dicarbonyls downregulate migration, invasion, and adhesion of liver cancer cells in a p53-dependent manner. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 45: 938–946.

Mège, R.M. and Ishiyama, N. (2017). Integration of Cadherin Adhesion and Cytoskeleton at Adherens Junctions. Cold Spring Harbor perspectives in biology 9.

Mendonsa, A.M., Na, T.-Y., and Gumbiner, B.M. (2018). E-cadherin in contact inhibition and cancer. Oncogene 37: 4769–4780.

Michaud, D.S., Bové, G., Gallo, V., Schlehofer, B., Tjønneland, A., Olsen, A., Overvad, K., Dahm, C.C., Teucher, B., and Boeing, H., et al. (2011). Anthropometric measures, physical activity, and risk of glioma and meningioma in a large prospective cohort study. Cancer prevention research (Philadelphia, Pa.) 4: 1385–1392.

Nayeri, A., Chotai, S., Prablek, M.A., Brinson, P.R., Douleh, D.G., Weaver, K.D., Thompson, R.C., and Chambless, L. (2016). Type 2 diabetes is an independent negative prognostic factor in patients undergoing surgical resection of a WHO grade I meningioma. Clinical neurology and neurosurgery 149: 6–10.

Niedermaier, T., Behrens, G., Schmid, D., Schlecht, I., Fischer, B., and Leitzmann, M.F. (2015). Body mass index, physical activity, and risk of adult meningioma and glioma: A meta-analysis. Neurology 85: 1342–1350.

Nigim, F., Kiyokawa, J., Gurtner, A., Kawamura, Y., Hua, L., Kasper, E.M., Brastianos, P.K., Cahill, D.P., Rabkin, S.D., and Martuza, R.L., et al. (2019). A Monoclonal Antibody Against β1 Integrin Inhibits Proliferation and Increases Survival in an Orthotopic Model of High-Grade Meningioma. Targeted oncology 14: 479–489.

Nokin, M.-J., Bellier, J., Durieux, F., Peulen, O., Rademaker, G., Gabriel, M., Monseur, C., Charloteaux, B., Verbeke, L., and van Laere, S., et al. (2019). Methylglyoxal, a glycolysis

metabolite, triggers metastasis through MEK/ERK/SMAD1 pathway activation in breast cancer. Breast cancer research : BCR 21: 11.

Ostrom, Q.T., Cioffi, G., Gittleman, H., Patil, N., Waite, K., Kruchko, C., and Barnholtz-Sloan, J.S. (2019). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012-2016. Neuro-oncology 21: v1-v100.

Ostrom, Q.T., Gittleman, H., Truitt, G., Boscia, A., Kruchko, C., and Barnholtz-Sloan, J.S. (2018). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. Neuro-oncology 20: iv1-iv86.

Paul, R.G. and Bailey, A.J. (1996). Glycation of collagen: the basis of its central role in the late complications of ageing and diabetes. The International Journal of Biochemistry & Cell Biology 28: 1297–1310.

Paul-Samojedny, M., Łasut, B., Pudełko, A., Fila-Daniłow, A., Kowalczyk, M., Suchanek-Raif, R., Zieliński, M., Borkowska, P., and Kowalski, J. (2016). Methylglyoxal (MGO) inhibits proliferation and induces cell death of human glioblastoma multiforme T98G and U87MG cells. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 80: 236–243.

Pedchenko, V.K., Chetyrkin, S.V., Chuang, P., Ham, A.-J.L., Saleem, M.A., Mathieson, P.W., Hudson, B.G., and Voziyan, P.A. (2005). Mechanism of perturbation of integrin-mediated cell-matrix interactions by reactive carbonyl compounds and its implication for pathogenesis of diabetic nephropathy. Diabetes 54: 2952–2960.

Rabbani, N. and Thornalley, P.J. (2008). The dicarbonyl proteome: proteins susceptible to dicarbonyl glycation at functional sites in health, aging, and disease. Annals of the New York Academy of Sciences 1126: 124–127.

Rabbani, N. and Thornalley, P.J. (2012). Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino acids 42: 1133–1142.

Rabbani, N. and Thornalley, P.J. (2015). Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. Biochemical and biophysical research communications 458: 221–226.

Rodriguez, F.J., Lewis-Tuffin, L.J., and Anastasiadis, P.Z. (2012). E-cadherin's dark side: possible role in tumor progression. Biochimica et biophysica acta 1826: 23–31.

Schalkwijk, C.G. (2015). Vascular AGE-ing by methylglyoxal: the past, the present and the future. Diabetologia 58: 1715–1719.

Scheer, M., Bork, K., Simon, F., Nagasundaram, M., Horstkorte, R., and Gnanapragassam, V.S. (2020). Glycation Leads to Increased Polysialylation and Promotes the Metastatic Potential of Neuroblastoma Cells. Cells 9.

Schneider, B., Pülhorn, H., Röhrig, B., and Rainov, N.G. (2005). Predisposing conditions and risk factors for development of symptomatic meningioma in adults. Cancer detection and prevention 29: 440–447.

Schwartzbaum, J., Jonsson, F., Ahlbom, A., Preston-Martin, S., Malmer, B., Lönn, S., Söderberg, K., and Feychting, M. (2005). Prior hospitalization for epilepsy, diabetes, and stroke and subsequent glioma and meningioma risk. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14: 643–650.

Suyama, K., Shapiro, I., Guttman, M., and Hazan, R.B. (2002). A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2: 301–314.

Takino, J.-I., Yamagishi, S.-i., and Takeuchi, M. (2010). Cancer malignancy is enhanced by glyceraldehyde-derived advanced glycation end-products. Journal of oncology 2010: 739852.

Thornalley, P.J. (1996). Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification-A role in pathogenesis and antiproliferative chemotherapy. General Pharmacology: The Vascular System 27: 565–573.

Utsuki, S., Oka, H., Sato, Y., Kawano, N., Tsuchiya, B., Kobayashi, I., and Fujii, K. (2005). Invasive meningioma is associated with a low expression of E-cadherin and beta-catenin. Clinical neuropathology 24: 8–12.

Walker, A., Frei, R., and Lawson, K.R. (2014). The cytoplasmic domain of N-cadherin modulates MMP-9 induction in oral squamous carcinoma cells. International journal of oncology 45: 1699–1706.

Wiedmann, M., Brunborg, C., Lindemann, K., Johannesen, T.B., Vatten, L., Helseth, E., and Zwart, J.A. (2013). Body mass index and the risk of meningioma, glioma and schwannoma in a large prospective cohort study (The HUNT Study). British journal of cancer 109: 289–294.

Yin, Q.-Q., Dong, C.-F., Dong, S.-Q., Dong, X.-L., Hong, Y., Hou, X.-Y., Luo, D.-Z., Pei, J.-J., and Liu, X.-P. (2012). AGEs induce cell death via oxidative and endoplasmic reticulum stresses in both human SH-SY5Y neuroblastoma cells and rat cortical neurons. Cellular and molecular neurobiology 32: 1299–1309.

6.2 Glycation interferes with the expression of sialyltransferases in meningiomas

Philipp Selke [1,*], Kaya Bork [1], Tao Zhang [3], Manfred Wuhrer [3], Christian Strauss [2], Rüdiger Horstkorte [1] and Maximilian Scheer [1,2]

1 Institute for Physiological Chemistry, Medical Faculty, Martin-Luther-University Halle-Wittenberg, 06114 Halle (Saale), Germany; philipp.selke@uk-halle.de (P.S.); kaya.bork@medizin.uni-halle.de (K.B.); maximilian.scheer@uk-halle.de (M.S.); Ruediger.horstkorte@medizin.uni-halle.de (R.H.)

2 Department of Neurosurgery, University Hospital Halle, 06120 Halle (Saale), Germany; Christian.strauss@uk-halle.de (C.S.)

3 Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands; m.wuhrer@lumc.nl (M.W.); t.zhang@lumc.nl (T.Z.)

* Correspondence: philipp.selke@uk-halle.de; Tel.: +49-0-345-557-3814

Citation: Selke, P.; Bork, K.; Zhang, T.; Wuhrer, M.; Strauss, C.; Horstkorte, R.; Scheer, M. Glycation Interferes with the Expression of Sialyltransferases in Meningiomas. Cells 2021, 10, 3298. https://doi.org/10.3390/cells10123298;

Received: 5 November 2021 Accepted: 22 November 2021 Published: 25 November 2021

Abstract: Meningiomas are the most common non-malignant intracranial tumors and prefer, like most tumors, anaerobic glycolysis for energy production (Warburg effect). This anaerobic glycolysis leads to an increased synthesis of the metabolite methylglyoxal (MGO) or glyoxal (GO), which is known to react with amino groups of proteins. This reaction is called glycation, thereby building advanced glycation endproducts (AGEs). In this study, we investigated the influence of glycation on sialylation in two meningioma cell lines, representing the WHO grade I (BEN-MEN-1) and the WHO grade III (IOMM-Lee). In the benign meningioma cell line, glycation led to differences in expression of sialyltransferases (ST3GAL1/2/3/5/6; ST6GAL1/2; ST6GALNAC2/6, and ST8SIA1/2), which are known to play a role in tumor progression. We could show that glycation of BEN-MEN-1 cells led to decreased expression of ST3Gal5. This resulted in decreased synthesis of the ganglioside GM3, the product of ST3Gal5. In the malignant meningioma cell line, we observed changes in expression of sialyltransferases (ST3GAL1/2/3; ST6GALNAC5 and ST8SIA1) after glycation which correlates with less aggressive behavior.

Keywords: intracranial tumor; methylglyoxal; MGO; Sialylation, tumorigenesis, posttranslational modification

Introduction

Meningiomas arise from the arachnoid and are the most common non-malignant intracranial tumor [1–5]. They are classified according to WHO (World Health Organization) in grades I, II, and III. The benign grade I represents the most frequent subtype (>80%), has a low risk of recurrence and slow growth [5,6]. As opposed to benign meningioma, grade III meningiomas (anaplastic, rhabdoid, and papillary subtype) are rare (1-3 %) and little is known about factors that influence their survival and malignity. The present surgical, medicinal, and radiotherapeutic treatments are not adequate to manage the morbidity and mortality in this subtype [7–10].

Like many other tumors, meningiomas use glucose as a primary energy source (Warburg effect), which is considered as one of the "hallmarks of cancer" [11–13].

During glycolysis, up to 0.4 % of the glucose is converted into methylglyoxal (MGO). MGO is a typical side product of glyceraldehyde-3-phosphate, which is generated by the aldolase reaction from fructose-1,6-bisphosphate. Please note that MGO is more than 20,000 times more reactive than glucose [11]. Previous studies showed that MGO concentrations are elevated in diabetic and or aged individuals [14]. Many studies suggest that diabetes is linked to an increased risk of cancer [15,16]. In line with this, there is a correlation between serum glucose levels and meningioma risk [17,18]. However, there are contrary data suggesting a positive [19,20] or inverse [21] correlation between diabetes and serum glucose levels and the risk of meningioma. For example, patients with type 2 diabetes have a decreased survival after surgical resection of a WHO grade I meningioma [22].

The dicarbonyl MGO reacts primarily with proteins (through arginine, lysine, and cysteine residues) or to a small extent also with DNA and lipids. This non-enzymatic reaction between the carbonyl groups of dicarbonyls (i.e. MGO) or monosaccharides (i.e. glucose) and the amino groups of proteins is called glycation [23,24]. Another important glycating agent is glyoxal (GO), which is formed by degradation of glucose or autoxidation of glycoaldehyde to glyoxal [25]. Glycation is much stronger with dicarbonyls than with monosaccharides [26]. The endproducts of this reaction are called advanced glycation endproducts (AGEs) [27–29]. Recently, we demonstrated that glycation through MGO led to an increased invasive behavior in benign meningioma cells [30]. Several other studies propose MGO as a tumor-promoting agent [31,32].

Another common posttranslational modification is glycosylation. In contrast to glycation, glycosylation is an enzymatic addition of carbohydrates, glycans to a non-carbohydrate-structure, commonly a lipid or protein in the endoplasmatic reticulum (ER)/Golgi. Sialylation is

of deep interest and describes the addition of sialic acids (Sia) to lipids (i.e. gangliosides) or proteins (i.e. neural cell adhesion molecule (NCAM)) through sialyltransferases (ST) [33].

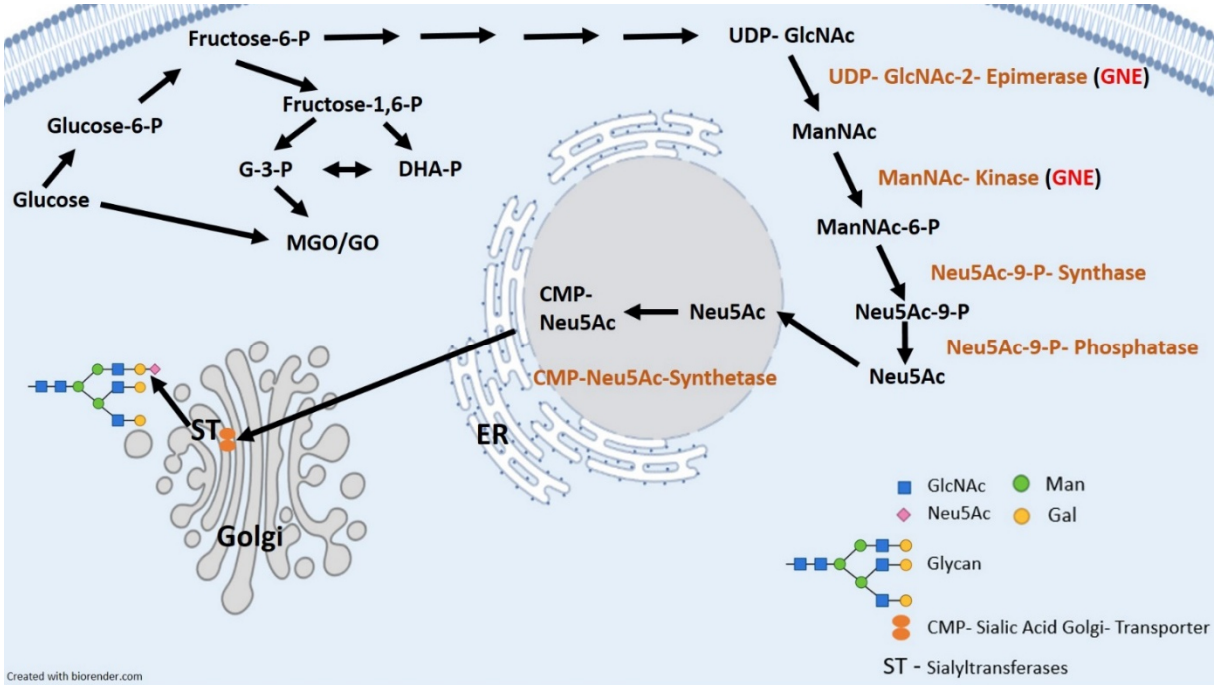

Figure 1: Schematic representation of the Sia biosynthesis from glucose to Sia and glycation agents (MGO/GO) and sialylation of glycoproteins (i.e. N-glycans, O-glycans or gangliosides) in the endoplasmatic reticulum and Golgi. G-3-P = glyceraldehyde-3-phosphate; DHA-P = Dihydroxyaceton phosphate; MGO = methylglyoxal; GO = glyoxal; GlcNAc = N-acetyl-glucosamine; Man =Mannose; Gal = Galactose; Neu5Ac = N-acetyl-neuraminic acid; GNE = UDP-N-acetyl glucosamine 2-epimerase/N-acetyl mannosamine kinase; ER = Endoplasmatic reticulum

N-acetyl neuraminic acid (Neu5Ac) represents the major Sia of mammals. It is synthesized from UDP-N-acetyl glucosamine (UDP-GlcNAc) in the cytosol [34]. The key enzyme of the Sia biosynthesis is the bifunctional UDP-N-acetyl glucosamine 2-epimerase/N-acetyl mannosamine kinase (GNE) [35]. Sialylation is taking place in the Golgi and is catalyzed by STs. They are 20 known STs in humans, which use CMP-activated Sia as substrate (figure 1). These STs are subdivided into 4 families dedicated to the carbohydrate linkages they synthesize: beta-galactoside alpha 2,3- sialyltransferases (ST3Gal1-6), beta-galactoside alpha 2,6- sialyltransferases (ST6Gal1-2), N-acetyl galactosamine (GalNAc) alpha 2,6-sialyltransferases (ST6GalNAc1-6) and alpha 2,8-sialyltransferases (ST8Sia1-6) [36,37]. The members of the ST3Gal family transfer Sia from CMP-Sia to terminal galactose residues

through 2,3 linkages, whereas the two known members of the ST6Gal family do this through 2,6 linkages. The six members of the ST6GalNAc family transfer Sia from CMP-Sia to GalNAc residues via 2,6 linkages. In addition, the ST8Sia-family transfer Sia from CMP-Sia to other terminal Sia residues by 2,8-linkages [36,37]. High blood glucose concentrations in individuals with diabetes result in a UDP-GlcNAc dependent change to more complex N-glycans [38]. Especially glycoproteins with only few N-glycosylation sites such as transforming growth factor β (TGFβ) or glucose transporter 4 (GLUT4) show rapid response to increasing GlcNAc concentrations causing complex glycan formation and branching [39]. Gangliosides are glycosphingolipids that contain Sias. The synthesis of gangliosides begins with ceramide (Cer) in the ER. During GM3-synthesis Cer will be glucosylated by the glucosylceramid synthase. After this step in the cis-golgi, glucosylceramide is converted in the trans-golgi to lactosylceramide [40]. This is the substrate for GM3-synthase (ST3Gal5). It is known that GM3 plays a role during several diseases (chronic inflammation, insulin resistance or cancer) [41–43].

In this study, we compared the expression of STs in benign and malignant meningioma cells and found significant differences between these two. Furthermore, we investigated the role of the glycating metabolite MGO on the expression of STs in both benign and malignant meningioma cells. Thereby we could show that glycation has a dramatic effect on the expression of STs and consequently on the GM3 expression. As a result, this could change sialylation-dependent tumor progression in meningioma.

Materials and Methods

Cell culture

The human benign meningioma cell line BEN-MEN-1 was obtained from Leibniz-Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and the human malignant meningioma cell line IOMM-Lee (ATCC® CRL-3370™) was obtained from American Type Culture Collection (ATCC, Manassas, USA). Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 µg/mL of streptomycin, 100 U/mL of penicillin, 4 mM glutamine, and 10 % fetal bovine serum (FBS, Sigma Aldrich, St. Louis, MO, USA) at 37 °C in a 5 % CO_2 incubator. The cell lines were split every 2 - 3 days with 0.1 % Trypsin-EDTA (Ethylenediaminetetraacetic acid) solution for 2 min.

Glycation and Real-Time PCR analysis

Cells were seeded in 12-well plates at a density of 3.95 x 10^4/ cm^2 in DMEM with 1 % FBS. After 2 h of attachment, the cells were treated with 0.3 mM MGO or GO. Controls (Ctrl) were cells (BEN-MEN-1, IOMM-Lee) without MGO or GO treatment. The cell lines were cultivated for 24 h. RNA was isolated using the Quick-RNA™ MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The quality and concentration of the RNA were analyzed using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). RNA (2 µg) was transcribed into cDNA using SuperScript™ II Reverse Transcriptase according to the manufacturer's instructions. PCR reactions were performed using DreamTaq DNA polymerase (Thermo Fisher Scientific), and products were separated on a 1.5 % agarose gel. The following conditions were used: initial denaturation for 2 min at 95 °C, 35 cycles (30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C), final elongation for 5 min at 72 °C. We use for all sialyltransferases the same primer pairs which were used in a previous study [44].

The sialyltransferase expression of untreated meningioma cell lines (BEN-MEN-1; IOMM- Lee) and after 24 h of glycation with 0.3 mM MGO were measured via quantitative real-time PCR (qPCR) using the iQ™ 5 Multicolor Real-Time PCR Detection System (Biorad, Hercules, CA, USA) and qPCR GreenMaster (Jena Bioscience, Jena, Germany) with the same primer pairs used for normal PCR. The following conditions were used for qPCR: initial denaturation for 1:30 min at 95 °C, 40 cycles (10 s at 95 °C, 10 s at 62 °C, 25 s at 72 °C), final elongation for 1 min at 72 °C followed by a melting curve analysis. The expression level of sialyltransferases in control and glycated cell lines was determined relatively to GAPDH (165 bp; fw: GGAGCGAGATCCCTCCAAA; rv: ATGACGAACATGGGGGCATC), calculated as ΔCT. The control was relatively computed to glycated cell line ($2^{-\Delta\Delta CT}$). All reactions were performed in triplicates.

Cultivation of BEN-MEN-1 cells and Preparation of GSL-glycan alditols released from BEN-MEN-1 cells

Extraction of GSLs and preparation of GSL-glycan alditols from cells were performed in triplicate as previously described [45]. The cells were cultivated until 80 % of confluence and followed by 24 h treatment with and without 0.3 mM MGO. Shortly, 2x 10^6 cells were harvested, washed and resuspended with 200 µL of water. The cell samples were lysed by vortexing and sonication for 30 min. In this step, 2.5 µL of 0.5 µM ganglioside GT1b in ethanol was added as a spiked internal standard to monitor sample preparation and to normalize roughly absolute quantification. Chloroform (550 µL) was added to the samples followed by 15 min sonication. Methanol (350 µL) was added to the cell pellets and incubated for 4 h with shaking at room

temperature. The upper phase containing GSLs was collected after centrifugation at 2700 × g for 20 min. Then, 400 µL of chloroform/methanol (2:1, v/v) was added, followed by adding 400 µL of methanol/water (1:1, v/v). After sonication and centrifugation, the upper phase was collected and pooled to the previous sample. The process of adding methanol/water (1:1, v/v), sonication, centrifugation and removing upper phase was repeated another two times. In each replicate, the upper phase was collected and replaced by the same volume of methanol/water (1:1, v/v). The combined upper phases were dried under vacuum in an Eppendorf Concentrator 5301 (Eppendorf, Hamburg, Germany) at 30°C.

Before the purification of the GSLs using reverse- phase (RP) SPE, the samples were dissolved in 100 µL methanol followed by the addition of 100 µL water. TC18-RP- cartridges were prewashed with 2 mL of chloroform/methanol (2:1, v/v), 2 mL of methanol followed by equilibration with 2 mL methanol/water (1:1, v/v). The extracted GSLs were loaded to the cartridge and washed with 2 mL methanol/water (1:1, v/v). The GSLs were eluted from the column with 2 mL methanol and 2 mL chloroform/methanol (2:1, v/v). The samples were dried under vacuum in an Eppendorf Concentrator at 30°C.

To release the glycans from the GSLs, a mixture of EGCase I (12 mU, 2 µL), EGCase I buffer (4 µL) and water (34 µL) (pH 5.2) was added to each sample and incubated for 36 h at 37°C. The released glycans were collected and loaded on TC18-RP-cartridges, which had been preconditioned with 2 mL of methanol and 2 mL of water. The samples were washed with 200 ul of water and residual glycans were loaded to the cartridge. Then, 500 µL of water was added to the cartridge to wash the glycans from the column. The flow-through and wash fractions were pooled and dried in an Eppendorf Concentrator at 30°C.

The reduction was carried out with slight modifications following the same procedure as described in previous work [45,46]. In brief, GSL-glycans were reduced to alditols in 20 µL of sodium borohydride (500 mM) in potassium hydroxide (50 mM) for 2 h at 50°C. Subsequently, 2 µL of glacial acetic acid was added to acidify the solution and quench the reaction. The desalting of GSL-glycans was performed as previously described. Glycan alditols were eluted with 50 µL of water twice. The combined flow-through and eluate were pooled and dried under vacuum in an Eppendorf Concentrator at 30°C. The carbon SPE clean-up was performed and the purified glycan alditols were re-suspended in 20 µL of water prior to *Porous Graphitized Carbon (PGC) nano-Liquid Chromatography (LC)- Electro Spray Ionization (ESI)-Mass Spectrometry (MS)/MS* /MS analysis.

Analysis of GSL-glycan alditols using PGC nano-LC-ESI-MS/MS

The analysis of glycan alditols was performed using PGC nano-LC-ESI-MS/MS following a method described previously [45,46]. Measurements were performed on an Ultimate 3000 Ultra High Performance Liquid Chromatography (UHPLC) system (Thermo) equipped with a home-packed PGC trap column (5 µm Hypercarb, 320 µm x 30 mm) and a home-packed PGC nano-column (3 µm Hypercarb 100 µm x 150 mm) coupled to an amaZon ETD speed ion trap (Bruker, Bremen, Germany). Mobile phase A consisted of 10 mM ABC, while mobile phase B was 60% (v/v) acetonitrile/10 mM ABC. The trap column was packed with 5 µm particle size PGC stationary phase from Hypercarb PGC analytical column (size 100 × 4.6 mm, 5 µm particle size, Thermo) while the PGC nano-column was packed with 3 µm particle size PGC stationary phase from Hypercarb PGC analytical column (size 30 × 4.6 mm, 3 µm particle size, Thermo).

To analyze glycans, 2 µL injections were performed and trapping was achieved on the trap column using a 6 µL/min loading flow in 1% solvent B for 5 min. Separation was achieved with a linear gradient from 1% to 50% solvent B over 73 min was applied followed by a 10 min wash step using 95% of B at a 0.6 µL/min flow rate. The column was held at a constant temperature of 35°C.

Ionization was achieved using the nanoBooster source (Bruker) with a capillary voltage of 1000 V applied and a dry gas temperature of 280°C at 3 L/min and isopropanol enriched nitrogen at 3 psi. MS spectra were acquired within an *m/z* range of 340-1850 in enhanced mode using negative ion mode, smart parameter setting was set to *m/z* 900. MS/MS spectra were recorded using the top 3 highest intensity peaks.

Structures of detected glycans were studied by MS/MS in negative mode. Glycan structures were assigned based on the known MS/MS fragmentation patterns in negative-ion mode [47,48], elution order, and general glycobiological knowledge, with help of Glycoworkbench [49] and Glycomod [50] software. To get an estimate of the glycan amount per cell, glycan intensity was normalized to the intensity of the internal standard GT1b. Then, assuming complete release of glycans and similar response factors between released glycan and GT1b standard, the number of glycans per cell was estimated.

Structures are depicted according to the Consortium of Functional Glycomics (CFG). Blue square is *N*-acetylglucosamine; yellow square is *N*-acetylgalactosamine; blue circle is glucose; yellow circle is galactose; red triangle is fucose; purple diamond is *N*-acetylneuraminic acid, grey diamond is *N*-glycolylneuraminic acid.

Statistical analysis

All analyses and visualizations were performed using OriginPro 2019 software (OriginLab Corporation, Northampton, USA). Paired student t-test against the control group, both cell lines of a theoretical value of 1 (due to data normalization) were executed. Figures show the average mean with standard deviation (SD) and levels of significance are represented within the figures.

Results

Expression of sialyltransferases in meningioma cell lines

Since there is evidence that sialyltransferases have an impact on tumorigenesis, we analyzed benign (BEN-MEN-1) and malignant (IOMM-Lee) meningioma cell lines regarding differences in expression of sialyltransferases (figure 2, table 1). Figure 2A shows the expression of ST3GAL1-6 in BEN-MEN-1 and IOMM-Lee. ST3GAL1-ST3GAL3 and ST3GAL5-ST3GAL6 were detected in both cell lines. The band intensity of ST3GAL2 was higher in the malignant cell line compared with the benign cell line whereas the ST3GAL3, ST3GAL5-6 band intensities were higher in BEN-MEN-1 compared to the malignant cell line. Agarose gel of ST6GAL1-2 is shown in figure 2B for both cell lines. ST6GAL2 was only expressed in the benign cell line. In contrast, no differences could be found in terms of band intensity of ST6GAL1 in both cell lines. The expression of ST6GALNAC1-6 for both cell lines is shown in figure 2C. In contrast to IOMM-Lee, a weak band in ST6GALNAC2 was detectable in BEN-MEN-1. Expression of ST6GALNAC4-6 was detectable in both cell lines. The band intensities of ST6GALNAC5 and ST6GALNAC6 were higher in IOMM-Lee compared to BEN-MEN-1. The expression of ST8SIA1-6 in both cell lines is shown in figure 2D. For ST8SIA1-2 and ST8SIA5-6, the expression has been detected in both meningioma cell lines. In BEN-MEN-1 we observed a higher expression of ST8SIA2 and ST8SIA6 compared to the malignant cell line. Again, the band intensity of ST8SIA5 was stronger in IOMM-Lee compared to the benign cell line.

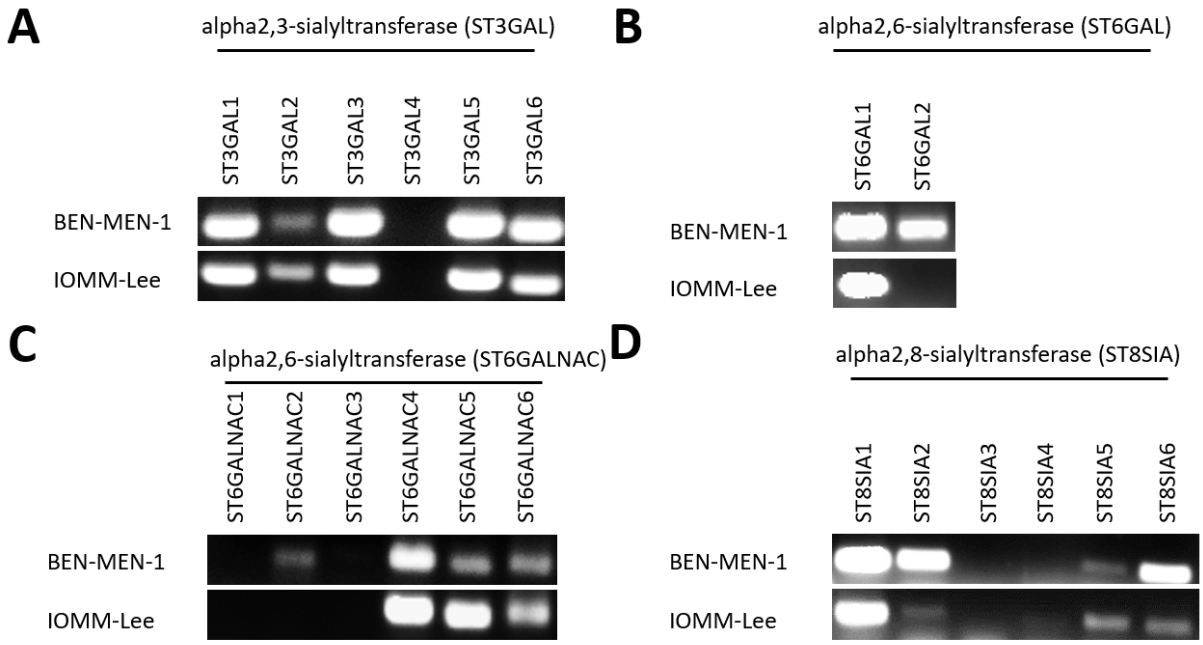

Figure 2: Expression of 20 Sialyltransferases (agarose gel) in BEN-MEN-1 and IOMM-Lee. Figure 2**A**: Expression of ST3GAL1-6. Figure 2**B:** Expression of ST6GAL1-2, Figure 2**C:** Expression of ST6GALNAC1-6, Figure 2**D:** Expression of ST8SIA1-6.

Table 1: Overview of sialyltransferase expressions in both meningioma cell lines

GENE	BEN-MEN-1	IOMM-Lee
ST3GAL1	+++	+++
ST3GAL2	+	++
ST3GAL3	+++	+++
ST3GaAL4	-	-
ST3GAL5	+++	+++
ST3GAL6	+++	+++
ST6GAL1	+++	+++
ST6GAL2	++	-
ST6GALNAC1	-	-
ST6GALNAC2	+	-

ST6GALNAC3	-	-
ST6GALNAC4	+++	+++
ST6GALNAC5	++	+++
ST6GALNAC6	++	++
ST8SIA1	+++	+++
ST8SIA2	+++	+
ST8SIA3	-	-
ST8SIA4	-	-
ST8SIA5	+	+
ST8SIA6	+++	+

Sialyltransferases are more affected by MGO in benign cell line

Since the expression of sialyltransferases is different in the benign and malignant meningioma cell lines, we quantified the sialyltransferase mRNA expression level after 24 h of glycation of the cells to verify the influence of glycation on sialylation. Figure 3 displays the different mRNA expressions of ST3GAL1-6 in BEN-MEN-1 (figure 3A) and IOMM-Lee (figure 3B). Glycation led to changes in ST expression. In the benign cell line, we observed an increased overall expression, whereas we noticed a decreased overall expression of STs in the malignant cell line. ST3GAL1 (1.4812 ± 0.115 fold change), ST3GAL2 (3.143 ± 0.476 fold change), and ST3GAL3 (1.28 ± 0.189 fold change) expression were increased in contrast to non-glycated cells in BEN-MEN-1. Furthermore, the relative expression of ST3GAL5 (0.7863 ± 0.0933 fold change) and ST3GAL6 (0.572 ± 0.126 fold change) were decreased in contrast to non-glycated cells. The relative expression of ST3GAL1 (0.601 ± 0.223 fold change), ST3GAL2 (0.288 ± 0.0535.fold change), ST3GAL3 (0.6175 ± 0.217 fold change), ST3GAL5 (0.4561 ± 0.1271 fold change), ST3GAL6 (0.502 ± 0.1325 fold change) were decreased in contrast to non-glycated cells in the malignant cell line.

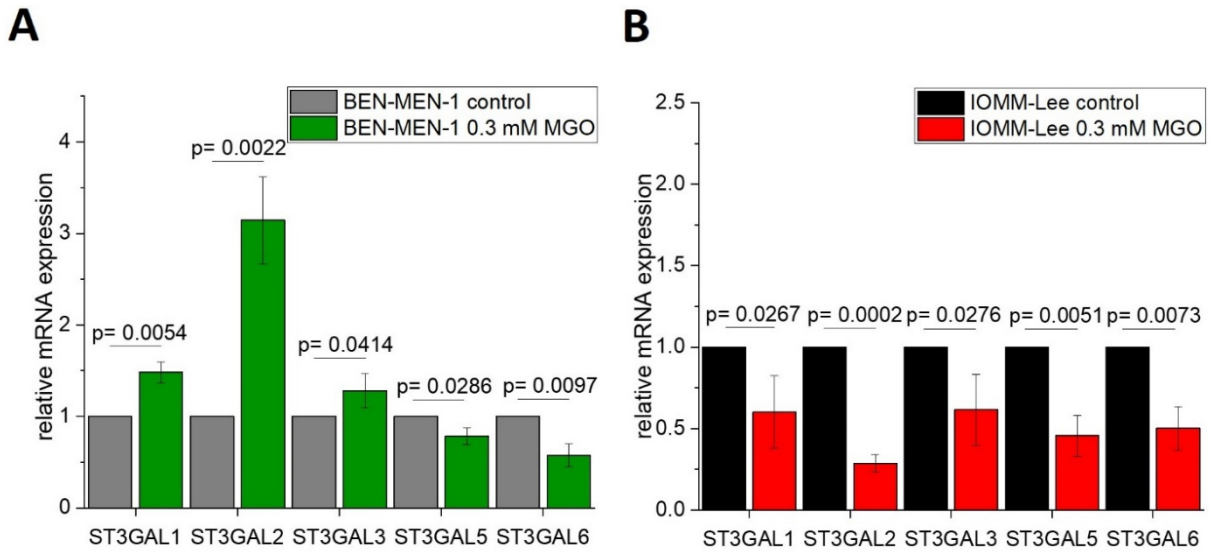

Figure 3: Relative mRNA expression of ST3GAL1-6 in BEN-MEN-1 (**A**) and IOMM- Lee (**B**). Figure 3**A**: Normalized control (**grey**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**green**). Figure 3**B:** Normalized control (**black**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**red**). Statistical analysis was performed using t-test and error bars represent SD (n= 4; (ST3GAL1: p = 0.0054 (**A**), p = 0.0267 (**B**); ST3GAL2: p = 0.0022 (**A**), p = 0.0002 (**B**); ST3GAL3: p = 0.0414 (**A**), p = 0.0276 (**B**); ST3GAL5: p = 0.0286 (**A**), p = 0.0051 (**B**); ST3GAL6: p = 0.0097 (**A**), p = 0.0073 (**B**)).

Figure 4 shows the expression of ST6GAL-family in BEN-MEN-1 (figure 4A) and IOMM-Lee (figure 4B). Glycation led to opposing changes in the expression of this sialyltransferase. We observed a higher expression in the benign cell line after treatment with MGO (3.2402 ± 0.962 fold change) whereas no changes could be measured in the glycated malignant cell line (1.018 ± 0.164 fold change). The expression of ST6GAL2 was only detected in BEN-MEN-1 and increased after treatment with MGO (1.624 ± 0.188 fold change).

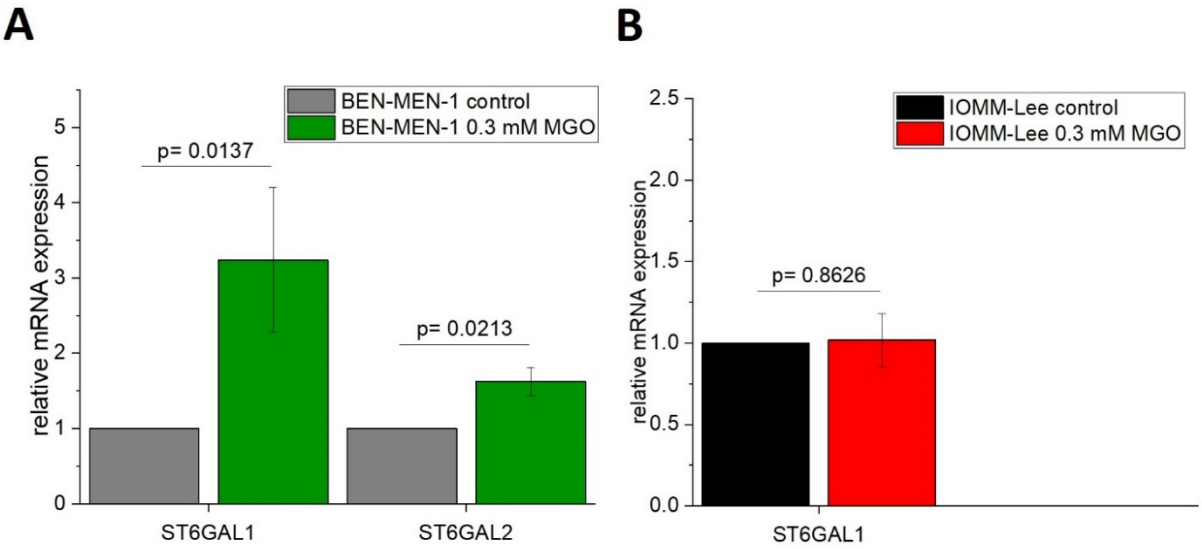

Figure 4: Relative mRNA expression of ST6GAL1-2 in BEN-MEN-1 (**A**) and IOMM- Lee (**B**). Figure 4**A**: Normalized control (**grey**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**green**). Figure 4**B**: Normalized control (**black**) and the mRNA expression after 24 h treatment with 0.3 mM MGO (**red**). Statistical analysis was performed using t-test and error bars represent SD (n= 4; (ST6GAL1: p = 0.0274 (**A**), p = 0.863 (**B**); ST6GAL2: p = 0.0213 (**A**)).

Moreover, the mRNA expression of ST6GALNAC1-6 in BEN-MEN-1 (figure 5A) and IOMM-Lee (figure 5B) is also differently altered after glycation. ST6GALNAC2 expression decreased after glycation in contrast to the untreated benign cell line (0.6807 ± 0.1106 fold change). ST6GALNAC4 expression is not affected in BEN-MEN-1 (2.556 ± 1.232 fold change) and IOMM-Lee (1.005 ± 0.2552 fold change), but glycation decreased the mRNA expression of ST6GALNAC5 in both cell lines (0.5575 ± 0.283; 0.5991 ± 0.2174). Glycation led to a higher expression of ST6GALNAC6 in the benign cell line (1.5141 ± 0.1999). ST6GALNAC6 expression is not influenced by glycation in the malignant cell line (0.839 ± 0.203).

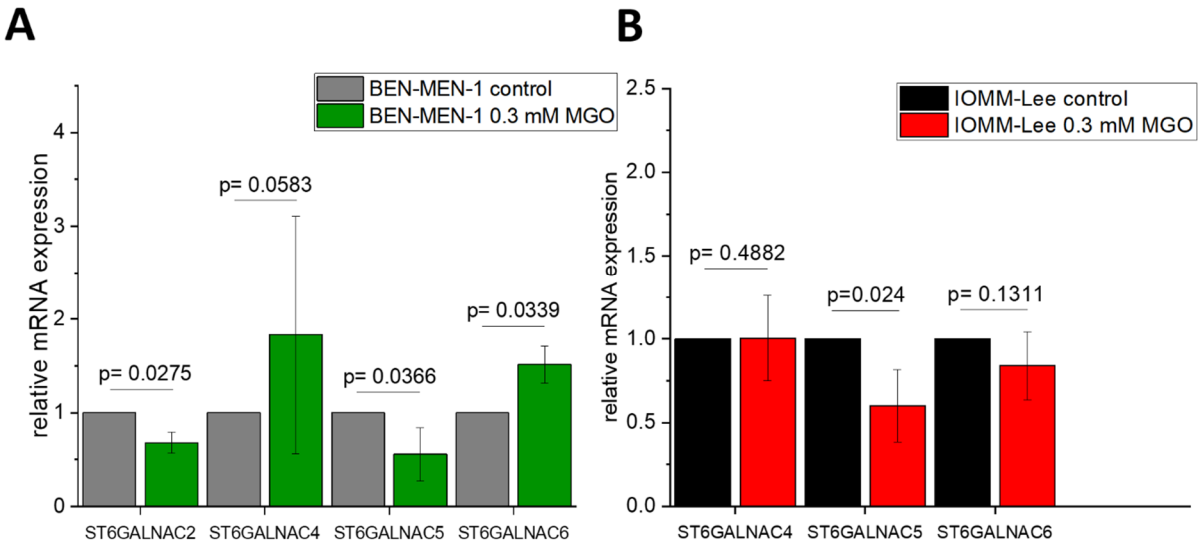

Figure 5: Relative mRNA expression of ST6GALNAC1-6 in BEN-MEN-1 (**A**) and IOMM- Lee (**B**). Figure 5**A:** Normalized control (**grey**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**green**). Figure 5**B:** Normalized control (**black**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**red**). Statistical analysis was performed using t-test and error bars represent SD (n= 4; (ST6GALNAC2: p = 0.0275 (**A**); ST6GALNAC4: p = 0.0583 (**A**), p = 0.4882 (**B**); ST6GALNAC5: p = 0.0366 (**A**), p = 0.024 (**B**); ST6GALNAC6: p = 0.0339 (**A**), p = 0.1311 (**B**)).

Finally, we quantified the expression of ST8SIA 1-6. Glycation influenced more strongly the expression level of these sialyltransferases in BEN-MEN-1 cells compared to the malignant IOMM-Lee cell line. The expression of ST8SIA1 was highly increased (2.696 ± 0.627 fold change) after glycation in the benign cell line (figure 6A) and decreased (0.744 ± 0.07712 fold change) in the glycated malignant cell line (figure 6B). The expression of ST8SIA2 (3.2171 ± 0.6837 fold change) and ST8SIA5 (1.696 ± 0.3475 fold change) were both increased in BEN-MEN-1 (figure 6A). ST8SIA5 expression was not influenced by glycation in IOMM-Lee cells. The expression of ST8SIA6 was not influenced by glycation in both cell lines.

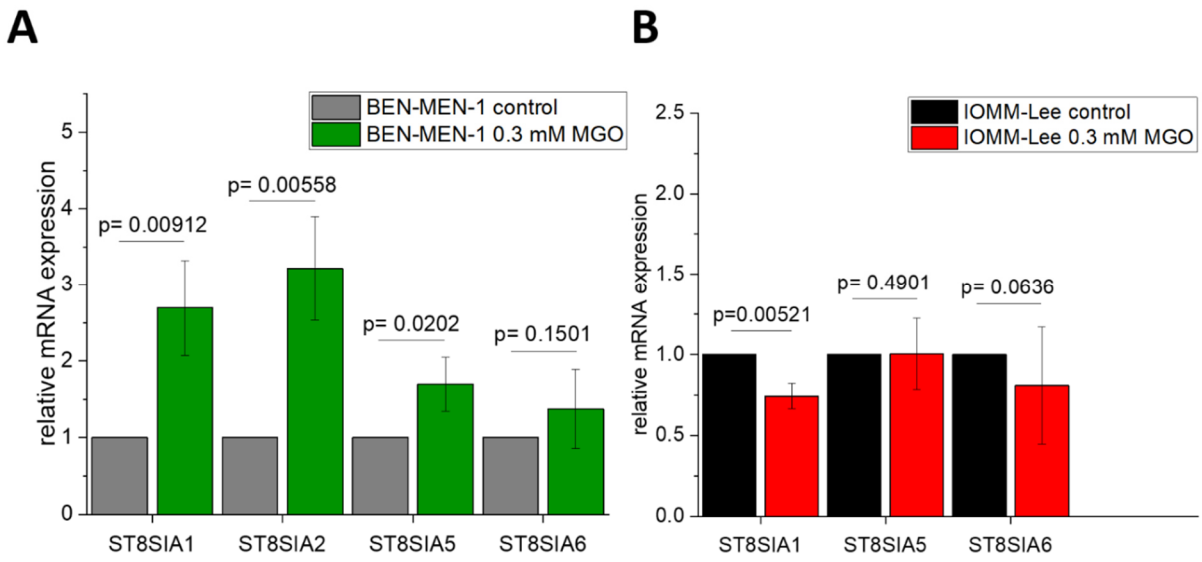

Figure 6: Relative mRNA expression of ST8SIA1-6 in BEN-MEN-1 (**A**) and IOMM- Lee (**B**). Figure 6**A**: Normalized control (**grey**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**green**). Figure 6**B:** Normalized control (**black**) and mRNA expression after 24 h treatment with 0.3 mM MGO (**red**). Statistical analysis was performed using t-test and error bars represent SD (n= 4; (ST8SIA1: p = 0.00912 (**A**), p = 0.00521 (**B**); ST8SIA2: p = 0.00558 (**A**); ST8SIA5: p = 0.0202 (**A**), p = 0.4901 (**B**); ST8SIA6: p = 0.1501 (**A**), p = 0.0636 (**B**)).

MGO-treatment decreases ganglioside GM3 expression in BEN-MEN-1

To proof, that MGO-induced reduction of ST3GAL5 expression has an impact on BEN-MEN-1 cells, we quantified ganglioside GM3 by PGC nano-LC-ESI-MS/MS. Figure 7A summarizes the biosynthesis of GM3. Figure 7B shows the signal intensity of GM3 before and after glycation in comparison to the internal standard of GT1b in BEN-MEN-1 cells. The absolute quantification of GM3 is shown in figure 7C. We could show a decreased GM3 expression after glycation (p= 0.00314), which is in line with the decreasing expression of ST3GAL5 (see: figure 3A). The normalized copy number of GM3 per cell in the untreated BEN-MEN-1 cell line ($1.32x10^8 \pm 9.15x10^6$) were decreased by 235% compared to the glycated cells ($5.61x10^7 \pm 3.89x10^6$).

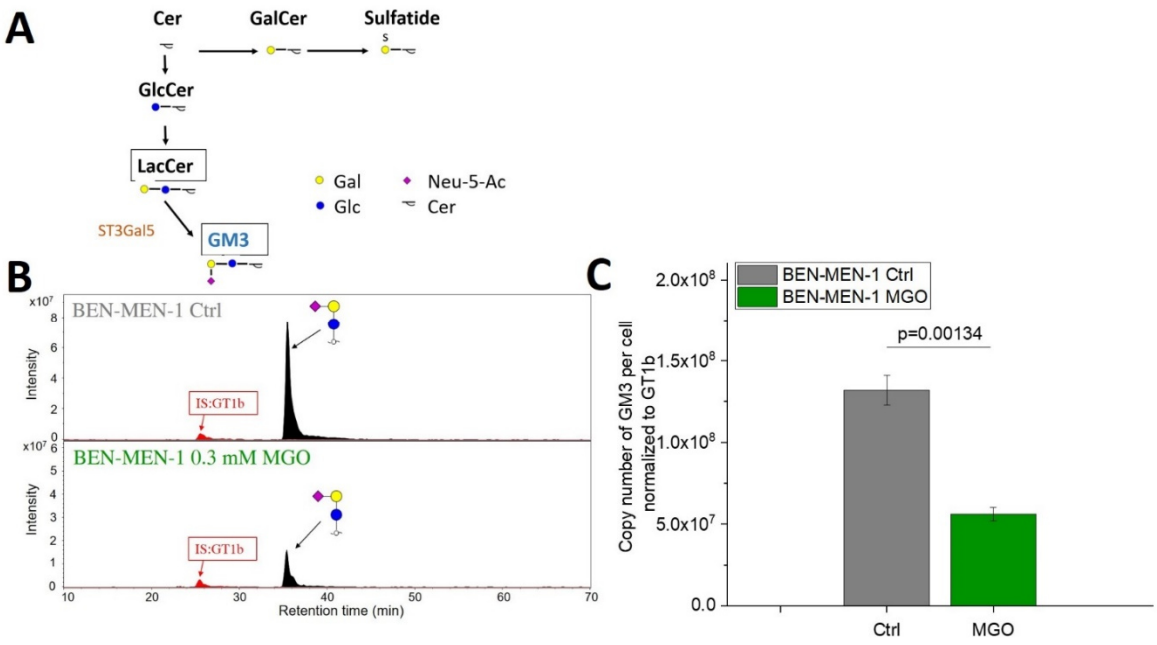

Figure 7: Absolute quantification of GM3 signal by PGC nano-LC-ESI-MS/MS. Figure 7**A**: Schematic representation of the GM3 biosynthesis. Cer = Ceramide; GalCer = Galactosylceramide; GlcCer = Glucosylceramide; LacCer = Lactosylceramide; GM3 = Monosialoganglioside 3. Figure 7**B**: Signal intensity of GM3 in BEN-MEN-1 Ctrl and BEN-MEN-1 0.3 mM MGO in comparison to the internal standard GT1b. Figure 7**C**: Absolute quantification of GM3 signal by PGC nano-LC-ESI-MS/MS of Ctrl (**grey**) and 0.3 mM MGO-treated BEN-MEN-1 (**green**). Statistical analysis was performed using t-test and error bars represent SD (n= 3; p = 0.00134).

Glyoxal-treatment has different effects in ST3GAL5

Finally, we analyzed whether another glycation agent than MGO has the same effect on ST3GAL5 expression as MGO. We could show by Westernblot analysis that 0.3 mM glyoxal (GO) leads to glycation in both cell lines (data not shown). Using qPCR of cDNA of BEN-MEN-1 cells (figure 8A) or IOMM-Lee (figure 8B), which were grown for 24 h in the presence of 0.3 mM GO, we could show that GO-induced glycation had the same effect on ST3GAL5 expression as MGO treatment in BEN-MEN-1 cells. However, GO did not alter the expression of ST3GAL5 in malignant IOMM-Lee cells, which is in contrast to MGO. This suggests a glycation agent-specific change of ST3GAL5 expression.

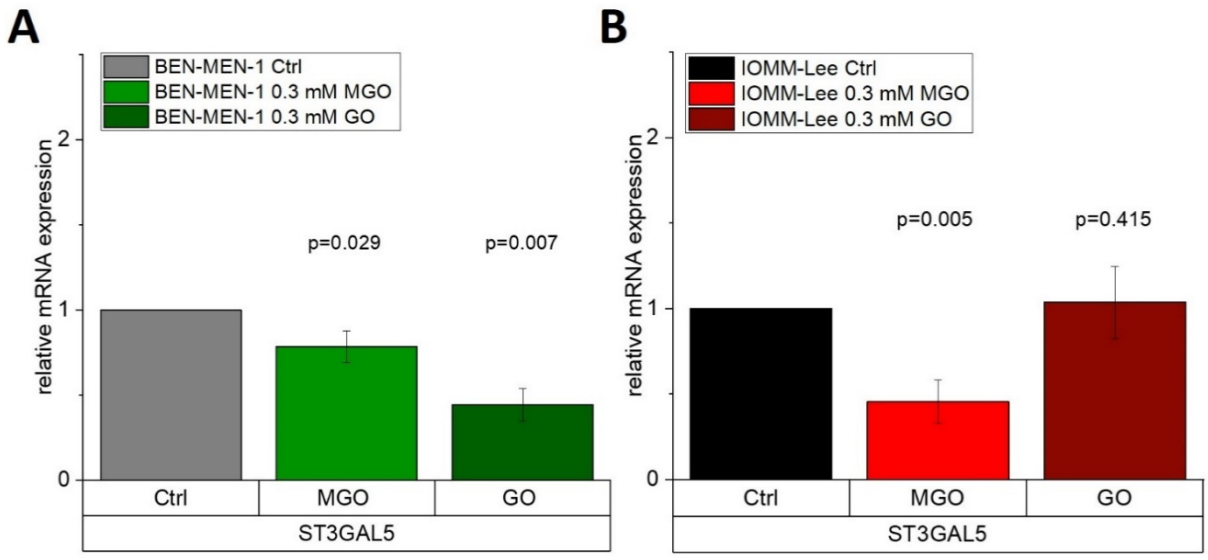

Figure 8: Relative mRNA expression of ST3GAL5 in BEN-MEN-1 (**A**) and IOMM-Lee (**B**). A shows the relative expression of ST3GAL5 in untreated (**grey**), 0.3mM MGO (green) and 0.3 mM GO (**dark green**) treated BEN-MEN-1. B shows the relative expression of ST3GAL5 in untreated (**black**), 0.3 mM MGO (**red**) and 0.3 mM GO (**dark red**) treated IOMM-Lee. Statistical analysis was performed using t-test and error bars represent SD (n= 4; ST3GAL5 GO (A) p = 0.007; (B) p = 0.415).

Discussion

Many studies demonstrated that sialylation has an impact on tumorigenesis [51–55]. Abnormal levels of different glycosyltransferases were found in different types of human cancers [56,57]. In addition, high serum levels of sialyltransferases are associated with the progression of advanced breast cancer [58].

But little is known about the influence on glycosylation by glycation, which is increased in several cancers because of the Warburg effect [59,60]. In this study, we could show, that glycation affects sialylation by modulating ST expression, which could have an impact on different ganglioside patterns and thereby on tumor development. Most of the STs were expressed in both, BEN-MEN-1 and IOMM-Lee, cell lines. Glycation of both cell lines resulted in an increasing level of STs in the benign meningioma cells and decreasing level in the malignant cells.

There are many reports of changes in ST expression in cancer. Overexpression of ST3GAL1 in ovarian cancer led to transforming growth factor (TGF)-β1-induced epithelial-mesenchymal-transition, migration, and invasion, and a knockdown resulted in the opposite [61]. Another study by Mehta et al. has revealed that ST3GAL2 and ST6GAL1 were significantly upregulated

in tumors with positive perineural invasion status [62], which we observed in the glycated benign cell line. ST3GAL3 was increased in the glycated BEN-MEN-1 cell line but decreased in glycated IOMM-Lee cells. Expression of ST3GAL3 is important for the regulation of biosynthesis of brain disialoganglioside (GD)1a and trisialoganglioside (GT)1b [57]. In several studies, the altered expression of ST3GAL3 has an impact on cell adhesion and invasion. Glycation of meningioma cell lines resulted in decreased ST3GAL5 expression in both cell lines. This sialyltransferase is also known as monosialoganglioside (GM)3 synthase [63] and suppresses the epidermal growth factor receptor (EGFR) phosphorylation, which influences the cell proliferation [64] and the cellular resistance to oxidative stress and radiation therapy through upregulation of extracellular signal-regulated kinases (ERK) [42]. The total amount of GM3 was decreased in BEN-MEN-1 cells after glycation. Yamashita and colleges reported that GM3 synthase knockout mice displayed enhanced ligand-induced insulin receptor phosphorylation. Furthermore, they could show that an increased sensitivity in glucose and insulin tolerance consequently results in an elevated insulin signaling response [65]. Other studies show that decreasing expression of GM3 leads to decreased cell motility and cell adhesion through ERK phosphorylation along with Ras upregulation. This regulates migration through mitogen-activated protein kinase (MAPK) [41,42,66,67]. The glycating agent GO has the same effect in BEN-MEN-1 cells (down-regulation of ST3GAL5) as MGO. However, in IOMM-Lee cells, we observed no effect after glycation with GO, which could be explained by higher glyoxalase 1 activity, which degradates dicarbonyls and has been described in many studies on cancer and glycation [68–70]. The expression of ST3GAL6 was reduced in both glycated meningioma cell lines, which is known to play a key role in the generation of functional Sialyl Lewis X [71]. Decreasing levels of ST3GAL6 can lead to decreasing migration and invasion in 5637 and J82 UBC cells as well as decreasing adhesion and migration in multiple myeloma cells [72,73].

Increased ST6GAL1 expression as we have shown in glycated BEN-MEN-1 cells, was also found in lung, colon, glioma, prostate, cervical, and breast cancer tissues [74–80]. The downregulation of ST6GAL1 decreased metalloproteinases (MMPs) expression and suppressed invasive potential of A549 and H1299 cells in vitro [79], whereas bladder cancer has ST6GAL1 upregulation a tumor-suppressive role [81]. The upregulation of ST6GAL2 was found in different types of cancer and was associated with breast cancer with higher expression of intracellular adhesion molecule (ICAM)-1, vascular adhesion molecule (VCAM)-1, CD24, MMP2, MMP9 and C-X-C motif chemokine receptor (CXCR)4 [82,83].

ST6GALNAC2 is known as a metastasis suppressor in breast cancer and a low expression of it, as we observed in glycated BEN-MEN-1 cells, is associated with a bad prognosis [84,85]. In Colorectal Carcinoma, Venkitachalam et al. have observed the same [86]. In contrast,

Schneider et al. could show, that a high expression of ST6GALNAC2 correlates with metastases to the lymph system [87]. The expression level may be a prognostic marker, but it seems that the mutation of the gene is more important. In our study, ST6GALNAC4 expression was elevated in glycated BEN-MEN-1. High expression of ST6GALNAC4 leads to the prevention of O-glycan chain elongation [88]. In another study of Follicular Thyroid Carcinoma (FTC)-238 cells the suppression of the ST6GALNAC4 gene led to an inhibition of invasive behavior *in vitro* and *in vivo* [89]. The lower expression of ST6GALNAC5 in both glycated meningioma cell lines in our study could be a sign of transformation, because it is restricted to the brain and synthesizes GD1alpha in the nervous tissues [90,91]. We have observed an increased expression of ST6GALNAC6 in glycated BEN-MEN-1 cells. A study in colon cancer has shown that ST6GALNAC6 is responsible for the synthesis of sialyl Lewis (a), which is a significant inductive mechanism in cancer progression [92,93].

The increased expression of ST8SIA1 in glycated BEN-MEN-1 cells could lead to a weak prognosis for patients. In contrast, we observed decreased expression of ST8SIA1 after glycation in IOMM-Lee cells. In melanoma brain metastases, it was shown that ST8SIA1 (GD3 synthases) is upregulated and the GD3 expression is increased, which was associated with a bad prognosis [94]. In gliomas, malignancy increased by higher GD3 and GD2 expression [95]. In addition, ST8SIA1 is one of the key drivers for malignancy in glioblastoma [96]. Mennel et al. reported on different expression levels of GD3 and GD2 in meningiomas, depending on the tumor origin [97]. A study for neuroblastoma and melanoma cells demonstrated that most neuroblastoma cells had a high expression of GD2 and melanoma cells had high expression of GD3 [98]. The increased expression of ST8SIA2, as we observed in glycated BEN-MEN-1 cells, plays a role in the invasive behavior and was significantly associated with the risk of relapse in non-small cell lung carcinoma [99]. The sialyltransferase ST8Sia5, which is increased in glycated BEN-MEN-1 cells, is known to synthesize GD1c/GT1a/Tetrasialogangliotetraosyl-ceramide (GQ)1b from GM1b/GD1a/GT1b. The group of Schiopu et al. has identified thirty-four distinct glycosphingolipid components (one GM4, nine GM3, two GM2, two GD3, nine GM1, and six GD1) differing in their ceramide compositions [100].

The glycation of meningioma cell lines has opposite effects in benign or malignant meningioma cells. Overall, glycated BEN-MEN-1 cells express more sialyltransferases than unglycated, whereas glycation of IOMM-Lee cells leads to a downregulation of the sialyltransferase expression. These observations support our recent observations that glycation of BEN-MEN-1 cells lead to increased invasive potential [30].

Conclusion

To sum up, glycation of meningioma cell lines has cell line-specific effects. The glycated BEN-MEN-1 cell line is affected in a different expression of ST3GAL1/2/3/5/6; ST6GAL1/2; ST6GALNAC2/6 and ST8SIA1/2. These STs have a direct or indirect impact on tumor progression. The decreased expression of ST3GAL5 after glycation results in a decreasing expression of GM3 in benign meningioma cells. The expression level of some sialyltransferases (ST3GAL1/2/3; ST6GALNAC5 and ST8SIA1) of glycated IOMM-Lee cell line were inhibited which indicates less aggressive behavior.

Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, P.S. ; methodology, P.S., K.B., M.W. and T.Z.; software, K.B and P.S.; validation, P.S.; formal analysis, P.S. , M.W. and T.Z.; investigation, P.S.; resources, P.S., K.B. and R.H.; data curation, P.S.; writing—original draft preparation, P.S.; writing—review and editing, M.S., R.H. and P.S.; visualization, P.S., M.S.; supervision, R.H., C.S.; project administration, R-H., C.S. and M.S. ; funding acquisition, R.H., M.S. and C.S. All authors have read and agreed to the published version of the manuscript.”

Funding

This research was funded by Wilhelm Roux-program, FKZ 31/21 and Deutsche Forschungsgemeinschaft (DFG, ProMoAge RTG 2155).

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Goldbrunner, R.; Minniti, G.; Preusser, M.; Jenkinson, M.D.; Sallabanda, K.; Houdart, E.; Deimling, A. von; Stavrinou, P.; Lefranc, F.; Lund-Johansen, M.; et al. EANO guidelines for the diagnosis and treatment of meningiomas. *The Lancet Oncology* **2016**, *17*, e383-e391, doi:10.1016/S1470-2045(16)30321-7.

2. Ostrom, Q.T.; Gittleman, H.; Truitt, G.; Boscia, A.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. *Neuro Oncol.* **2018**, *20*, iv1-iv86, doi:10.1093/neuonc/noy131.

3. Holleczek, B.; Zampella, D.; Urbschat, S.; Sahm, F.; Deimling, A. von; Oertel, J.; Ketter, R. Incidence, mortality and outcome of meningiomas: A population-based study from Germany. *Cancer Epidemiol.* **2019**, *62*, 101562, doi:10.1016/j.canep.2019.07.001.

4. Ostrom, Q.T.; Cioffi, G.; Gittleman, H.; Patil, N.; Waite, K.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012-2016. *Neuro Oncol.* **2019**, *21*, v1-v100, doi:10.1093/neuonc/noz150.

5. Maier, A.D.; Bartek, J.; Eriksson, F.; Ugleholdt, H.; Juhler, M.; Broholm, H.; Mathiesen, T.I. Clinical and histopathological predictors of outcome in malignant meningioma. *Neurosurg. Rev.* **2020**, *43*, 643–653, doi:10.1007/s10143-019-01093-5.

6. Buerki, R.A.; Horbinski, C.M.; Kruser, T.; Horowitz, P.M.; James, C.D.; Lukas, R.V. An overview of meningiomas. *Future Oncol.* **2018**, *14*, 2161–2177, doi:10.2217/fon-2018-0006.

7. Champeaux, C.; Jecko, V. World Health Organization grade III meningiomas. A retrospective study for outcome and prognostic factors assessment. *Neurochirurgie.* **2016**, *62*, 203–208, doi:10.1016/j.neuchi.2016.05.001.

8. Peyre, M.; Gauchotte, G.; Giry, M.; Froehlich, S.; Pallud, J.; Graillon, T.; Bielle, F.; Cazals-Hatem, D.; Varlet, P.; Figarella-Branger, D.; et al. De novo and secondary anaplastic meningiomas: a study of clinical and histomolecular prognostic factors. *Neuro Oncol.* **2018**, *20*, 1113–1121, doi:10.1093/neuonc/nox231.

9. Claus, E.B.; Bondy, M.L.; Schildkraut, J.M.; Wiemels, J.L.; Wrensch, M.; Black, P.M. Epidemiology of intracranial meningioma. *Neurosurgery* **2005**, *57*, 1088-95; discussion 1088-95, doi:10.1227/01.neu.0000188281.91351.b9.

10. Pasquier, D.; Bijmolt, S.; Veninga, T.; Rezvoy, N.; Villa, S.; Krengli, M.; Weber, D.C.; Baumert, B.G.; Canyilmaz, E.; Yalman, D.; et al. Atypical and malignant meningioma: outcome and prognostic factors in 119 irradiated patients. A multicenter, retrospective study of the Rare Cancer Network. *Int. J. Radiat. Oncol. Biol. Phys.* **2008**, *71*, 1388–1393, doi:10.1016/j.ijrobp.2007.12.020.

11. Allaman, I.; Bélanger, M.; Magistretti, P.J. Methylglyoxal, the dark side of glycolysis. *Front. Neurosci.* **2015**, *9*, 23, doi:10.3389/fnins.2015.00023.

12. Bharadwaj, S.; Venkatraghavan, L.; Mariappan, R.; Ebinu, J.; Meng, Y.; Khan, O.; Tung, T.; Reyhani, S.; Bernstein, M.; Zadeh, G. Serum lactate as a potential biomarker of non-glial brain tumors. *J. Clin. Neurosci.* **2015**, *22*, 1625–1627, doi:10.1016/j.jocn.2015.05.009.

13. Gill, K.S.; Fernandes, P.; O'Donovan, T.R.; McKenna, S.L.; Doddakula, K.K.; Power, D.G.; Soden, D.M.; Forde, P.F. Glycolysis inhibition as a cancer treatment and its role in an anti-tumour immune response. *Biochim. Biophys. Acta* **2016**, *1866*, 87–105, doi:10.1016/j.bbcan.2016.06.005.

14. Rabbani, N.; Thornalley, P.J. Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. *Biochem. Biophys. Res. Commun.* **2015**, *458*, 221–226, doi:10.1016/j.bbrc.2015.01.140.

15. Giovannucci, E.; Harlan, D.M.; Archer, M.C.; Bergenstal, R.M.; Gapstur, S.M.; Habel, L.A.; Pollak, M.; Regensteiner, J.G.; Yee, D. Diabetes and cancer: a consensus report. *CA Cancer J. Clin.* **2010**, *60*, 207–221, doi:10.3322/caac.20078.

16. Chowdhury, T.A. Diabetes and cancer. *QJM* **2010**, *103*, 905–915, doi:10.1093/qjmed/hcq149.

17. Michaud, D.S.; Bové, G.; Gallo, V.; Schlehofer, B.; Tjønneland, A.; Olsen, A.; Overvad, K.; Dahm, C.C.; Teucher, B.; Boeing, H.; et al. Anthropometric measures, physical activity, and risk of glioma and meningioma in a large prospective cohort study. *Cancer Prev. Res. (Phila)* **2011**, *4*, 1385–1392, doi:10.1158/1940-6207.CAPR-11-0014.

18. Edlinger, M.; Strohmaier, S.; Jonsson, H.; Bjørge, T.; Manjer, J.; Borena, W.T.; Häggström, C.; Engeland, A.; Tretli, S.; Concin, H.; et al. Blood pressure and other metabolic syndrome factors and risk of brain tumour in the large population-based Me-Can cohort study. *J. Hypertens.* **2012**, *30*, 290–296, doi:10.1097/HJH.0b013e32834e9176.

19. Schneider, B.; Pülhorn, H.; Röhrig, B.; Rainov, N.G. Predisposing conditions and risk factors for development of symptomatic meningioma in adults. *Cancer Detect. Prev.* **2005**, *29*, 440–447, doi:10.1016/j.cdp.2005.07.002.

20. Schwartzbaum, J.; Jonsson, F.; Ahlbom, A.; Preston-Martin, S.; Malmer, B.; Lönn, S.; Söderberg, K.; Feychting, M. Prior hospitalization for epilepsy, diabetes, and stroke and subsequent glioma and meningioma risk. *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14*, 643–650, doi:10.1158/1055-9965.EPI-04-0119.

21. Bernardo, B.M.; Orellana, R.C.; Weisband, Y.L.; Hammar, N.; Walldius, G.; Malmstrom, H.; Ahlbom, A.; Feychting, M.; Schwartzbaum, J. Association between prediagnostic glucose,

triglycerides, cholesterol and meningioma, and reverse causality. *Br. J. Cancer* **2016**, *115*, 108–114, doi:10.1038/bjc.2016.157.

22. Nayeri, A.; Chotai, S.; Prablek, M.A.; Brinson, P.R.; Douleh, D.G.; Weaver, K.D.; Thompson, R.C.; Chambless, L. Type 2 diabetes is an independent negative prognostic factor in patients undergoing surgical resection of a WHO grade I meningioma. *Clin. Neurol. Neurosurg.* **2016**, *149*, 6–10, doi:10.1016/j.clineuro.2016.07.015.

23. Ahmed, N. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res. Clin. Pract.* **2005**, *67*, 3–21, doi:10.1016/j.diabres.2004.09.004.

24. Rabbani, N.; Thornalley, P.J. The dicarbonyl proteome: proteins susceptible to dicarbonyl glycation at functional sites in health, aging, and disease. *Ann. N. Y. Acad. Sci.* **2008**, *1126*, 124–127, doi:10.1196/annals.1433.043.

25. Thornalley, P.J.; Langborg, A.; Minhas, H.S. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* **1999**, *344 Pt 1*, 109–116.

26. Brings, S.; Fleming, T.; Freichel, M.; Muckenthaler, M.U.; Herzig, S.; Nawroth, P.P. Dicarbonyls and Advanced Glycation End-Products in the Development of Diabetic Complications and Targets for Intervention. *Int. J. Mol. Sci.* **2017**, *18*, doi:10.3390/ijms18050984.

27. Kalapos, M.P. Methylglyoxal and glucose metabolism: a historical perspective and future avenues for research. *Drug Metabol. Drug Interact.* **2008**, *23*, 69–91, doi:10.1515/dmdi.2008.23.1-2.69.

28. Falone, S.; D'Alessandro, A.; Mirabilio, A.; Petruccelli, G.; Cacchio, M.; Di Ilio, C.; Di Loreto, S.; Amicarelli, F. Long term running biphasically improves methylglyoxal-related metabolism, redox homeostasis and neurotrophic support within adult mouse brain cortex. *PLoS One* **2012**, *7*, e31401, doi:10.1371/journal.pone.0031401.

29. Schalkwijk, C.G. Vascular AGE-ing by methylglyoxal: the past, the present and the future. *Diabetologia* **2015**, *58*, 1715–1719, doi:10.1007/s00125-015-3597-5.

30. Selke, P.; Rosenstock, P.; Bork, K.; Strauss, C.; Horstkorte, R.; Scheer, M. Glycation of benign meningioma cells leads to increased invasion. *Biol. Chem.* **2021**, doi:10.1515/hsz-2020-0376.

31. Bellahcène, A.; Nokin, M.-J.; Castronovo, V.; Schalkwijk, C. Methylglyoxal-derived stress: An emerging biological factor involved in the onset and progression of cancer. *Semin. Cancer Biol.* **2018**, *49*, 64–74, doi:10.1016/j.semcancer.2017.05.010.

32. Antognelli, C.; Moretti, S.; Frosini, R.; Puxeddu, E.; Sidoni, A.; Talesa, V.N. Methylglyoxal Acts as a Tumor-Promoting Factor in Anaplastic Thyroid Cancer. *Cells* **2019**, *8*, doi:10.3390/cells8060547.

33. Takashima, S.; Tsuji, S.; Tsujimoto, M. Characterization of the second type of human beta-galactoside alpha 2,6-sialyltransferase (ST6Gal II), which sialylates Galbeta 1,4GlcNAc structures on oligosaccharides preferentially. Genomic analysis of human sialyltransferase genes. *J. Biol. Chem.* **2002**, *277*, 45719–45728, doi:10.1074/jbc.M206808200.

34. Comb D. G.; Roseman S. The sialic acids. I. The structure and enzymatic synthesis of N-acetylneuraminic acid. *J. Biol. Chem.* **1960**, *235*, 2529–2537.

35. Stäsche, R.; Hinderlich, S.; Weise, C.; Effertz, K.; Lucka, L.; Moormann, P.; Reutter, W. A bifunctional enzyme catalyzes the first two steps in N-acetylneuraminic acid biosynthesis of rat liver. Molecular cloning and functional expression of UDP-N-acetyl-glucosamine 2-epimerase/N-acetylmannosamine kinase. *J. Biol. Chem.* **1997**, *272*, 24319–24324, doi:10.1074/jbc.272.39.24319.

36. Harduin-Lepers, A.; Vallejo-Ruiz, V.; Krzewinski-Recchi, M.-A.; Samyn-Petit, B.; Julien, S.; Delannoy, P. The human sialyltransferase family. *Biochimie* **2001**, *83*, 727–737, doi:10.1016/S0300-9084(01)01301-3.

37. Datta, A.K. Comparative sequence analysis in the sialyltransferase protein family: analysis of motifs. *Curr. Drug Targets* **2009**, *10*, 483–498, doi:10.2174/138945009788488422.

38. Lau, K.S.; Partridge, E.A.; Grigorian, A.; Silvescu, C.I.; Reinhold, V.N.; Demetriou, M.; Dennis, J.W. Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. *Cell* **2007**, *129*, 123–134, doi:10.1016/j.cell.2007.01.049.

39. Taganna, J.; Boer, A.R. de; Wuhrer, M.; Bouckaert, J. Glycosylation changes as important factors for the susceptibility to urinary tract infection. *Biochem. Soc. Trans.* **2011**, *39*, 349–354, doi:10.1042/BST0390349.

40. Uemura, S.; Go, S.; Shishido, F.; Inokuchi, J.-i. Expression machinery of GM4: the excess amounts of GM3/GM4S synthase (ST3GAL5) are necessary for GM4 synthesis in mammalian cells. *Glycoconj. J.* **2014**, *31*, 101–108, doi:10.1007/s10719-013-9499-1.

41. Hakomori, S.-I.; Handa, K. GM3 and cancer. *Glycoconj. J.* **2015**, *32*, 1–8, doi:10.1007/s10719-014-9572-4.

42. Shimizu, T.; Nagane, M.; Suzuki, M.; Yamauchi, A.; Kato, K.; Kawashima, N.; Nemoto, Y.; Maruo, T.; Kawakami, Y.; Yamashita, T. Tumor hypoxia regulates ganglioside GM3

synthase, which contributes to oxidative stress resistance in malignant melanoma. *Biochim. Biophys. Acta Gen. Subj.* **2020**, *1864*, 129723, doi:10.1016/j.bbagen.2020.129723.

43. Inokuchi, J.-i.; Inamori, K.-I.; Kabayama, K.; Nagafuku, M.; Uemura, S.; Go, S.; Suzuki, A.; Ohno, I.; Kanoh, H.; Shishido, F. Biology of GM3 Ganglioside. *Prog. Mol. Biol. Transl. Sci.* **2018**, *156*, 151–195, doi:10.1016/bs.pmbts.2017.10.004.

44. Rosenstock, P.; Bork, K.; Massa, C.; Selke, P.; Seliger, B.; Horstkorte, R. Sialylation of Human Natural Killer (NK) Cells is Regulated by IL-2. *J. Clin. Med.* **2020**, *9*, doi:10.3390/jcm9061816.

45. Zhang, T.; van Die, I.; Tefsen, B.; van Vliet, S.J.; Laan, L.C.; Zhang, J.; Dijke, P. ten; Wuhrer, M.; Belo, A.I. Differential O- and Glycosphingolipid Glycosylation in Human Pancreatic Adenocarcinoma Cells With Opposite Morphology and Metastatic Behavior. *Front. Oncol.* **2020**, *10*, 732, doi:10.3389/fonc.2020.00732.

46. Jensen, P.H.; Karlsson, N.G.; Kolarich, D.; Packer, N.H. Structural analysis of N- and O-glycans released from glycoproteins. *Nat. Protoc.* **2012**, *7*, 1299–1310, doi:10.1038/nprot.2012.063.

47. Anugraham, M.; Everest-Dass, A.V.; Jacob, F.; Packer, N.H. A platform for the structural characterization of glycans enzymatically released from glycosphingolipids extracted from tissue and cells. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 545–561, doi:10.1002/rcm.7130.

48. Karlsson, N.G.; Wilson, N.L.; Wirth, H.-J.; Dawes, P.; Joshi, H.; Packer, N.H. Negative ion graphitised carbon nano-liquid chromatography/mass spectrometry increases sensitivity for glycoprotein oligosaccharide analysis. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2282–2292, doi:10.1002/rcm.1626.

49. Ceroni, A.; Maass, K.; Geyer, H.; Geyer, R.; Dell, A.; Haslam, S.M. GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *J. Proteome Res.* **2008**, *7*, 1650–1659, doi:10.1021/pr7008252.

50. Cooper, C.A.; Gasteiger, E.; Packer, N.H. GlycoMod - A software tool for determining glycosylation compositions from mass spectrometric data. *Proteomics* **2001**, *1*, 340–349, doi:10.1002/1615-9861(200102)1:2<340:AID-PROT340>3.0.CO;2-B.

51. Pihikova, D.; Kasak, P.; Kubanikova, P.; Sokol, R.; Tkac, J. Aberrant sialylation of a prostate-specific antigen: Electrochemical label-free glycoprofiling in prostate cancer serum samples. *Anal. Chim. Acta* **2016**, *934*, 72–79, doi:10.1016/j.aca.2016.06.043.

52. Yoneyama, T.; Ohyama, C.; Hatakeyama, S.; Narita, S.; Habuchi, T.; Koie, T.; Mori, K.; Hidari, K.I.P.J.; Yamaguchi, M.; Suzuki, T.; et al. Measurement of aberrant glycosylation of prostate specific antigen can improve specificity in early detection of prostate cancer. *Biochem. Biophys. Res. Commun.* **2014**, *448*, 390–396, doi:10.1016/j.bbrc.2014.04.107.

53. Vučković, F.; Theodoratou, E.; Thaçi, K.; Timofeeva, M.; Vojta, A.; Štambuk, J.; Pučić-Baković, M.; Rudd, P.M.; Đerek, L.; Servis, D.; et al. IgG Glycome in Colorectal Cancer. *Clin. Cancer Res.* **2016**, *22*, 3078–3086, doi:10.1158/1078-0432.CCR-15-1867.

54. Fleming, S.C.; Smith, S.; Knowles, D.; Skillen, A.; Self, C.H. Increased sialylation of oligosaccharides on IgG paraproteins--a potential new tumour marker in multiple myeloma. *J. Clin. Pathol.* **1998**, *51*, 825–830, doi:10.1136/jcp.51.11.825.

55. Saldova, R.; Wormald, M.R.; Dwek, R.A.; Rudd, P.M. Glycosylation changes on serum glycoproteins in ovarian cancer may contribute to disease pathogenesis. *Dis. Markers* **2008**, *25*, 219–232, doi:10.1155/2008/601583.

56. Suzuki, O.; Abe, M.; Hashimoto, Y. Sialylation by β-galactoside α-2,6-sialyltransferase and N-glycans regulate cell adhesion and invasion in human anaplastic large cell lymphoma. *Int. J. Oncol.* **2015**, *46*, 973–980, doi:10.3892/ijo.2015.2818.

57. Cui, H.-X.; Wang, H.; Wang, Y.; Song, J.; Tian, H.; Xia, C.; Shen, Y. ST3Gal III modulates breast cancer cell adhesion and invasion by altering the expression of invasion-related molecules. *Oncol. Rep.* **2016**, *36*, 3317–3324, doi:10.3892/or.2016.5180.

58. Dao, T.L.; Ip, C.; Patel, J. Serum sialyltransferase and 5'-nucleotidase as reliable biomarkers in women with breast cancer. *J. Natl. Cancer Inst.* **1980**, *65*, 529–534.

59. Ahmad, S.; Khan, H.; Siddiqui, Z.; Khan, M.Y.; Rehman, S.; Shahab, U.; Godovikova, T.; Silnikov, V.; Moinuddin. AGEs, RAGEs and s-RAGE; friend or foe for cancer. *Semin. Cancer Biol.* **2018**, *49*, 44–55, doi:10.1016/j.semcancer.2017.07.001.

60. Bellier, J.; Nokin, M.-J.; Lardé, E.; Karoyan, P.; Peulen, O.; Castronovo, V.; Bellahcène, A. Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. *Diabetes Res. Clin. Pract.* **2019**, *148*, 200–211, doi:10.1016/j.diabres.2019.01.002.

61. Wu, X.; Zhao, J.; Ruan, Y.; Sun, L.; Xu, C.; Jiang, H. Sialyltransferase ST3GAL1 promotes cell migration, invasion, and TGF-β1-induced EMT and confers paclitaxel resistance in ovarian cancer. *Cell Death Dis.* **2018**, *9*, 1102, doi:10.1038/s41419-018-1101-0.

62. Mehta, K.A.; Patel, K.A.; Pandya, S.J.; Patel, P.S. "Aberrant sialylation plays a significant role in oral squamous cell carcinoma progression". *J. Oral Pathol. Med.* **2020**, *49*, 253–259, doi:10.1111/jop.12976.

63. Trinchera, M.; Parini, R.; Indellicato, R.; Domenighini, R.; dall'Olio, F. Diseases of ganglioside biosynthesis: An expanding group of congenital disorders of glycosylation. *Mol. Genet. Metab.* **2018**, *124*, 230–237, doi:10.1016/j.ymgme.2018.06.014.

64. Kawashima, N.; Nishimiya, Y.; Takahata, S.; Nakayama, K.-I. Induction of Glycosphingolipid GM3 Expression by Valproic Acid Suppresses Cancer Cell Growth. *J. Biol. Chem.* **2016**, *291*, 21424–21433, doi:10.1074/jbc.M116.751503.

65. Yamashita, T.; Hashiramoto, A.; Haluzik, M.; Mizukami, H.; Beck, S.; Norton, A.; Kono, M.; Tsuji, S.; Daniotti, J.L.; Werth, N.; et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3445–3449, doi:10.1073/pnas.0635898100.

66. Kojima, N.; Hakomori, S. Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid-glycolipid interaction. *J. Biol. Chem.* **1991**, *266*, 17552–17558.

67. Hashiramoto, A.; Mizukami, H.; Yamashita, T. Ganglioside GM3 promotes cell migration by regulating MAPK and c-Fos/AP-1. *Oncogene* **2006**, *25*, 3948–3955, doi:10.1038/sj.onc.1209416.

68. Hu, X.; Yang, X.; He, Q.; Chen, Q.; Yu, L. Glyoxalase 1 is up-regulated in hepatocellular carcinoma and is essential for HCC cell proliferation. *Biotechnol. Lett.* **2014**, *36*, 257–263, doi:10.1007/s10529-013-1372-6.

69. Peng, H.-T.; Chen, J.; Liu, T.-Y.; Wu, Y.-Q.; Lin, X.-H.; Lai, Y.-H.; Huang, Y.-F. Up-regulation of the tumor promoter Glyoxalase-1 indicates poor prognosis in breast cancer. *Int. J. Clin. Exp. Pathol.* **2017**, *10*, 10852–10862.

70. Burdelski, C.; Shihada, R.; Hinsch, A.; Angerer, A.; Göbel, C.; Friedrich, E.; Hube-Magg, C.; Burdak-Rothkamm, S.; Kluth, M.; Simon, R.; et al. High-Level Glyoxalase 1 (GLO1) expression is linked to poor prognosis in prostate cancer. *Prostate* **2017**, *77*, 1528–1538, doi:10.1002/pros.23431.

71. Chachadi, V.B.; Bhat, G.; Cheng, P.-W. Glycosyltransferases involved in the synthesis of MUC-associated metastasis-promoting selectin ligands. *Glycobiology* **2015**, *25*, 963–975, doi:10.1093/glycob/cwv030.

72. Dalangood, S.; Zhu, Z.; Ma, Z.; Li, J.; Zeng, Q.; Yan, Y.; Shen, B.; Yan, J.; Huang, R. Identification of glycogene-type and validation of ST3GAL6 as a biomarker predicts clinical outcome and cancer cell invasion in urinary bladder cancer. *Theranostics* **2020**, *10*, 10078–10091, doi:10.7150/thno.48711.

73. Glavey, S.V.; Manier, S.; Natoni, A.; Sacco, A.; Moschetta, M.; Reagan, M.R.; Murillo, L.S.; Sahin, I.; Wu, P.; Mishima, Y.; et al. The sialyltransferase ST3GAL6 influences homing and survival in multiple myeloma. *Blood* **2014**, *124*, 1765–1776, doi:10.1182/blood-2014-03-560862.

74. Lin, S.; Kemmner, W.; Grigull, S.; Schlag, P.M. Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. *Exp. Cell Res.* **2002**, *276*, 101–110, doi:10.1006/excr.2002.5521.

75. Lu, J.; Isaji, T.; Im, S.; Fukuda, T.; Hashii, N.; Takakura, D.; Kawasaki, N.; Gu, J. β-Galactoside α2,6-sialyltranferase 1 promotes transforming growth factor-β-mediated epithelial-mesenchymal transition. *J. Biol. Chem.* **2014**, *289*, 34627–34641, doi:10.1074/jbc.M114.593392.

76. Swindall, A.F.; Bellis, S.L. Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. *J. Biol. Chem.* **2011**, *286*, 22982–22990, doi:10.1074/jbc.M110.211375.

77. Wang, P.-H.; Lee, W.-L.; Lee, Y.-R.; Juang, C.-M.; Chen, Y.-J.; Chao, H.-T.; Tsai, Y.-C.; Yuan, C.-C. Enhanced expression of α 2,6-sialyltransferase ST6Gal I in cervical squamous cell carcinoma. *Gynecologic Oncology* **2003**, *89*, 395–401, doi:10.1016/s0090-8258(03)00127-6.

78. Wei, A.; Fan, B.; Zhao, Y.; Zhang, H.; Wang, L.; Yu, X.; Yuan, Q.; Yang, D.; Wang, S. ST6Gal-I overexpression facilitates prostate cancer progression via the PI3K/Akt/GSK-3β/β-catenin signaling pathway. *Oncotarget* **2016**, *7*, 65374–65388, doi:10.18632/oncotarget.11699.

79. Yuan, Q.; Chen, X.; Han, Y.; Lei, T.; Wu, Q.; Yu, X.; Wang, L.; Fan, Z.; Wang, S. Modification of α2,6-sialylation mediates the invasiveness and tumorigenicity of non-small cell lung cancer cells in vitro and in vivo via Notch1/Hes1/MMPs pathway. *Int. J. Cancer* **2018**, *143*, 2319–2330, doi:10.1002/ijc.31737.

80. Yamamoto, H.; Oviedo, A.; Sweeley, C.; Saito, T.; Moskal, J.R. Alpha2,6-sialylation of cell-surface N-glycans inhibits glioma formation in vivo. *Cancer Res.* **2001**, *61*, 6822–6829.

81. Antony, P.; Rose, M.; Heidenreich, A.; Knüchel, R.; Gaisa, N.T.; Dahl, E. Epigenetic inactivation of ST6GAL1 in human bladder cancer. *BMC Cancer* **2014**, *14*, 901, doi:10.1186/1471-2407-14-901.

82. Cheng, J.; Wang, R.; Zhong, G.; Chen, X.; Cheng, Y.; Li, W.; Yang, Y. ST6GAL2 Downregulation Inhibits Cell Adhesion and Invasion and is Associated with Improved Patient

Survival in Breast Cancer. *Onco. Targets. Ther.* **2020**, *13*, 903–914, doi:10.2147/OTT.S230847.

83. Xu, G.; Chen, J.; Wang, G.; Xiao, J.; Zhang, N.; Chen, Y.; Yu, H.; Wang, G.; Zhao, Y. Resveratrol Inhibits the Tumorigenesis of Follicular Thyroid Cancer via ST6GAL2-Regulated Activation of the Hippo Signaling Pathway. *Mol. Ther. Oncolytics* **2020**, *16*, 124–133, doi:10.1016/j.omto.2019.12.010.

84. Ferrer, C.M.; Reginato, M.J. Sticking to sugars at the metastatic site: sialyltransferase ST6GalNAc2 acts as a breast cancer metastasis suppressor. *Cancer Discov.* **2014**, *4*, 275–277, doi:10.1158/2159-8290.CD-14-0075.

85. Murugaesu, N.; Iravani, M.; van Weverwijk, A.; Ivetic, A.; Johnson, D.A.; Antonopoulos, A.; Fearns, A.; Jamal-Hanjani, M.; Sims, D.; Fenwick, K.; et al. An in vivo functional screen identifies ST6GalNAc2 sialyltransferase as a breast cancer metastasis suppressor. *Cancer Discov.* **2014**, *4*, 304–317, doi:10.1158/2159-8290.CD-13-0287.

86. Venkitachalam, S.; Revoredo, L.; Varadan, V.; Fecteau, R.E.; Ravi, L.; Lutterbaugh, J.; Markowitz, S.D.; Willis, J.E.; Gerken, T.A.; Guda, K. Biochemical and functional characterization of glycosylation-associated mutational landscapes in colon cancer. *Sci. Rep.* **2016**, *6*, 23642, doi:10.1038/srep23642.

87. Schneider, F.; Kemmner, W.; Haensch, W.; Franke, G.; Gretschel, S.; Karsten, U.; Schlag, P.M. Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. *Cancer Res.* **2001**, *61*, 4605–4611.

88. Reticker-Flynn, N.E.; Bhatia, S.N. Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. *Cancer Discov.* **2015**, *5*, 168–181, doi:10.1158/2159-8290.CD-13-0760.

89. Miao, X.; Jia, L.; Zhou, H.; Song, X.; Zhou, M.; Xu, J.; Zhao, L.; Feng, X.; Zhao, Y. miR-4299 mediates the invasive properties and tumorigenicity of human follicular thyroid carcinoma by targeting ST6GALNAC4. *IUBMB Life* **2016**, *68*, 136–144, doi:10.1002/iub.1467.

90. Bos, P.D.; Zhang, X.H.-F.; Nadal, C.; Shu, W.; Gomis, R.R.; Nguyen, D.X.; Minn, A.J.; van de Vijver, M.J.; Gerald, W.L.; Foekens, J.A.; et al. Genes that mediate breast cancer metastasis to the brain. *Nature* **2009**, *459*, 1005–1009, doi:10.1038/nature08021.

91. Okajima, T.; Fukumoto, S.; Ito, H.; Kiso, M.; Hirabayashi, Y.; Urano, T.; Furukawa, K. Molecular cloning of brain-specific GD1alpha synthase (ST6GalNAc V) containing

CAG/Glutamine repeats. *J. Biol. Chem.* **1999**, *274*, 30557–30562, doi:10.1074/jbc.274.43.30557.

92. Kannagi, R. Carbohydrate antigen sialyl Lewis a--its pathophysiological significance and induction mechanism in cancer progression. *Chang Gung Med. J.* **2007**, *30*, 189–209.

93. Tsuchida, A.; Okajima, T.; Furukawa, K.; Ando, T.; Ishida, H.; Yoshida, A.; Nakamura, Y.; Kannagi, R.; Kiso, M.; Furukawa, K. Synthesis of disialyl Lewis a (Le(a)) structure in colon cancer cell lines by a sialyltransferase, ST6GalNAc VI, responsible for the synthesis of alpha-series gangliosides. *J. Biol. Chem.* **2003**, *278*, 22787–22794, doi:10.1074/jbc.M211034200.

94. Ramos, R.I.; Bustos, M.A.; Wu, J.; Jones, P.; Chang, S.C.; Kiyohara, E.; Tran, K.; Zhang, X.; Stern, S.L.; Izraely, S.; et al. Upregulation of cell surface GD3 ganglioside phenotype is associated with human melanoma brain metastasis. *Mol. Oncol.* **2020**, *14*, 1760–1778, doi:10.1002/1878-0261.12702.

95. Iwasawa, T.; Zhang, P.; Ohkawa, Y.; Momota, H.; Wakabayashi, T.; Ohmi, Y.; Bhuiyan, R.H.; Furukawa, K.; Furukawa, K. Enhancement of malignant properties of human glioma cells by ganglioside GD3/GD2. *Int. J. Oncol.* **2018**, *52*, 1255–1266, doi:10.3892/ijo.2018.4266.

96. Yeh, S.-C.; Wang, P.-Y.; Lou, Y.-W.; Khoo, K.-H.; Hsiao, M.; Hsu, T.-L.; Wong, C.-H. Glycolipid GD3 and GD3 synthase are key drivers for glioblastoma stem cells and tumorigenicity. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 5592–5597, doi:10.1073/pnas.1604721113.

97. Mennel, H.D.; Bosslet, K.; Geissel, H.; Bauer, B.L. Immunohistochemically visualized localisation of gangliosides Glac2 (GD3) and Gtri2 (GD2) in cells of human intracranial tumors. *Experimental and Toxicologic Pathology* **2000**, *52*, 277–285, doi:10.1016/S0940-2993(00)80046-9.

98. Ruan, S.; Raj, B.K.; Lloyd, K.O. Relationship of glycosyltransferases and mRNA levels to ganglioside expression in neuroblastoma and melanoma cells. *J. Neurochem.* **1999**, *72*, 514–521, doi:10.1046/j.1471-4159.1999.0720514.x.

99. Hao, J.; Zeltz, C.; Pintilie, M.; Li, Q.; Sakashita, S.; Wang, T.; Cabanero, M.; Martins-Filho, S.N.; Wang, D.Y.; Pasko, E.; et al. Characterization of Distinct Populations of Carcinoma-Associated Fibroblasts from Non-Small Cell Lung Carcinoma Reveals a Role for ST8SIA2 in Cancer Cell Invasion. *Neoplasia* **2019**, *21*, 482–493, doi:10.1016/j.neo.2019.03.009.

100. Schiopu, C.; Vukelić, Z.; Capitan, F.; Kalanj-Bognar, S.; Sisu, E.; Zamfir, A.D. Chip-nanoelectrospray quadrupole time-of-flight tandem mass spectrometry of meningioma

gangliosides: a preliminary study. *Electrophoresis* **2012**, *33*, 1778–1786, doi:10.1002/elps.201200044.

Erklärungen

(1) Ich erkläre, dass ich mich an keiner anderen Hochschule einem Promotionsverfahren unterzogen bzw. eine Promotion begonnen habe.

(2) Ich erkläre, die Angaben wahrheitsgemäß gemacht und die wissenschaftliche Arbeit an keiner anderen wissenschaftlichen Einrichtung zur Erlangung eines akademischen Grades eingereicht zu haben.

(3) Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Alle Regeln der guten wissenschaftlichen Praxis wurden eingehalten; es wurden keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht.

Datum, Unterschrift

Danksagung

Als erstes möchte ich mich bei Herrn Prof. Dr. Rüdiger Horstkorte und Dr. Maximilian Scheer für die Bereitstellung des Themas und der Unterstützung während dieser Zeit bedanken.

Darüber hinaus möchte ich mich bei der gesamten Arbeitsgruppe für das gute Arbeitsklima sowie der Unterstützung im Labor und den guten Gesprächen in nicht fachlicher Hinsicht bedanken.

Als nächstes möchte ich mich bei meiner Freundin, meinen Eltern und allen Freunden bedanken, die an mich geglaubt und in allem unterstützt haben.