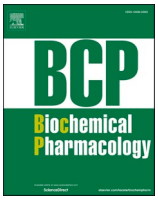
Biochemical Pharmacology 217 (2023) 115837

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm



Review

The role of EGFR in vascular AT1R signaling: From cellular mechanisms to systemic relevance

Michael Gekle [a,*], Virginie Dubourg [a], Gerald Schwerdt [a], Ralf A. Benndorf [b], Barbara Schreier [a]

[a] Julius-Bernstein-Institute of Physiology, Martin-Luther-University Halle-Wittenberg, Magdeburger Str. 6, D-06112 Halle (Saale), Germany
[b] Institute of Pharmacy, Martin-Luther-University, Halle, Germany

ARTICLE INFO

Keywords:
AT1 receptor
Epidermal growth factor receptor
EGFR
Transactivation
Vascular pathophysiology

ABSTRACT

The epidermal growth factor receptor (EGFR) belongs to the ErbB-family of receptor tyrosine kinases that are of importance in oncology. During the last years, substantial evidence accumulated for a crucial role of EGFR concerning the action of the angiotensin II type 1 receptor (AT1R) in blood vessels, resulting form AT1R-induced EGFR transactivation. This transactivation occurs through the release of membrane-anchored EGFR-ligands, cytosolic tyrosine kinases, heterocomplex formation or enhanced ligand expression. AT1R-EGFR crosstalk amplifies the signaling response and enhances the biological effects of angiotensin II. Downstream signaling cascades include ERK1/2 and p38 MAPK, PLCγ and STAT. AT1R-induced EGFR activation contributes to vascular remodeling and hypertrophy via e.g. smooth muscle cell proliferation, migration and extracellular matrix production. EGFR transactivation results in increased vessel wall thickness and reduced vascular compliance. AT1R and EGFR signaling pathways are also implicated the induction of vascular inflammation. Again, EGFR transactivation exacerbates the effects, leading to endothelial dysfunction that contributes to vascular inflammation, dysfunction and remodeling. Dysregulation of the AT1R-EGFR axis has been implicated in the pathogenesis of various cardiovascular diseases and inhibition or prevention of EGFR signaling can attenuate part of the detrimental impact of enhanced renin-angiotensin-system (RAAS) activity, highlighting the importance of EGFR for the adverse consequences of AT1R activation. In summary, EGFR plays a critical role in vascular AT1R action, enhancing signaling, promoting remodeling, contributing to inflammation, and participating in the pathogenesis of cardiovascular diseases. Understanding the interplay between AT1R and EGFR will foster the development of effective therapeutic strategies of RAAS-induced disorders.

1. Introduction

In light of the knowledge gained concerning cardiovascular epidermal growth factor receptor (EGFR), a more intense attention on this tyrosine kinase receptor from the cardiovascular perspective - and not only from the oncological viewpoint - is necessary. Vascular EGFR expression and activity under physiological conditions as well as alterations in certain pathophysiological is now well established. Beyond this correlative knowledge, there is substantial evidence for the involvement of EGFR in the regulation of vascular function as well as vascular dysfunction and structural remodeling. In this context, it is important to consider that EGFR contributes to signaling networks of several vasoactive substances, representing a central regulatory hub. In this review we will address mechanisms and consequences of the angiotensin II receptor type 1 (AT1R) crosstalk with EGFR for the vascular system.

1.1. EGF receptors

ErbB receptors are a family of four receptor tyrosine kinases (Fig. 1) that have received attention as promoters of tumor growth, e.g. in breast

Abbreviations: ADAM, a disintegrin and metalloproteinase; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; cSRC, tyrosine kinase Src; EC, endothelial cell; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK1/2, extracellular-signal regulated kinases 1/2; ErbB, tyrosine kinases homologue to erythroblastic leukemia viral oncogene; GPCR, G-protein coupled receptor; Grb2, growth factor receptor-bound protein 2; HB-EGF, heparin binding EGF like factor; JAK2, janus kinase 2; PGE, prostaglandin E; PLC, phospholipase C; RAAS, renin, angiotensin, aldosterone system; STAT, signal transducers and activators of transcription; VSMC, vascular smooth muscle cell.

* Corresponding author.
E-mail address: michael.gekle@medizin.uni-halle.de (M. Gekle).

https://doi.org/10.1016/j.bcp.2023.115837
Received 28 July 2023; Received in revised form 25 September 2023; Accepted 27 September 2023
Available online 29 September 2023

cancer, ovarian carcinoma, lung tumors, colon carcinoma, and pancreatic carcinoma [1]. In addition to ErbB2 (Her-2), ErbB3 and ErbB4, the ErbB receptor family includes the EGFR (=ErbB1) [2]. In the meantime, a pathogenetic significance of the EGFR in non-neoplastic diseases such as polycystic kidney disease [3], psoriasis [4,5], bronchial asthma [6] and vascular diseases [7,8] has been described. Since the EGFR was proposed as a target structure for tumor therapy more than 30 years ago [9–11], drugs against ErbB-receptor tyrosine kinases have been developed and successfully integrated into the treatment of cancer patients [1,9]. However, the ErbB receptors are almost ubiquitously expressed and their physiological significance has not yet been fully elucidated, partly because mice with global deletion of the receptors usually die in utero or shortly after birth [12]. It is therefore also not surprising that side effects occur during the treatment with some of the antitumor therapeutics. For example, cardiac hypertrophy and cardiac failure have been observed as side effects in patients receiving trastuzumab, especially when taken concomitantly with cytostatic drugs [13]. In addition to the findings during tumor therapy, it was shown that the EGFR contributes to pathophysiological effects of the mineralocorticoid receptor and the AT1R, i.e. of the renin-angiotensin-aldosterone system (RAAS), in vascular cells [14]. In large part, the insights concerning the cardiovascular system have been obtained in studies with cultured primary cells or cell lines.

EGFR-dependent effects include excessive growth of cells, cell migration and disorders of connective tissue homeostasis (fibrosis), but also physiological processes such as smooth muscle function [15–20]. Furthermore, there is evidence that the EGFR also plays a role in cellular aging processes [21] and supports the development of a pro-inflammatory milieu. However, there are also data indicating that the EGFR develops a protective effect in the cardiovascular system under certain - not yet precisely known - conditions [8]. Yet, the postnatal importance of EGFR for AT1R-related effects in vivo has not yet been finally clarified, since the EGFR also plays an essential role for cardiovascular development. Consequently, the involvement of the EGFR in physiological or pathophysiological effects of AT1R in vivo could not be investigated for a long time. Previous investigations addressing this question have applied either pharmacological inhibitors or rodent models with global reduction in EGFR activity. However, these approaches have the disadvantage that the results are difficult to interpret due to ubiquitous EGFR expression and the lack of cell specificity of the approaches. For example, detrimental effects on the heart could mask positive effects on blood vessel function during EGFR inhibition.

2. EGFR as signaling hub

EGFR belongs to the group of membrane-bound receptor tyrosine kinases (Fig. 1) and can be activated by different ligands with high or low affinities [22]. EGF and HB-EGF are high affinity ligands [23]. In addition, the EGFR also serves as a relay station for heterologous signal transduction [24–27], being activated by signals that are not direct ligands for it, such as angiotensin II, aldosterone, endothelin-1 or catecholamines or their receptors as well as metabolic and mechanical factors [25,28–31]. This transactivation is necessary for some of the actions of these mediators, although this has not yet been investigated systematically in all cases. But, during the last years, the fundamental importance of EGFR as signaling hub in the context of angiotensin II-induced effects has been increasingly worked out pharmacologically, without identifying the contribution of different cell types in vivo [32,33].

2.1. Canonical pathways of EGFR signaling

ErbB receptors can form receptor homo- and hetero-dimers, whose composition will influence the nature of the activated signaling pathways (Fig. 1). The type of activated signaling pathways will then

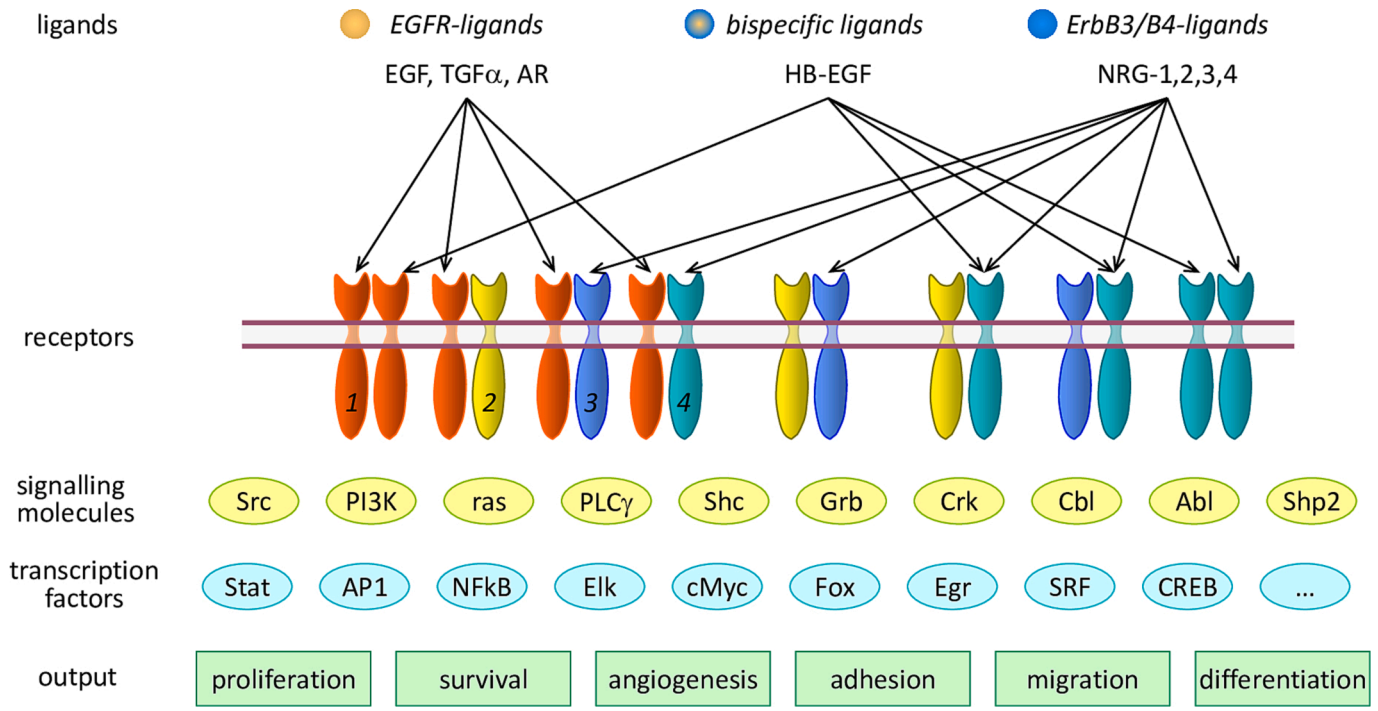


Fig. 1. Overview showing possible ErbB dimers, their ligands and the downstream cellular pathways affected. ErbB1 and ErbB4 form homodimers and dimers with all other family members. ErbB2 and ErbB3 form only heterodimers, because there is no ligand for ErbB2 and ErbB3 has no cytosolic kinase activity. There are various ligands for ErB receptors, These ligand vary in terms of receptor specificity as well as in terms of binding affinity (high and low affinity binding). The receptor dimers interact with various signaling molecules that mediate the activation signaling networks and finally modulate the activity of transcription factors. Transcription regulation can affect various cellular traits (=output). The pattern of signaling network activation and the subsequent output depend on the ligands and receptor dimers involved as well as on the cellular context.

determine the impact of ErbB in vivo and in vitro. However, the identification of ErbB subtypes constituting receptor heterodimers necessary for the activation of the different signaling pathways is complex. ErbB receptors (a) can form dimers with all other members, (b) some of the signaling pathways can be activated by several ErbB receptors [2,34] and (c) there are signaling molecules that are activated at low ligand concentrations, while others are only activated at high ligand concentrations [35].

Signaling pathways activated by low EGFR ligand concentrations include ERK1/2 (Extracellular signal regulated kinase), protein kinase B (Akt), Shc1, the adaptor protein CrkL, the E3 ubiquitin protein ligase Cbl and the GRB2-associated Binding protein 1 (Gab1). STAT1, STAT3, STAT5 and phospholipase C (PLC) γ1 are switched to an active state by the EGFR only at high ligand concentrations [35]. This could be the result of two EGF receptors with different ligand affinities, both derived from the same transcript [35]. The differences in receptor ligand affinities appear to cause different cellular effects. Thus, at low ligand concentrations, the high-affinity receptors activate signaling pathways that lead to e.g. proliferation, while at high ligand concentrations, the low-affinity receptors inhibit proliferation and promote the formation of cell clusters [35]. EGFRs interact with downstream proteins via their SH2 or PTB domains. Over a hundred proteins have been described that interact with the EGFR itself and over 200 proteins that are modified in an EGFR-dependent manner [36]. Furthermore, when examining the signaling pathways induced by EGFR, it should be considered that the EGFR can be internalized and some proteins mainly interact with the internalized receptors to promote their signal transmission [23]. The four major signaling pathways activated by EGFR (Figs. 1 and 2) include 1) the Ras-Raf-MEK-ERK signaling pathway, 2) the PI3-kinase-Akt signaling pathway, 3) the PLCγ and 4) the STAT signaling pathway [37]. Beyond the type of signaling pathway activated, the subcellular localization of signal transduction affects the cellular outcome [38]. EGFR activation and subsequent information transfer can occur at the cell membrane, at endocytic vesicles, in late endosomes and in the nucleus. At least at certain microdomains in the cell membrane and in endocytic vesicles the interaction with AT1R is possible.

2.2. Transactivation

Transactivation of the EGFR was first described in 1994 [39]. Through transactivation, the EGFR can transmit signals from other mediators such as endothelin [40–42], norepinephrine [2], prostaglandin E2 (PGE2) [43] or angiotensin II [44]. But also glucose [45], reactive oxygen species [46], oxidized lipids [47], ultraviolet light [39], changes in cell volume [48] and stretching [49] can activate cellular signaling pathways by transactivation of the EGFR and thus enable the organism to adapt to changing environmental conditions. Through this mechanism, the EGFR seems to be involved in pathological processes such as damage to the heart by ischemia and reperfusion, atherosclerosis, kidney disease, hypertension and bronchial asthma. Four mechanisms of EGFR transactivation (Fig. 3) can be distinguished [50–53].

2.3. EGFR ligand-independent transactivation

In this mechanism, EGFR is activated without any specific ligand. Activation of cytosolic signaling networks led to EGFR phosphorylation. This was first described for growth hormone [54] and prolactin [55]. The signaling networks involved comprise cSRC, protein kinase C, protein tyrosine kinase 2 beta (PYK2), Ca^{2+}, reactive oxygen species and JAK2 [31,56]. During ligand-independent transactivation, EGFR serves as a scaffold and activation of its kinase domain is not necessary for signaling [57]. EGFR phosphorylation on tyrosine residues that are not the canonical autophosphorylation sites, leads to activation of downstream signaling pathways, such as PI3 kinase [57–60]. Yamauchi et al [54] were the first to show that growth hormone leads to tyrosine phosphorylation of EGFR in mouse liver and in cultured cells. In this case EGFR phosphorylation was dependent on the kinase JAK2 but not on EGFR kinase activity [54,61]. Growth hormone may induce EGFR phosphorylation at Y1068, which is part of a growth factor receptor-bound protein 2 (Grb2) binding motif. Subsequently, EGFR and Grb2 associate, finally leading to ERK1/2 activation [61]. Growth hormone leads to simultaneous serine/threonine phosphorylation of both EGFR and ErbB2 [61,62] inactivating ErbB-2 [62] but enhancing EGFR signaling after its internalization [61], indicating the complexity of the functional signaling networks.

G protein-coupled receptors (GPCR) can induce ligand-independent

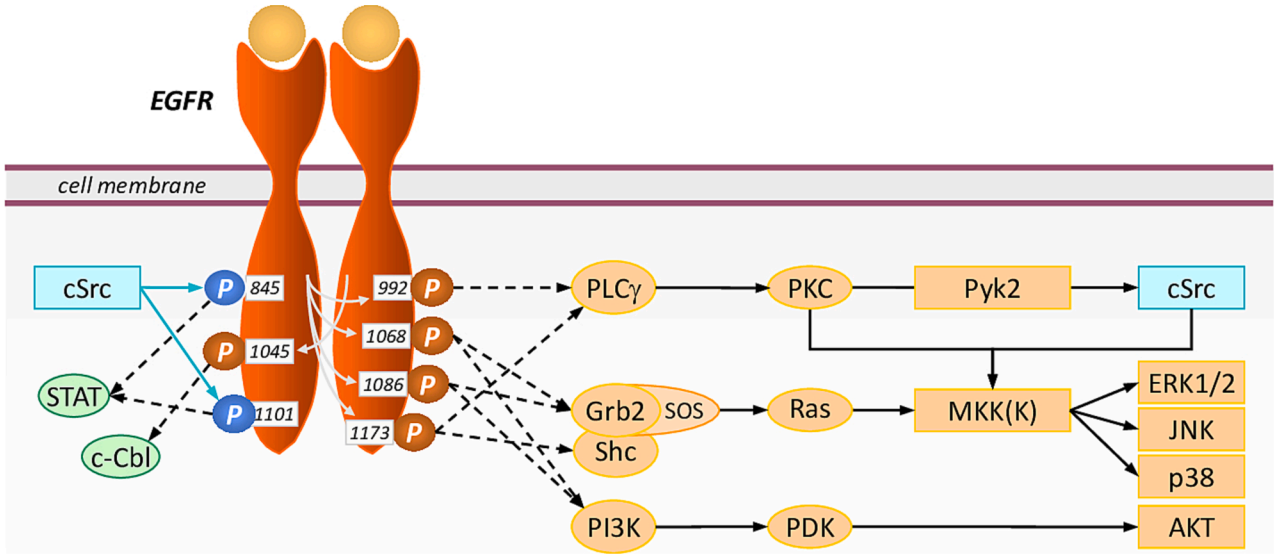


Fig. 2. Scheme of important EGFR (=ErbB1) tyrosine phosphorylation sites and their functional role in cell signaling. There are tyrosine residues autophosphorylation sites that get more intensely phosphorylated by the tyrosine kinase activity of an activated EGFR. Other tyrosine residues get phosphorylation by cytosolic kinases (mostly cSrc). In both cases phosphorylation serves as an on-switch for downstream signaling pathways. For this pur-pose adapter (Grb2, SOS, Shc) or executer (STAT, PLCγ, phosphoinositide 3-kinase) proteins are recruited and activated by phosphorylated tyrosines. As an exception, c-Cbl recruitment and activation serves as regulator of EGFR retrival from the membrane and degradation.

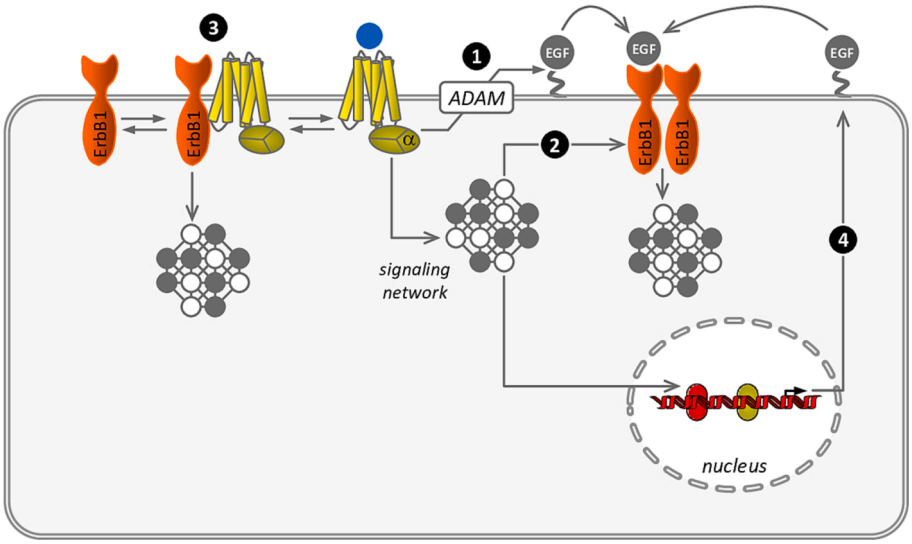


Fig. 3. Four possible mechanisms of AT1R-EGFR crosstalk, leading to EGFR transactivation resp. enhanced EGFR activity are shown. 1) AT1R activates a membrane metalloproteinase (ADAM), which cleaves membrane-anchored EGFR ligand. This ligand activates EGFR of the same (autocrine) or adjacent (paracrine) cells. 2) AT1R activates, via some intermediate steps, cytosolic tyrosine kinases (mostly of the cSrc family) that phosphorylate cytosolic EGFR tyrosine residues thereby activating the receptor. The evidence level for these two mechanisms and their relevance for vascular cell signaling is very high. This is not the case for the other two mechanisms, which are still controversially discussed. 3) AT1R may directly and physical interact with EGFR, forming heterocomplexes. This interaction is supposed to induces alteration of the cytoplasmic part of EGFR that finally result in enhanced kinase activity. The available data do not allow to conclude that heterodimers are formed but also could be explained by the formation of complexes consisting of several proteins. 4) The last and most indirect transactivation, results from AT1R-indcued expression of EGFR ligand. In this case EGFR activation occurs with a substantial temporal delay after AT1R activation. Thus this mechanism would not explain an AT1R-EGFR crosstalk during fast effects, like vasoconstriction but could be relevant for vascular remodeling.

EGFR transactivation via the recruitment of β-arrestins or cSrc through their βγ-subunits [63–65]. cSrc will activate the kinase domain of EGFR [44,65,66]. The target structures of cSrc are tyrosine residues Y845 and Y1101 [59,67,68]. Transactivation of EGFR by cSrc induces MAPK [45,46], PI3-kinase [41,45,46,58,59,69] and STAT signaling. Stimuli reported to transactivate EGFR via cSrc include rapamycin [70], pervanadate [71], hydrogen peroxide [46], D-glucose [45], PGE2 [43], activation of proteinase-activated receptor-2 (PAR2) [72], endothelin-1 [41] and angiotensin II in VSMC [44]. For AT1R additional putative mediators with guanine nucleotide exchange function or kinase function for ligand-independent EGFR transactivation have been identified. These include TRIO (triple functional domain), BMX (BMX nonreceptor tyrosine kinase) and CHKA (choline kinase alpha) [53].

2.4. EGFR ligand-dependent transactivation

GPCR exert a large part of their influence on cell proliferation, cell survival and migration by this mechanism. During ligand-dependent EGFR transactivation, vasoactive substances bind to their canonical receptor and activate cytosolic signaling pathways that enhance the activity of membrane-anchored proteases, mainly ADAM-metalloproteinases [24,73–75]. The latter promote the release of ligands that subsequently bind to and activate the EGFR. Since the signal passes through the cell membrane three times during ligand-dependent transactivation, it is referred to as the "triple membrane spanning" mechanism and was first described for carbachol by the group of Axel Ullrich [24]. Angiotensin II, for example, induces the release of membrane-anchored EGFR ligands from the cell surface mainly via the metalloproteinase ADAM17 [7,76]. The substrates for ADAM17 are very often HB-EGF or transforming growth factor α [77].

The ligands released upon GPCR-activation bind to the EGFR in an auto- or paracrine fashion, promote its activation [42,65,78] and thus the activity of the EGFR's canonical signaling pathways, such as MAPK and PLC-γ1. Given the great number of different GPCRs and their variable expression depending on the type of tissue, this mechanism is most probably of great physiological and pathophysiological importance in several circumstances. Ligands of GPCR known to transactivate EGFR via this mechanism include acetylcholine [37,79], oxidized phospholipids [47], serotonin [80,81] endothelin-1 [81], thrombin [81], angiotensin II [82], lysophosphatidic acid [2] or adrenergic agonists [2].

The exact molecular mechanism for each GPCR has not yet been finally elucidated. Neither the ligand-releasing proteases nor the intracellular signaling pathways have been identified for all transactivating substances. The AT1R induces ligand-dependent transactivation by coupling to the Gq/11-pathway [7,83]. Gαq/11 engages different signaling modules (Ca^{2+}, protein kinase C, reactive oxygen species, cSRC) to activate the metalloproteinase ADAM17, which clusters with EGFR and HB-EGF in caveolae [84]. In addition, the contribution of PLA_2 and p38 kinase in this process has been suggested [85,86]. In these cell membrane microdomains locally released HB-EGF binds to and activates adjacent EGFR. Activation of ADAM17 involves, at least in part, its phosphorylation at Y702 [84,87,88]. Activated EGFR stimulates the downstream pathways ERK1/2, p38, AKT, p70S6K [31]. There is now experimental evidence for the pathophysiological in vivo relevance of this mechanism [87,89–92], e.g. during hypertension, vascular remodeling or aortic aneurysm formation. In addition to ADAM17, MMP14/Membrane Type-1 Matrix Metalloprotease has been shown to contribute to AT1R-induced EGFR transactivation. This mechanism appears to be mediated via the direct activation of MMP-14 by Gβγ and subsequent release of HB-EGF [65]. Transactivation of EGFR by oxidized phospholipids is the result of lipid binding to a free cysteine residue of ADAM10 or ADAMTS4, thereby enhancing their activity [47]. In endothelial cells (EC), this results in an increased release of HB-EGF leading to enhanced expression of IL-8 [47].

Apparently, coupling of AT1R to Gα12/13 does not contribute to EGFR transactivation but stimulates the RhoA/Rock pathway [7]. By contrast, the βγ-subunits of the heterotrimeric G-proteins can induce ligand-independent EGFR transactivation, as described above [89,93]. Thus, activating AT1R may induce a network-like signal transduction due to the simultaneous recruitment of several interacting signaling pathways. This implies that network analysis instead of linear pathway analysis is required to unveil the full AT1R impact on cellular signaling.

Other GPCR, like those for serotonin and endothelin-1 also mediate the transactivation of EGFR via Gαq/11 subunits [81].

It can be concluded that ligand-dependent transactivation of EGFR depends on the inducing ligand, its receptor, the cell type and the actual status of the cell. Furthermore, transactivation can lead to signaling divergence that finally results in activity modulation of intracellular signaling networks. The physiological and pathophysiological significance of these events for angiotensin II have not yet been clarified conclusively, but more and more evidence for a physiological and pathophysiological relevance is provided [7,8,76].

2.5. Heterocomplex formation

A third mechanism for EGFR transactivation is the direct protein–-protein interaction between GPCR and EGFR, i.e. a heterocomplex formation. There are now various studies that provide evidence for several GPCRs, including the AT1R, concerning EGFR heterocomplex formation [27,50,94–98]. Thereby, it has been recently proposed that AT1R-EGFR heteromerization leads to the recruitment of Grb2, which may result in the activation of downstream pathways [98]. The evidence for such interactions was obtained from different experimental approaches, like FRET, BRET, immunoprecipitation or proximity labelling mediated by APEX proximity labeling [99]. Heterocomplexes can be constitutive and ligand-independent or exist in a ligand-dependent equilibrium between association and dissociation. In this case, ligands may favor either dissociation or association. Furthermore, heterocomplex formation is thought to depend on the subcellular location of the interaction partner. The above mentioned protein TRIO has been identified as interaction partner of AT1R-EGFR heteromers contributing to the transactivation process and connecting the heteromer to several downstream signaling modules [100]. Finally, competition of GPCR for heterocomplex formation with EGFR has been shown [94], although not yet for AT1R. By such a competition, one GPCR could influence the EGFR-dependent signaling of a second GPCR and finally lead to weaker or stronger activation of the downstream pathways, depending on the relative "strength" of the two GPCRs.

Although, there is evidence for heteromer formation, the issue is still discussed controversially and further molecular and biophysical investigations are required. Furthermore, it is not clear whether real dimers or protein multimers (microdomains) are formed [53,98,100,101].

Heterocomplex formation could also enable a bidirectional information exchange and explain the modulation of GPCR activity by activated EGFR [95]. However, this direction of crosstalk is less well investigated and therefore the relevance for AT1R signaling cannot be assessed. In general, AT1R heterocomplex formation can result in pathologic aggregation, as shown for the AT1R-B2R heteromer [102]. Whether this is also the case for AT1R-EGFR heteromers has to be investigated in the future.

2.6. Induction of EGFR ligands

Finally, AT1R can modulate EGFR activity on a different time scale by enhancing the expression of membrane-anchored EGFR ligands. Such a mechanism was proposed for angiotensin II in murine vascular smooth muscle cells (VSMC) [103]. Increased abundance of HB-EGF was required for the induction of transforming growth factor β and connective tissue growth factor expression. Angiotensin II-induced HB-EGF upregulation was also shown for bladder smooth muscle cells and in a model of nephrotoxicity [104,105]. Assuming that AT1R activation would also stimulate HB-EGF shedding, angiotensin II acts via two mechanisms with different time scales, whereby the second mechanism enforces the first one.

3. Cellular consequences of EGFR-AT1R crosstalk

Canonical, EGFR-independent, angiotensin II signaling is mediated by AT1R and AT2R [7]. Vasoconstriction and pathological vascular effect are mediated mainly by the AT1R. Both receptors couple to heterotrimeric G-proteins, whereby downstream signaling of AT2R is less well understood. AT1R activates either Gq or G12/13 subunits of heterotrimeric G-proteins. Subsequently, either the phospholipase Cβ or the RhoA/ROCK pathway are engaged, both leading to vasoconstriction. The protein tyrosine kinase 2 beta (PYK2), JAK2 and Ca^{2+}/calmodulin-dependent protein kinase II can support this process.

3.1. Nuclear information transfer

Although AT1R-EGFR interaction is well studied with respect to proximal signaling in cells, the consequences of this interaction in terms of information transfer to the nucleus, transcription regulation and finally the transcriptome are less well understood (Fig. 4). It is possible that AT1R-EGFR-transctivation leads to (i) a linear, EGFR-triggered, nuclear signaling or whether transactivation induces (ii) parallel AT1R and EGFR signaling leading to (iii) synergistic effects, as it would be also the case during separate but simultaneous activation by external ligands (EGF and angiotensin II). Deeper understanding of these mechanisms is of importance because nuclear information transfer affects gene expression with major impact on cell fate [99] and is therefore of potential physiological and pathophysiological relevance (Fig. 4). In this regard, the question arised whether a potential synergistic information transfer leads to quantitative, qualitative or temporal variations relevant for gene expression and environmental interaction [99].

The influence of AT1R and EGFR on SRF (serum response factor), activator protein 1 and early growth response protein 1 transcriptional activity, and transcriptome regulation were investigated in HEK293 cells, HK-2 cells and VSMC in combination with RNA sequencing and comprehensive bioinformatic analysis [99,106]. The data showed that AT1R and EGFR synergistically activate SRF via the ERK1/2-TCF and the actin-MRTF pathway. This synergism, consisting of switch-like and graded single-cell reaction, converged at least partially on the transcription factors activator protein 1 and early growth response protein 1 and led to substantial transcriptome changes, qualitatively (number of affected genes), quantitatively (expression level of individual genes) and temporal (later onset and longer-expressed genes). Bioinfomatic analyses pointed to persistent cell stress and consequences for vascular biology. The synergism occurred during separate but simultaneous activation of both receptors and during AT1R-induced transactivation of EGFR. EGFR and AT1R thus synergistically regulate gene expression in qualitative, quantitative and temporal terms with (patho)physiological relevance. An AT1R-EGFR synergism regarding nuclear signaling was also described for intestinal epithelial cells, albeit it involved the transcription factor CREB [107].

3.2. Vascular smooth muscle cells

In VSMC, the activation of the following signaling modules are described after EGFR stimulation: ERK1/2 [19,108–110], STAT3 [19], JAK [19], phosphoinositide 3-kinase [109], PLC-γ [111], GIT-1 [111] and light chain of myosin [19]. However, it should be noted that VSMC differ in the expression of e.g. receptors or ion channels, depending among other things on the circulatory segment from which they originate. For example, a stretch-dependent vasoconstriction can be induced in VSMC of resistance vessels, but not in those of large arteries. So far, little attention has been paid to the difference in VSMC depending on the vessel type of origin.

Substances that transactivate EGFR in VSMC include angiotensin II [44,110,112] aldosterone [113], adrenergic agonists [114,115], endothelin 1 [116–118] and ATP [119]. Ligand-dependent and ligand-independent transactivation have been described in VSMC, and it is possible that both pathways can be activated by the same substance. Angiotensin II is said to activate the EGFR in a ligand-dependent [112] but also in a ligand-independent [44,110] manner. Whether the

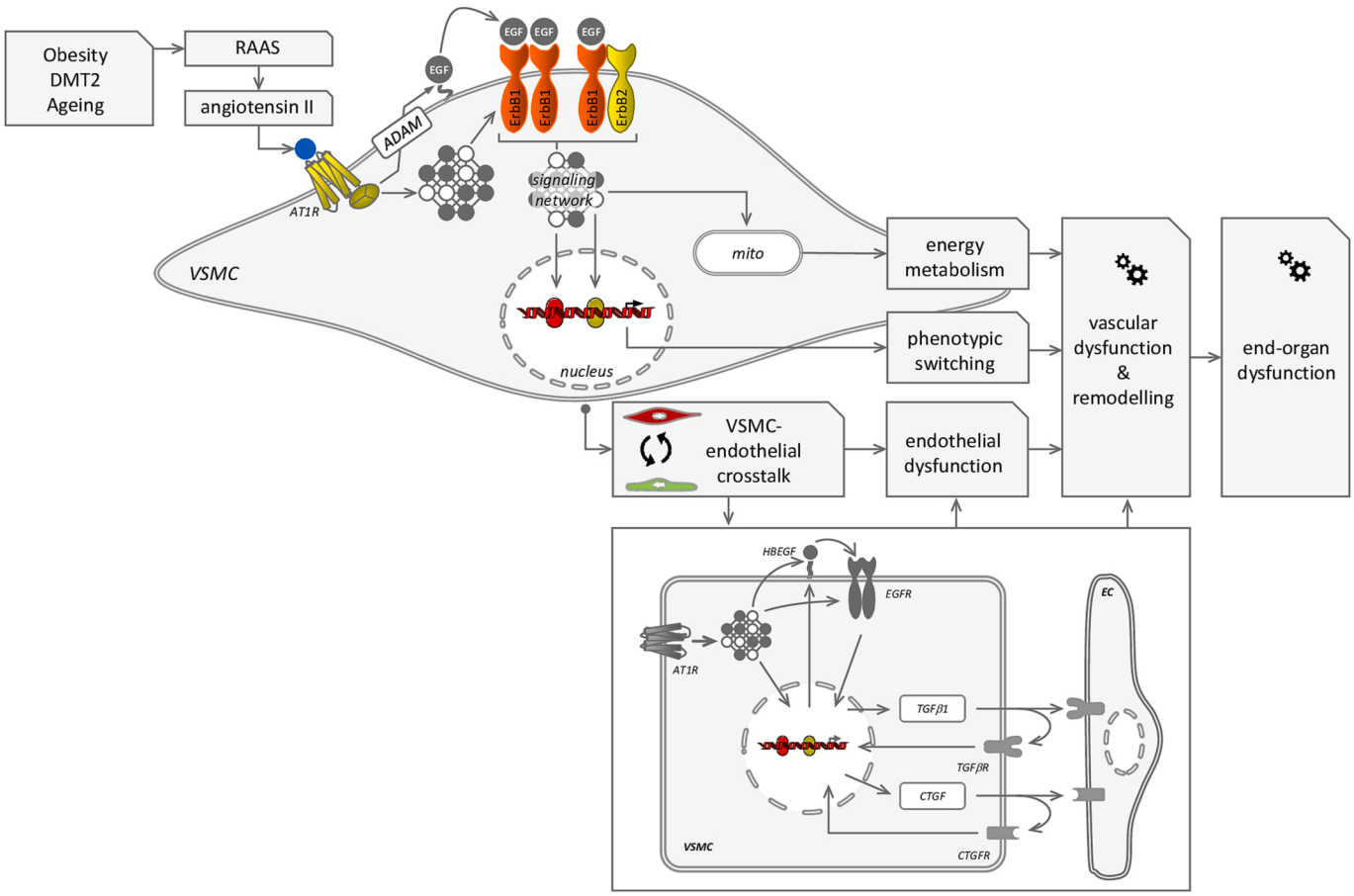


Fig. 4. Overview of vascular consequences resulting from AT1R-induced EGFR transactivation in vascular smooth muscle cells (VSMC) and a subsequent crosstalk with endothelial cells (EC). RAAS = renin-angiotensin-aldosterone system. CTGF = Connecting tissue growth factor. Mito = mitochondria. DMT2 = diabetes mellitus type 2.

activation of the EGFR in VSMC of different arteries occurs with the help of the same mechanism has not yet been investigated in detail. The ligand that mediates EGFR transactivation in VSMC is mostly HB-EGF [114,114,120,121], although transactivation by transforming growth factor α [122] has also been described.

EGFR transactivation by angiotensin II in VSMC is mediated predominantly by AT1R that couples to Gαq/11 or Gα12/13 [7] and can involve EGFR transactivation via ADAM metalloproteinase domain 17 or cSrc kinase [31,124]. In the case of ADAM17, EGFR is activated by shedding and binding of HB-EGF whereas cSrc leads to direct EGFR phosphorylation [56]. AT1R-EGFR heteromerization leading to recruitment of Grb2, has also been proposed [98]. Besides, AT1R-EGFR synergy independent of transactivation or receptor interaction has been described [123–126], although the post-receptor steps involved are not well characterized.

In addition to the previously described signaling pathways of EGFR transactivation through activation of c-Src or Jak, activation of EGFR through changes in ion currents has also been described in VSMC [49,116,117], with non-voltage-dependent Ca^{2+} channels playing the crucial role. Thus, mechanical stretch in VSMC leads to the opening of stretch-dependent Ca^{2+} channels and thereby to the activation of EGFR and ERK1/2 [49]. Endothelin-1 also induces EGFR transactivation via activation of Ca^{2+} channels [116,117].

The importance of EGFR for VSMC survival, pentose phosphate pathway activity, matrix homeostasis, activation of mitogen-activated kinases (ERK1/2) and interference with cellular calcium homeostasis was established in murine primary VSMC from the aorta of mice with conditional VSMC-EGFR deletion [127]. EGFR deletion enhances spontaneous cell death, reduced pentose phosphate pathway activity, disrupted cellular matrix homeostasis (collagen III and fibronectin), motility, and switched off EGF sensitivity. In addition, endothelin-1-, phenylephrine-, ATP- and H_2O_2-induced ERK1/2 phosphorylation was significantly reduced in VSMC from knockout animals, as was the calcium response to endothelin-1 and phenylephrine. These findings demonstrate the importance of the VSMC-EGFR for (i) basal VSMC cell homeostasis, (ii) ERK1/2 activation by G-protein coupled receptors and oxygen radical stress, and (iii) calcium signaling [127,128].

Being a relevant signaling hub, its expression level influences the importance of VSMC-EGFR. Concerning the crosstalk of EGFR with the RAAS [21] in VSMC, an ageing-related EGFR-induction has been shown [21]. EGFR expression in VSMC is stimulated by aging and aldosterone. Inhibition of EGFR reduced age-related expression of pro-inflammatory genes. Parallel to EGFR, the expression of the mineralocorticoid receptor and the aldosterone sensitivity of the cells to EGFR-dependent ERK1/2 phosphorylation increased with age. Aldosterone also resulted in increased expression of Transforming Growth Factor-ß, Intercellular Adhesion Molecule 1 and Procollagen 1, depending on age and the EGFR-ERK1/2 axis. These data indicate that during ageing the RAAS enhances EGFR expression, making VSMC more sensitive to angiotensin II. This enhanced RAAS sensitivity supports the pro-inflammatory VSMC phenotype during ageing in an EGFR-dependent manner [21]. Further investigations showed that the induction of EGFR expression in VSMC occurs via a new, SP1-dependent, response element in the EGFR promoter [129].

The relevance of transcriptional signaling synergy based on the interaction of EGFR with AT1R and in parallel with the thromboxane A2 receptor (TBXA2R) has been investigated in primary murine aortic vascular muscle cells [106]. Transcriptome analysis revealed that

simultaneous EGFR-AT1R or EGFR-TBXA2R activation led to significantly different gene expression patterns compared to single substance exposure (=qualitative synergy). In addition, simultaneous EGFR-TBXA2R activation led to an over-additive increase in the amplitude of expression changes of a group of genes (=quantitative synergy) including Klf15 and Spp1, which are relevant in vascular damage. Furthermore, Gene Ontology enrichment analyses showed that synergy-dependent changes in gene expression affect processes critical to vascular integrity, such as cell cycle regulation and senescence. These data show that the activation of GPCRs in parallel to EGFR induces synergistic regulation of gene expression in VSMC and requires extensive network analyses to evaluate functional relevance of altered signaling. Investigating the effect of individual receptors often does not provide a sufficient knowledge for assessing physiological or pathological effects [106].

3.3. Endothelial cells

Less is known about the importance of EGFR in EC. However, several factors mediating at least part of their effect via the EGFR were described. Substances that contribute to vascular damage in diseases such as diabetes mellitus or atherosclerosis and may transactivate endothelial EGFR include advanced glycation end products (AGE) [130], hydrogen peroxide [46], oxidized lipids [47], endothelin-1 [41,131] and angiotensin II [132]. Possible consequences of EGFR transactivation in EC have not yet been assessed conclusively. However, there is increasing evidence that activation of EGFR stimulates EC proliferation, promotes angiogenesis, induces adhesion of leukocytes, increases permeability and affects vascular reactivity [8,27,41,133]. In line, angiotensin II stimulates angiogenesis via transactivation of EC EGFR in a cell culture model [132]. With regard to the signaling pathways activated by the EGFR in EC, systematic descriptions are still lacking. In addition to the “triple membrane spanning” mechanism, ligand-independent EGFR transactivation [46] has been described.

3.4. Intercellular communication

Using murine VSMC, it was shown that VSMC-EGFR can act as a relay station for pathologically relevant paracrine communication (Fig. 4). Angiotensin II increased HB-EGF expression in primary VSMC with a subsequent, EGFR-mediated expression and secretion increase of transforming growth factor ß followed by enhanced connective tissue growth factor expression. Both mediators can lead to pathological changes in VSMC as well as in EC, so that there is a possible mechanism of VSMC-EGFR-dependent changes in endothelial function [99,103,134]. In addition, it was shown that angiotensin II-induced HB-EGF release leads to an activation of the SRF signaling pathway through paracrine binding to the EGFR of neighboring cells, synergistically with the AT1R. This indicates that the EGFR signaling pathway enables communication between neighbouring vascular cells and mediates their adaptation to changing environmental conditions [99,103].

4. EGFR and vasculature

Vessel function and morphology are under the control of numerous humoral and local mediators that affect interacting cellular signaling cascades forming functional networks, with integrated signaling changes ultimately critical to understanding (patho)physiological processes. Here, the EGFR plays an important role as an integrator of numerous signaling pathways [8,99,135].

The importance of ErbB receptors in cardiovascular function and homeostasis was first recognized in women who developed cardiac hypertrophy during a cancer therapy with pharmacological EGFR inhibitors. There are now numerous reports emphasizing the importance of all four members of the ErbB family in the development of the cardiovascular system [9,136]. A review by Forrester et al [31] gives an overview of the cardiovascular phenotype in different mouse lines with reduced EGFR activity, either due to mutation-induced changes or pharmacological inhibition of the receptor.

Transactivation of the EGFR could be demonstrated in blood vessels in vivo as well as ex vivo and is supposed to be involved in vascular remodeling and the development of high blood pressure. The influence on angiogenesis, restenosis [137,138], atherosclerosis, vascular dysfunction [139] and vascular fibrosis has been described. Furthermore, EGFR is responsible for some of the vascular changes during diabetes mellitus [140] and ageing [21]. Finally yet importantly, transactivation of the EGFR in blood vessels has been described for angiotensin II [32,141–144]. However, the in vivo relevance of EGFR for vascular alterations was mainly assessed by pharmacological inhibition, global partial reduction of its activity due to mutations or indirectly by interference with putative ligands [31]. It was therefore not possible to distinguish whether, for example, a reduced blood pressure-increasing effect of angiotensin II when EGFR is inhibited results from a reduced contraction of the VSMC or is caused by increased NO synthesis in the endothelium [145].

Under resting conditions, the EGFR seemed to have only a small effect on vessel structure, since no macro- or microscopic differences could be observed in mice with a hypomorphic EGFR [15]. In the carotid artery, it could be shown that the lumen-reducing vascular remodeling (inward remodeling) can be reduced by inhibition of EGFR, either via AG1478 [137] or an inhibitory antibody [138]. The physiological importance of the EGFR for maintaining blood pressure was also unclear. There is no difference in systolic blood pressure in Wa-2 mice compared to wild-type animals [15,146]. Furthermore, the administration of AG1478 for five hours did not change neither systolic, diastolic nor mean blood pressure in rats [145]. Altogether, these findings did not allow conclusive assessment of EGFR importance during the development of high blood pressure.

In mice with a global hypoactive EGFR (Wa-2, mouse waved-2 phenotype with a point mutation in the EGFR tyrosine kinase), endothelium-dependent vasodilation of abdominal aortic rings was slightly reduced [15]. However, the endothelium-independent vasorelaxation is unchanged, suggesting that the differences were caused by EC, possibly due to the reduced expression of endothelial NO synthase (eNOS) in these animals [15]. This finding could be reproduced in the pathophysiological relevant model of aldosterone/salt treatment in combination with 5/6 nephrectomy [15]. This treatment sensitized blood vessels to angiotensin II in an EGFR-dependent manner [147]. These results are at least in part contradictory to findings in rat thoracic aortic rings [141], which describe an EGFR-dependent desensitization for angiotensin II in 5/6 nephrectomy. However, the EGFR promoted sensitization of the myogenic response. The importance of the vascular EGFR has so far been examined focusing on VSMC, whereby there is also evidence for a role in EC (e.g. NO homeostasis, endothelial dysfunction, effect of angiotensin II), which however requires further functional confirmation or pathophysiological evaluation [8,31,131,148,149]. Thus, data availability for vascular muscle cells is far more comprehensive and has recently been expanded by in vivo and ex vivo investigations on genetic mouse models [15,127,150,151], with regard to the physiological significance and pathophysiological relevance.

Beyond their cell-biological importance, vascular EGFR are also of pathological and clinical relevance. Their involvement in pathogenic vascular processes during atherosclerosis, in obesity and DMT2 as well as systemic and pulmonary hypertension is postulated with substantial evidence [8,131,140,152,153]. Examining genetic models with cell-specific EGFR deletion, it could be shown in vivo that the smooth muscle EGFR contributes to the setting of a physiological vascular tone and thus blood pressure and supports the effect of vasoactive hormones (see below) [127,150,151]. Furthermore, these investigations showed that the smooth muscle EGFR contributes to the maintenance of vascular wall homeostasis. Overall, the findings show that the EGFR plays a more complex role in the signaling network of the vessel wall in vivo and that

cell-unspecific reductions in its activity (by inhibitors or the Wa-2 mouse strain) cannot provide conclusive answers. The picture of a Janus-faced EGFR emerges, since on the one hand, EGFR contributes to physiological vascular function and homeostasis, but on the other hand it also supports the development of pathological vascular changes, so that it plays an important role in two different contexts. The role of the EGFR is thus complex, since it can have both protective and damaging effects and these may depend on the strength or circumstance of activation.

Vascular EGFR has been linked to inflammatory processes in the vessel wall, also in connection with angiotensin II, as reviewed before [31,56]. These inflammatory processes are most probably the result of EC activation that can result from endothelial EGFR or smooth muscle EGFR signaling. In the latter case, an EGFR-dependent VSMC-to-EC crosstalk induces inflammation, as indicated in an obesity model [154]. Signaling pathways that with proinflammatory transcription-al impact include ER-stress, HIF1α, CREB and NFkB [56,155]. The relevance for angiotensin II-induced inflammation has been shown in a mouse model with endothelial-specific HB-EGF deletion. Knock-out animals showed significantly reduced angiotensin II-induced renal inflammation, indicated by lower IL6 and MCP1 expression [156]. Evidence for a systemic pathophysiological relevance was proved by a study showing that EGFR inhibitors decrease inflammation markers (TNFα, IL6) in atherosclerotic plaques of mice and their macrophages, as well as in in human VSMC [157].

In a mouse model of SM22 promoter dependent EGFR deletion, the basal, cell-specific importance of the EGFR in VSMC and cardiomyocytes could be investigated in vivo for the first time [150]. Plethysmographic and intravascular blood pressure measurements as well as echocardiography showed a reduced peripheral vascular resistance, reduced diastolic and mean blood pressure with unchanged systolic blood pressure. Loss of VSMC-EGFR resulted in a dilated vascular phenotype with low levels of fibrosis and inflammation and reduced angiotensin II reactivity. Echocardiography, necropsy and histology revealed dramatic eccentric cardiac hypertrophy in constitutive conditional knockout mice with greater stroke and cardiac output, left ventricular volume and thickened ventricular wall. Cardiac hypertrophy is accompanied by an increase in cardiomyocyte volume and the expression of hypertrophy markers, without a significant increase in profibrotic or proinflammatory parameters. Furthermore, an increased mRNA expression of NADPH oxidase 4 (NOX4), but not NOX2, as well as increased NOX activity in the heart and in isolated cardiomyocytes could be detected. Thus, the EGFR appears to counteract cardiac growth, possibly by affecting oxygen radical homeostasis. These changes correspond mechanistically to those that occur in cardiomyocytes as part of aging [158]. The blood pressure changes were confirmed in the inducible model of SMMHC-CreERT2-driven EGFR knockout in VSMC [134,159]. Since cardiac output was not increased in this model, systolic blood pressure was also reduced. Thus, VSMC-EGFR contributes to the maintenance of the vessel wall architecture as well as vessel reactivity and physiological vasotonus [150].

At the cellular level (i.e. studies using cultured cells) the mechanisms of GPCR-EGFR crosstalk seem to be similar and in many cases transactivation is dependent on the activation of ADAMs, irrespective of the stimulus. However, the cell type under investigation may play a role for the mode of transactivation, because the expression of membrane-bound EGFR-ligands can differ. Ex vivo studies with isolated vessels showed that the importance of EGFR-transactivation by vasoconstrictor differs. Whereas vascular reactivity to AII, phenylephrine or U46619 is at least partially EGFR-dependent, reactivity to KCl (depolarization), serotonin or endothelin-1 was not [160]. Since vascular reactivity to e.g. KCl was not affected in EGFR-KO animals, a basal impairment of contractile function is not likely.

5. EGFR-AT1R crosstalk in vivo

One of the better-studied roles of vascular EGFR in vivo is its contribution to the blood pressure-increasing effect of angiotensin II. However, the data were inconsistent for a long time. For example, the angiotensin II-induced increase in blood pressure could be reduced by AG1478 administration to rats [145]. On the other hand, Chan et al. [32] were unable to observe an effect on the hypertension induced by angiotensin II either with genetic mutation (Wa-2 mouse strain, strong reduction in EGFR activity) or with inhibition of EGFR (AG1478) [32]. The contractile response of murine aortic rings [15] by angiotensin II was altered by a global but partial reduction of EGFR activity. Contraction of human coronary vessels was unaffected by EGFR blockade when stimulated with angiotensin II alone, whereas an angiotensin-aldosterone synergism was prevented [161].

Concerning the acute angiotensin II-induced blood pressure burden, an essential role for VSMC-EGFR was observed in a transgenic mouse model with inducible and conditional EGFR knockout [159]. As in the constitutive-conditional VSMC-KO model [127,150], a dilated vascular phenotype was also present. Morphometry and gene expression analysis on aortic rings from wild type and VSMC-EGFR-KO animals showed no significant basal differences. The same holds true for strain-wall stress behavior in the relevant working range, determined by myography. The response to KCl, serotonin and endothelin-1 as well as the carbachol- or NO-induced relaxation were virtually not changed. In contrast, angiotensin II-induced force development was significantly reduced in constitutive and inducible conditional transgenic models. Further investigation showed that AT1R signaling was reduced; homologous desensitization was accelerated but receptor mRNA expression was unaltered. The involvement of AT2 receptors could be ruled out pharmacologically. These data demonstrated for the first time the differential role of the VSMC-EGFR in the regulation of vascular tone by stimuli capable of EGFR transactivation [160]. Furthermore, the KO animals were protected from the age-dependent increase in heart weight and also showed no increase in aortic and cardiac inflammatory markers (Ccl2, Serpine 1). These data suggest that the VSMC-EGFR is involved in basal blood pressure homeostasis and acute blood pressure regulation by angiotensin II and contributes to age-related cardiovascular remodeling [160].

Concerning chronic angiotensin II-induced alterations in blood pressure and vascular dysfunction, three-week infusion of angiotensin II via osmotic minipumps in wild-type animals resulted in an increase in blood pressure and aortic wall thickness. These effects were not observed in animals with inducible VSMC-EGFR-KO (aortic wall thickness) or were significantly reduced (blood pressure increase) [134]. There were no differences in water and food intake. Parallel to the aortic wall thickening, the fibrosis markers collagen-1, fibronectin-1 and collagen-3 were induced by angiotensin II infusion in wild-type animals but not in KO animals. These data demonstrate that the VSMC-EGFR is involved not only in acute physiological, but also the chronic, pathological actions of angiotensin II in the cardiovascular system, thus complementing pharmacological studies on the action of EGFR antagonists in the cardiovascular system [33,134].

A more complex, angiotensin-dependent clinical-pathological scenario is obesity-induced diabetes mellitus type 2 (DMT2). Vascular dysfunctions in this context depends to a major part on an overactive RAAS. The role of the EGFR in these situations is of mechanistic and clinical relevance, since previous studies prove the fundamental importance of the EGFR without specifying the cell types involved or the pathomechanisms affected [31]. For example, increased vascular EGFR expression or increased EGFR phosphorylation has been described in hyperglycemia and in insulin-resistant animals. Furthermore, vascular function and wall homeostasis improve in the presence of EGFR kinase inhibitors [8,140,152,153]. Since a relevant role of angiotensin II in the context of vascular damage during obesity and DMT2 has been shown [162,163] and vascular EGFR is necessary for the full effect of angiotensin II on vessel structure and function, a fundamental pathogenic significance of vascular EGFRs for vascular dysfunction/wall remodeling under the conditions mentioned is conceivable. This role may turn

vascular EGFR, for which pharmacological tools already exist, into a relevant therapeutic target. However, for a long time it was not possible to test the hypothesis of a cell-specific EGFR involvement in vascular dysfunction or damage in the context of obesity/DMT2 in vivo.

The role of vascular EGFR in high-fat diet (HFD)-induced DMT2 was studied in mouse models with induced VSMC-EGFR-KO or with EC-EGFR-KO [154,162]. The results show that the VSMC-EGFR mediates obesity/DMT2-induced vascular dysfunction, remodeling and transcriptional dysregulation. These precede kidney damage. Furthermore, they identify an EGFR-glucose synergism with regard to serum response factor (SRF; main regulator of VSMC differentiation and glucose sensor) activation, matrix dysregulation and mitochondrial dysfunction. VSMC-EGFR-KO protects the animals from HFD-induced endothelial dysfunction (thus there is a VSMC-EGFR-dependent interaction between VSMC and endothelium), creatininemia and albuminuria. Furthermore, the HFD-induced changes of the vascular transcriptome were prevented. These findings suggest EGFR-dependent SRF activation, matrix dysregulation and mitochondrial dysfunction. At the cellular level, hyperglycaemia was shown to increase EGFR/ErbB2-induced stimulation of SRF activity via the EGFR/ErbB2 ROCK-actin-MRTF signaling pathway and amplify mitochondrial dysfunction. Thus, it was shown that the VSMC-EGFR contributes to HFD-induced vascular and ultimately renal changes. The potentiation of the EGFR/ErbB2-ROCK-MRTF-SRF signaling axis and mitochondrial dysfunction plays an important role here. [154].

The results with the EC-EGFR-KO model on vascular and renal function under control and HFD (i.e. obesity) conditions showed a very different role and importance of EC-EGFR in vivo [164]. Heart and lung weights, blood pressure and aortic transcriptome were unaffected by EC-EGFR-KO, as was aortic contractile response to α1-adrenergic stimuli, in contrast to VSMC-EGFR-KO [134,150,159]. Yet, the endothelium-dependent relaxation of the abdominal aorta of EC-EGFR-KO animals was reduced. The results for mesenteric arteries were in part different, indicating a vessel type specific role of EC-EGFR. Mesenteric arteries from EC-EGFR-KO animals were more sensitive to α1-adrenergic stimulation than wild-type animals, while endothelium-dependent relaxation and vessel wall morphology were unchanged. The obesity-induced changes are comparable in EC-EGFR-WT and -KO animals. HFD-induced aortic endothelial dysfunction, which is not additive to EC-EGFR-KO-induced dysfunction, indicating a negative effect of obesity on the protective role of EC-EGFR. Only HFD-induced albuminuria is attenuated in EC-EGFR-KO animals. These data suggest that the EC-EGFR, compared to the VSMC-EGFR, is of lesser and sometimes opposite importance for vascular function itself and for obesity-induced damage [164].

6. Perspective

The importance of EGFR has expanded over the field of tumour biology and is now well documented for the cardiovascular system, especially for angiotensin II. For a long time our knowledge was based mainly on studies with primary cells, cell lines or heterologous expression systems and cell non-specific in vivo interventions. The generation of cell-specific EGFR-KO mouse models in recent years made the translation to in vivo models possible, giving the opportunity to dissect the importance of EGFR in different cell types. Combining cellular, ex vivo and in vivo data teaches us a complex and cell-specific role of vascular EGFR as a central hub in intracellular and intercellular signaling networks. This complex role comprises physiological functions and tissue homeostasis as well as pathological vascular alterations. We now need to deepen our understanding on the one hand of its role in cellular signaling and transcription networks by the appropriate bioinformatic analyses and modelling approaches. On the other hand we need to deepen our understanding of its systemic and cell-specific role under physiological and pathophysiological conditions to enable the development of rationale therapeutic approach with vascular EGFR as drug target.

Rating the future impact of our improved knowledge, concerning vascular EGFR, beyond the basal mechanistic understanding, is complex. We now understand that EGFR is an important signaling hub with respect to the balance and dysbalance of vascular wall functional and structural homeostasis. Because there are several clinical EGFR inhibitors available, it is tempting to speculate about their use in patients with vascular diseases. However, systemic application of EGFR inhibitors could interfere with its beneficial functions, like epithelial repair mechanisms. Furthermore, application ErbB inhibitor during cancer treatment showed cardiac side effects. Thus, while EGFR inhibitors may not be an additional option for the reduction of an enhanced vasotonus (there are very efficient drugs on the market), they may well add a valuable layer to the therapeutic strategies concerning vascular remodeling, stiffening and even atherosclerosis. For this purpose, inhibitors that act mainly on vascular cells are desirable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are funded by the Deutsche Forschungsgemeinschaft (DFG).

References

[1] D.N. Amin, K. Hida, D.R. Bielenberg, M. Klagsbrun, Tumor Endothelial Cells Express Epidermal Growth Factor Receptor (EGFR) but not ErbB3 and Are Responsive to EGF and to EGFR Kinase Inhibitors, Cancer Res. 66 (2006) 2173–2180.
[2] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell Biol. 2 (2001) 127–137.
[3] W.E. Sweeney, Y. Chen, K. Nakanishi, P. Frost, E.D. Avner, Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor, Kidney Int. 57 (2000) 33–40.
[4] P.W. Cook, M. Piepkorn, C.H. Clegg, G.D. Plowman, J.M. DeMay, J.R. Brown, M. R. Pittelkow, Transgenic expression of the human amphiregulin gene induces a psoriasis-like phenotype, J. Clin. Invest. 100 (1997) 2286–2294.
[5] J.T. Elder, G.J. Fisher, P.B. Lindquist, G.L. Bennett, M.R. Pittelkow, R. J. Coffey Jr., L. Ellingsworth, R. Derynck, J.J. Voorhees, Overexpression of transforming growth factor alpha in psoriatic epidermis, Science 243 (1989) 811–814.
[6] A. Wissner, E. Overbeek, M.F. Reich, M.B. Floyd, B.D. Johnson, N. Mamuya, E. C. Rosfjord, C. Discafani, R. Davis, X. Shi, S.K. Rabindran, B.C. Gruber, F. Ye, W. A. Hallett, R. Nilakantan, R. Shen, Y.F. Wang, L.M. Greenberger, H.R. Tsou, Synthesis and structure-activity relationships of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design of an orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2), J. Med. Chem. 46 (2003) 49–63.
[7] S.J. Forrester, G.W. Booz, C.D. Sigmund, T.M. Coffman, T. Kawai, V. Rizzo, R. Scalia, S. Eguchi, Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology, Physiol. Rev. 98 (2018) 1627–1738.
[8] B. Schreier, M. Gekle, C. Grossmann, Role of epidermal growth factor receptor in vascular structure and function, Curr. Opin. Nephrol. Hypertens. 23 (2014) 113–121.
[9] C.J. Barrick, M. Yu, H.H. Chao, D.W. Threadgill, Chronic pharmacologic inhibition of EGFR leads to cardiac dysfunction in C57BL/6J mice, Toxicol. Appl. Pharmacol. (2008). In Press, Accepted Manuscript.
[10] G.N. Gill, T. Kawamoto, C. Cochet, A. Le, J.D. Sato, H. Masui, C. McLeod, J. Mendelsohn, Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity, J. Biol. Chem. 259 (1984) 7755–7760.
[11] H. Masui, T. Kawamoto, J.D. Sato, B. Wolf, G. Sato, J. Mendelsohn, Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies, Cancer Res. 44 (1984) 1002–1007.
[12] P.J. Miettinen, J.E. Berger, J. Meneses, Y. Phung, R.A. Pedersen, Z. Werb, R. Derynck, Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor, Nature 376 (1995) 337–341.
[13] A. Seidman, C. Hudis, M.K. Pierri, S. Shak, V. Paton, M. Ashby, M. Murphy, S. J. Stewart, D. Keefe, Cardiac dysfunction in the trastuzumab clinical trials experience, J. Clin. Oncol. 20 (2002) 1215–1221.

[14] C. Grossmann, M. Gekle, Non-classical actions of the mineralocorticoid receptor: Misuse of EGF receptors? Mol. Cell. Endocrinol. 277 (2007) 6–12.
[15] V. Griol-Charhbili, C. Fassot, S. Messaoudi, C. Perret, V. Agrapart, F. Jaisser, Epidermal growth factor receptor mediates the vascular dysfunction but not the remodeling induced by aldosterone/salt, Hypertension 57 (2011) 238–244.
[16] N. Ulu, H. Gurdal, S.W. Landheer, M. Duin, M.O. Guc, H. Buikema, R.H. Henning, a1-Adrenoceptor-mediated contraction of rat aorta is partly mediated via transactivation of the epidermal growth factor receptor, Br. J. Pharmacol. 161 (2010) 1301–1310.
[17] Q. Che, P.K. Carmines, Angiotensin II triggers EGFR tyrosine kinase-dependent Ca2+ influx in afferent arterioles, Hypertension 40 (2002) 700–706.
[18] D. Chansel, M. Ciroldi, S. Vandermeersch, L.F. Jackson, A.M. Gomez, D. Henrion, D.C. Lee, T.M. Coffman, S. Richard, J.C. Dussaule, P.L. Tharaux, Heparin binding EGF is necessary for vasospastic response to endothelin, FASEB J. 20 (2006) 1936–1938.
[19] A.H. Amin, Z.Y. Abd Elmageed, M. Partyka, K. Matrougui, Mechanisms of myogenic tone of coronary arteriole: Role of down stream signaling of the EGFR tyrosine kinase, Microvasc. Res. 81 (2011) 135–142.
[20] J. Kim, C.K. Lee, H.J. Park, H.J. Kim, H.H. So, K.S. Lee, H.M. Lee, H.Y. Roh, W. S. Choi, T.K. Park, B. Kim, Epidermal growth factor induces vasoconstriction through the phosphatidylinositol 3-kinase-mediated mitogen-activated protein kinase pathway in hypertensive rats, J. Pharmacol. Sci. 101 (2006) 135–143.
[21] A.W. Krug, L. Allenhofer, R. Monticone, G. Spinetti, M. Gekle, M. Wang, E. G. Lakatta, Elevated mineralocorticoid receptor activity in aged rat vascular smooth muscle cells promotes a proinflammatory phenotype via extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase and epidermal growth factor receptor-dependent pathways, Hypertension 55 (2010) 1476–1483.
[22] J.M. Sanders, M.E. Wampole, M.L. Thakur, E. Wickstrom, Molecular determinants of epidermal growth factor binding: a molecular dynamics study, PLoS One 8 (2013) e54136.
[23] R.B. Jones, A. Gordus, J.A. Krall, G. MacBeath, A quantitative protein interaction network for the ErbB receptors using protein microarrays, Nature 439 (2006) 168–174.
[24] N. Prenzel, E. Zwick, H. Daub, M. Leserer, R. Abraham, C. Wallasch, A. Ullrich, EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF, Nature 402 (1999) 884–888.
[25] P.O. Hackel, E. Zwick, N. Prenzel, A. Ullrich, Epidermal growth factor receptors: critical mediators of multiple receptor pathways, Curr. Opin. Cell Biol. 11 (1999) 184–189.
[26] R. Wetzker, F.D. Bohmer, Transactivation joins multiple tracks to the ERK/MAPK cascade, Nat. Rev. Mol. Cell Biol. 4 (2003) 651–657.
[27] L.E. Kilpatrick, S.J. Hill, Transactivation of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs): Recent insights using luminescence and fluorescence technologies, Curr Opin Endocr Metab Res 16 (2021) 102–112.
[28] B.H. Shah, K.J. Catt, A central role of EGF receptor transactivation in angiotensin II -induced cardiac hypertrophy, Trends Pharmacol. Sci. 24 (2003) 239–244.
[29] R.M. Touyz, Recent advances in intracellular signalling in hypertension, Curr. Opin. Nephrol. Hypertens. 12 (2003) 165–174.
[30] P.J. Little, GPCR responses in vascular smooth muscle can occur predominantly through dual transactivation of kinase receptors and not classical Gq protein signalling pathways, Life Sci. 92 (2013) 951–956.
[31] S.J. Forrester, T. Kawai, S. O'Brien, W. Thomas, R.C. Harris, S. Eguchi, Epidermal growth factor receptor transactivation: mechanisms, pathophysiology, and potential therapies in the cardiovascular system, Annu. Rev. Pharmacol. Toxicol. 56 (2016) 627–653.
[32] S.L. Chan, S. Umesalma, G.L. Baumbach, Epidermal growth factor receptor is critical for angiotensin II mediated hypertrophy in cerebral arterioles, Hypertension 65 (2015) 806–812.
[33] T. Takayanagi, T. Kawai, S.J. Forrester, T. Obama, T. Tsuji, Y. Fukuda, K.J. Elliott, D.G. Tilley, R.L. Davisson, J.Y. Park, S. Eguchi, Role of epidermal growth factor receptor and endoplasmic reticulum stress in vascular remodeling induced by angiotensin II, Hypertension 65 (2015) 1349–1355.
[34] S.P. Soltoff, L.C. Cantley, p120cbl is a cytosolic adapter protein that associates with phosphoinositide 3-kinase in response to epidermal growth factor in PC12 and other cells, J. Biol. Chem. 271 (1996) 563–567.
[35] J.A. Krall, E.M. Beyer, G. MacBeath, High- and low-affinity epidermal growth factor receptor-ligand interactions activate distinct signaling pathways, PLoS One 6 (2011) e15945.
[36] S. Morandell, T. Stasyk, S. Skvortsov, S. Ascher, L.A. Huber, Quantitative proteomics and phosphoproteomics reveal novel insights into complexity and dynamics of the EGFR signaling network, Proteomics 8 (2008) 4383–4401.
[37] M.K. Nyati, M.A. Morgan, F.Y. Feng, T.S. Lawrence, Integration of EGFR inhibitors with radiochemotherapy, Nat. Rev. Cancer 6 (2006) 876–885.
[38] J.C. Weddell, P.I. Imoukhuede, Integrative meta-modeling identifies endocytic vesicles, late endosome and the nucleus as the cellular compartments primarily directing RTK signaling, Integr Biol (camb) 9 (2017) 464–484.
[39] C. Sachsenmaier, A. Radler-Pohl, R. Zinck, A. Nordheim, P. Herrlich, H. J. Rahmsdorf, Involvement of growth factor receptors in the mammalian UVC response, Cell 78 (1994) 963–972.
[40] H. Kodama, K. Fukuda, T. Takahashi, M. Sano, T. Kato, S. Tahara, D. Hakuno, T. Sato, T. Manabe, F. Konishi, S. Ogawa, Role of EGF Receptor and Pyk2 in endothelin-1-induced ERK activation in rat cardiomyocytes, J. Mol. Cell. Cardiol. 34 (2002) 139–150.
[41] H.L. Hsieh, C.C. Lin, H.J. Chan, C.M. Yang, C.M. Yang, c-Src-dependent EGF receptor transactivation contributes to ET-1-induced COX-2 expression in brain microvascular endothelial cells, J. Neuroinflammation 9 (2012) 152.
[42] H. Daub, F.U. Weiss, C. Wallasch, A. Ullrich, Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors, Nature 379 (1996) 557–560.
[43] F.G. Buchanan, D. Wang, F. Bargiacchi, R.N. DuBois, Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor, J. Biol. Chem. 278 (2003) 35451–35457.
[44] D. Bokemeyer, U. Schmitz, H.J. Kramer, Angiotensin II-induced growth of vascular smooth muscle cells requires an Src-dependent activation of the epidermal growth factor receptor, Kidney Int. 58 (2000) 549–558.
[45] S. Akhtar, M.H.M. Yousif, G.S. Dhaunsi, B. Chandrasekhar, O. Al-Farsi, I.F. Benter, Angiotensin-(1–7) inhibits epidermal growth factor receptor transactivation via a Mas receptor-dependent pathway, Br. J. Pharmacol. no (2011).
[46] K. Chen, J.A. Vita, B.C. Berk, J.F. Keaney Jr., c-Jun N-terminal kinase activation by hydrogen peroxide in endothelial cells involves SRC-dependent epidermal growth factor receptor transactivation, J. Biol. Chem. 276 (2001) 16045–16050.
[47] S. Lee, J.R. Springstead, B. Parks, C.E. Romanoski, R. Palvolgyi, T. Ho, P. Nguyen, A.J. Lusis, J.A. Berliner, Metalloproteinase processing of HBEGF is a proximal event in the response of human aortic endothelial cells to oxidized phospholipids, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1246–1254.
[48] R. Lezama, A. Diaz-Tellez, G. Ramos-Mandujano, L. Oropeza, H. Pasantes-Morales, Epidermal growth factor receptor is a common element in the signaling pathways activated by cell volume changes in isosmotic, hyposmotic or hyperosmotic conditions, Neurochem. Res. 30 (2005) 1589–1597.
[49] H. Iwasaki, S. Eguchi, H. Ueno, F. Marumo, Y. Hirata, Mechanical stretch stimulates growth of vascular smooth muscle cells via epidermal growth factor receptor, Am. J. Phys. Heart Circ. Phys. 278 (2000) H521–H529.
[50] L. Di, V, Mudo G, Belluardo N, Crosstalk between receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCR) in the brain: Focus on heteroreceptor complexes and related functional neurotrophic effects, Neuropharmacology 152 (2019) 67–77.
[51] L.A. Grisanti, S. Guo, D.G. Tilley, Cardiac GPCR-mediated EGFR transactivation: impact and therapeutic implications, J. Cardiovasc. Pharmacol. 70 (2017) 3–9.
[52] C. Crudden, T. Shibano, D. Song, N. Suleymanova, A. Girnita, L. Girnita, Blurring boundaries: receptor tyrosine kinases as functional G protein-coupled receptors, Int. Rev. Cell Mol. Biol. 339 (2018) 1–40.
[53] A.J. George, B.W. Purdue, C.M. Gould, D.W. Thomas, Y. Handoko, H. Qian, G. A. Quaife-Ryan, K.A. Morgan, K.J. Simpson, W.G. Thomas, R.D. Hannan, A functional siRNA screen identifies genes modulating angiotensin II-mediated EGFR transactivation, J. Cell Sci. 126 (2013) 5377–5390.
[54] T. Yamauchi, K. Ueki, K. Tobe, H. Tamemoto, N. Sekine, M. Wada, M. Honjo, M. Takahashi, T. Takahashi, H. Hirai, T. Tushima, Y. Akanuma, T. Fujita, I. Komuro, Y. Yazaki, T. Kadowaki, Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone, Nature 390 (1997) 91–96.
[55] T. Yamauchi, N. Yamauchi, K. Ueki, T. Sugiyama, H. Waki, H. Miki, K. Tobe, S. Matsuda, T. Tsushima, T. Yamamoto, T. Fujita, Y. Taketani, M. Fukayama, S. Kimura, Y. Yazaki, R. Nagai, T. Kadowaki, Constitutive tyrosine phosphorylation of ErbB-2 via Jak2 by autocrine secretion of prolactin in human breast cancer, J. Biol. Chem. 275 (2000) 33937–33944.
[56] J. Chen, F. Zeng, S.J. Forrester, S. Eguchi, M.Z. Zhang, R.C. Harris, Expression and function of the epidermal growth factor receptor in physiology and disease, Physiol. Rev. 96 (2016) 1025–1069.
[57] T. Holbro, N.E. Hynes, ERBB receptors: directing key signaling networks throughout life, Annu. Rev. Pharmacol. Toxicol. 44 (2004) 195–217.
[58] R.N. Jorissen, F. Walker, N. Pouliot, T.P. Garrett, C.W. Ward, A.W. Burgess, Epidermal growth factor receptor: mechanisms of activation and signalling, Exp. Cell Res. 284 (2003) 31–53.
[59] D.R. Stover, M. Becker, J. Liebetanz, N.B. Lydon, Src phosphorylation of the epidermal growth factor receptor at novel sites mediates receptor interaction with Src and P85 alpha, J. Biol. Chem. 270 (1995) 15591–15597.
[60] J.S. Biscardi, M.C. Maa, D.A. Tice, M.E. Cox, T.H. Leu, S.J. Parsons, c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function, J. Biol. Chem. 274 (1999) 8335–8343.
[61] Y. Huang, S.O. Kim, J. Jiang, S.J. Frank, Growth hormone-induced phosphorylation of epidermal growth factor (EGF) receptor in 3T3-F442A cells. Modulation of EGF-induced trafficking and signaling, J. Biol. Chem. 278 (2003) 18902–18913.
[62] S.O. Kim, J.C. Houtman, J. Jiang, J.M. Ruppert, P.J. Bertics, S.J. Frank, Growth hormone-induced alteration in ErbB-2 phosphorylation status in 3T3-F442A fibroblasts, J. Biol. Chem. 274 (1999) 36015–36024.
[63] T. Noma, A. Lemaire, S.V. Naga Prasad, L. Barki-Harrington, D.G. Tilley, J. Chen, P. Le Corvoisier, J.D. Violin, H. Wei, R.J. Lefkowitz, H.A. Rockman, beta-Arrestin-mediated b1-adrenergic receptor transactivation of the EGFR confers cardioprotection, J. Clin. Invest. 117 (2007) 2445–2458.
[64] J. Kim, S. Ahn, K. Rajagopal, R.J. Lefkowitz, Independent beta-arrestin2 and Gq/protein kinase Czeta pathways for ERK stimulated by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor, J. Biol. Chem. 284 (2009) 11953–11962.
[65] A.C. Overland, P.A. Insel, Heterotrimeric G Proteins Directly Regulate MMP14/Membrane Type-1 Matrix Metalloprotease: a novel mechanism for GPCR-EGFR transactivation, J. Biol. Chem. 290 (2015) 9941–9947.
[66] G.P. Amorino, P.D. Deeble, S.J. Parsons, Neurotensin stimulates mitogenesis of prostate cancer cells through a novel c-Src/Stat5b pathway, Oncogene 26 (2007) 745–756.

[67] S. Sebastian, J. Settleman, S.J. Reshkin, A. Azzariti, A. Bellizzi, A. Paradiso, The complexity of targeting EGFR signalling in cancer: from expression to turnover, Biochim. Biophys. Acta 1766 (2006) 120–139.
[68] C.R. Lombardo, T.G. Consler, D.B. Kassel, In vitro phosphorylation of the epidermal growth factor receptor autophosphorylation domain by c-src: identification of phosphorylation sites and c-src SH2 domain binding sites, Biochemistry 34 (1995) 16456–16466.
[69] M.T. Kloth, K.K. Laughlin, J.S. Biscardi, J.L. Boerner, S.J. Parsons, C.M. Silva, STAT5b, a mediator of synergism between c-Src and the epidermal growth factor receptor, J. Biol. Chem. 278 (2003) 1671–1679.
[70] D. Chaturvedi, X. Gao, M.S. Cohen, J. Taunton, T.B. Patel, Rapamycin induces transactivation of the EGFR and increases cell survival, Oncogene 28 (2009) 1187–1196.
[71] Y.R. Kim, H.Y. Cha, K. Lim, B.D. Hwang, K.L. Hoe, U. Namgung, S.K. Park, Activation of epidermal growth factor receptor is responsible for pervanadate-induced phospholipase D activation, Exp. Mol. Med. 35 (2003) 118–124.
[72] B. Al-Ani, P.W. Hewett, M.J. Cudmore, T. Fujisawa, M. Saifeddine, H. Williams, W. Ramma, S. Sissaoui, P.S. Jayaraman, M. Ohba, S. Ahmad, M.D. Hollenberg, A. Ahmed, Activation of proteinase-activated receptor 2 stimulates soluble vascular endothelial growth factor receptor 1 release via epidermal growth factor receptor transactivation in endothelial cells, Hypertension 55 (2010) 689–697.
[73] G. Carpenter, EGF receptor transactivation mediated by the proteolytic production of EGF-like agonists, Sci. STKE 2000 (2000) e1.
[74] A. Gschwind, N. Prenzel, A. Ullrich, Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation, Cancer Res. 62 (2002) 6329–6336.
[75] A. Gschwind, E. Zwick, N. Prenzel, M. Leserer, A. Ullrich, Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission, Oncogene 20 (2001) 1594–1600.
[76] T. Kawai, K.J. Elliott, R. Scalia, S. Eguchi, Contribution of ADAM17 and related ADAMs in cardiovascular diseases, Cell. Mol. Life Sci. 78 (2021) 4161–4187.
[77] D.F. McCole, A. Truong, M. Bunz, K.E. Barrett, Consequences of direct vs. indirect activation of EGFR in intestinal epithelial cells are dictated by protein tyrosine phosphatase 1B, J. Biol. Chem. (2007) M700424200.
[78] H. Daub, C. Wallasch, A. Lankenau, A. Herrlich, A. Ullrich, Signal characteristics of G protein-transactivated EGF receptor, EMBO J. 16 (1997) 7032–7044.
[79] D.F. McCole, S.J. Keely, R.J. Coffey, K.E. Barrett, Transactivation of the epidermal growth factor receptor in colonic epithelial cells by carbachol requires extracellular release of transforming growth factor-alpha, J. Biol. Chem. 277 (2002) 42603–42612.
[80] A.L. Teixeira, M.N. Gomes, R. Medeiros, EGFR signaling pathway and related-miRNAs in age-related diseases: the example of miR-221 and miR-222, Front. Genet. 3 (2012).
[81] Q. Li, T. Hosaka, Y. Shikama, Y. Bando, C. Kosugi, N. Kataoka, Y. Nakaya, M. Funaki, Heparin-binding EGF-like growth factor (HB-EGF) mediates 5-HT-induced insulin resistance through activation of EGF receptor-ERK1/2-mTOR pathway, Endocrinology 153 (2012) 56–68.
[82] S. Eguchi, T. Inagami, Signal transduction of angiotensin II type 1 receptor through receptor tyrosine kinase, Regul. Pept. 91 (2000) 13–20.
[83] R. Eckenstaler, J. Sandori, M. Gekle, R.A. Benndorf, Angiotensin II receptor type 1 - an update on structure, expression and pathology, Biochem. Pharmacol. 114673 (2021).
[84] T. Takayanagi, K.J. Crawford, T. Kobayashi, T. Obama, T. Tsuji, K.J. Elliott, T. Hashimoto, V. Rizzo, S. Eguchi, Caveolin 1 is critical for abdominal aortic aneurysm formation induced by angiotensin II and inhibition of lysyl oxidase, Clin. Sci. (Lond.) 126 (2014) 785–794.
[85] F. Li, K.U. Malik, Angiotensin II-induced Akt activation through the epidermal growth factor receptor in vascular smooth muscle cells is mediated by phospholipid metabolites derived by activation of phospholipase D, J. Pharmacol. Exp. Ther. 312 (2005) 1043–1054.
[86] X.L. Cui, Y. Ding, L.D. Alexander, C. Bao, O.K. Al-Khalili, M. Simonson, D. C. Eaton, J.G. Douglas, Oxidative signaling in renal epithelium: critical role of cytosolic phospholipase A2 and p38(SAPK), Free Radic. Biol. Med. 41 (2006) 213–221.
[87] K.J. Elliott, A.M. Bourne, T. Takayanagi, A. Takaguri, T. Kobayashi, K. Eguchi, S. Eguchi, ADAM17 silencing by adenovirus encoding miRNA-embedded siRNA revealed essential signal transduction by angiotensin II in vascular smooth muscle cells, J. Mol. Cell. Cardiol. 62 (2013) 1–7.
[88] T. Takayanagi, S.J. Forrester, T. Kawai, T. Obama, T. Tsuji, K.J. Elliott, E. Nuti, A. Rossello, H.F. Kwok, R. Scalia, V. Rizzo, S. Eguchi, Vascular ADAM17 as a novel therapeutic target in mediating cardiovascular hypertrophy and perivascular fibrosis induced by angiotensin II, Hypertension 68 (2016) 949–955.
[89] A.J. George, R.D. Hannan, W.G. Thomas, Unravelling the molecular complexity of GPCR-mediated EGFR transactivation using functional genomics approaches, FEBS J. 280 (2013) 5258–5268.
[90] J.M. Spin, M. Hsu, J. Azuma, M.M. Tedesco, A. Deng, J.S. Dyer, L. Maegdefessel, R.L. Dalman, P.S. Tsao, Transcriptional profiling and network analysis of the murine angiotensin II-induced abdominal aortic aneurysm, Physiol. Genomics 43 (2011) 993–1003.
[91] T. Obama, T. Tsuji, T. Kobayashi, Y. Fukuda, T. Takayanagi, Y. Taro, T. Kawai, S. J. Forrester, K.J. Elliott, E.T. Choi, A. Daugherty, V. Rizzo, S. Eguchi, Epidermal growth factor receptor inhibitor protects abdominal aortic aneurysm in a mouse model, Clin. Sci. (Lond.) (2014).
[92] T. Obama, T. Takayanagi, T. Kobayashi, A.M. Bourne, K.J. Elliott, M. Charbonneau, C.M. Dubois, S. Eguchi, Vascular induction of a disintegrin and metalloprotease 17 by angiotensin II through hypoxia inducible factor 1a, Am. J. Hypertens. 28 (2015) 10–14.
[93] L.M. Luttrell, G.J. Della Rocca, B.T. Van, D.K. Luttrell, R.J. Lefkowitz, Gbetagamma subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. a scaffold for G protein-coupled receptor-mediated Ras activation, J. Biol. Chem. 272 (1997) 4637–4644.
[94] M.M. Hopkins, Z. Liu, K.E. Meier, Positive and negative cross-talk between lysophosphatidic acid receptor 1, free fatty acid receptor 4, and epidermal growth factor receptor in human prostate cancer cells, J. Pharmacol. Exp. Ther. 359 (2016) 124–133.
[95] J.A. Garcia-Sainz, M.T. Romero-Avila, L.C. Medina, Dissecting how receptor tyrosine kinases modulate G protein-coupled receptor function, Eur. J. Pharmacol. 648 (2010) 1–5.
[96] L.A. Stoddart, L.E. Kilpatrick, S.J. Hill, NanoBRET approaches to study ligand binding to GPCRs and RTKs, Trends Pharmacol. Sci. 39 (2018) 136–147.
[97] S.J. Sanni, N. Kulahin, R. Jorgensen, C. Lyngso, S. Gammeltoft, J.L. Hansen, A bioluminescence resonance energy transfer 2 (BRET2) assay for monitoring seven transmembrane receptor and insulin receptor crosstalk, J. Recept. Signal Transduct. Res. 37 (2017) 590–599.
[98] S.L. O'Brien, E.K.M. Johnstone, D. Devost, J. Conroy, M.E. Reichelt, B.W. Purdue, M.A. Ayoub, T. Kawai, A. Inoue, S. Eguchi, T.E. Hebert, K.D.G. Pfleger, W. G. Thomas, BRET-based assay to monitor EGFR transactivation by the AT(1)R reveals G(q/11) protein-independent activation and AT(1)R-EGFR complexes, Biochem. Pharmacol. 158 (2018) 232–242.
[99] B. Schreier, V. Dubourg, S. Hübschmann, S. Rabe, S. Mildenberger, M. Gekle, Synergy of epidermal growth factor (EGFR) and angiotensin II (AT1R) receptor determines composition and temporal pattern of transcriptome variation, Cell. Mol. Life Sci. 79 (2021) 57.
[100] E.K.M. Johnstone, R.S. Abhayawardana, H.B. See, R.M. Seeber, S.L. O'Brien, W. G. Thomas, K.D.G. Pfleger, Complex interactions between the angiotensin II type 1 receptor, the epidermal growth factor receptor and TRIO-dependent signaling partners, Biochem. Pharmacol. 188 (2021), 114521.
[101] N.J. Smith, H.W. Chan, H. Qian, A.M. Bourne, K.M. Hannan, F.J. Warner, R. H. Ritchie, R.B. Pearson, R.D. Hannan, W.G. Thomas, Determination of the exact molecular requirements for type 1 angiotensin receptor epidermal growth factor receptor transactivation and cardiomyocyte hypertrophy, Hypertension 57 (2011) 973–980.
[102] U. Quitterer, S. AbdAlla, Discovery of pathologic GPCR aggregation, Front Med (Lausanne) 6 (2019) 9.
[103] A. Nolze, C. Köhler, S. Ruhs, K. Quarch, N. Strätz, M. Gekle, C. Grossmann, Calcineurin (PPP3CB) regulates angiotensin II-dependent vascular remodelling by potentiating EGFR signalling in mice, Acta Physiol. (2021) e13715.
[104] C.W. Yang, H.J. Ahn, W.Y. Kim, M.J. Shin, S.K. Kim, J.H. Park, Y.O. Kim, Y. S. Kim, J. Kim, B.K. Bang, Influence of the renin-angiotensin system on epidermal growth factor expression in normal and cyclosporine-treated rat kidney, Kidney Int. 60 (2001) 847–857.
[105] J.M. Park, J.G. Borer, M.R. Freeman, C.A. Peters, Stretch activates heparin-binding EGF-like growth factor expression in bladder smooth muscle cells, Am. J. Phys. Anthropol. 275 (1998) C1247–C1254.
[106] V. Dubourg, B. Schreier, G. Schwerdt, S. Rabe, R.A. Benndorf, M. Gekle, The functional interaction of EGFR with AT1R or TP in primary vascular smooth muscle cells triggers a synergistic regulation of gene expression, Cells (2022) 11.
[107] H. Pham, B. Chong, R. Vincenti, L.W. Slice, Ang II and EGF synergistically induce COX-2 expression via CREB in intestinal epithelial cells, J. Cell. Physiol. 214 (2008) 96–109.
[108] Y. Li, L.O. Levesque, M.B. Anand-Srivastava, Epidermal growth factor receptor transactivation by endogenous vasoactive peptides contributes to hyperproliferation of vascular smooth muscle cells of SHR, Am. J. Phys. Heart Circ. Phys. 299 (2010) H1959–H1967.
[109] B.T. Andresen, J.J. Linnoila, E.K. Jackson, G.G. Romero, Role of EGFR transactivation in angiotensin II signaling to extracellular regulated kinase in preglomerular smooth muscle cells, Hypertension 41 (2003) 781–786.
[110] M.C. Lauzier, E.L. Page, M.D. Michaud, D.E. Richard, Differential regulation of hypoxia-inducible factor-1 through receptor tyrosine kinase transactivation in vascular smooth muscle cells, Endocrinology 148 (2007) 4023–4031.
[111] J. Haendeler, G. Yin, Y. Hojo, Y. Saito, M. Melaragno, C. Yan, V.K. Sharma, M. Heller, R. Aebersold, B.C. Berk, GIT1 mediates Src-dependent activation of phospholipase Cgamma by angiotensin II and epidermal growth factor, J. Biol. Chem. 278 (2003) 49936–49944.
[112] S. Eguchi, P.J. Dempsey, G.D. Frank, E.D. Motley, T. Inagami, Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metalloprotease-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK, J. Biol. Chem. 276 (2001) 7957–7962.
[113] R. Limor, M. Kaplan, O. Sharon, E. Knoll, M. Naidich, G. Weisinger, S. Keidar, N. Stern, Aldosterone up-regulates 12- and 15-lipoxygenase expression and LDL oxidation in human vascular smooth muscle cells, J. Cell. Biochem. (2009).
[114] L. Hao, M. Du, A. Lopez-Campistrous, C. Fernandez-Patron, Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway, Circ. Res. 94 (2004) 68–76.
[115] L. Hao, T. Nishimura, H. Wo, C. Fernandez-Patron, Vascular responses to alpha1-adrenergic receptors in small rat mesenteric arteries depend on mitochondrial reactive oxygen species, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 819–825.
[116] Y. Kawanabe, K. Nozaki, N. Hashimoto, T. Masaki, Involvement of extracellular Ca2+ influx and epidermal growth factor receptor tyrosine kinase transactivation in endothelin-1-induced arachidonic acid release, Br. J. Pharmacol. 139 (2003) 1516–1522.

[117] Y. Kawanabe, N. Hashimoto, T. Masaki, Characterization of Ca2+ channels involved in ET-1-induced transactivation of EGF receptors, Am. J. Phys. Heart Circ. Phys. 283 (2002) H2671–H2675.
[118] H. Iwasaki, S. Eguchi, F. Marumo, Y. Hirata, Endothelin-1 stimulates DNA synthesis of vascular smooth-muscle cells through transactivation of epidermal growth factor receptor, J. Cardiovasc. Pharmacol. 31 (Suppl 1) (1998) S182–S184.
[119] C.C. Lin, W.N. Lin, S.E. Cheng, W.H. Tung, H.H. Wang, C.M. Yang, Transactivation of EGFR/PI3K/Akt involved in ATP-induced inflammatory protein expression and cell motility, J. Cell. Physiol. 227 (2012) 1628–1638.
[120] A.M. Bakken, C.D. Protack, E. Roztocil, S.M. Nicholl, M.G. Davies, Cell migration in response to the amino-terminal fragment of urokinase requires epidermal growth factor receptor activation through an ADAM-mediated mechanism, J. Vasc. Surg. 49 (2009) 1296–1303.
[121] M. Koide, P.L. Penar, B.I. Tranmer, G.C. Wellman, Heparin-binding EGF-like growth factor mediates oxyhemoglobin-induced suppression of voltage-dependent potassium channels in rabbit cerebral artery myocytes, Am. J. Phys. Heart Circ. Phys. 293 (2007) H1750–H1759.
[122] D. Dreymueller, C. Martin, J. Schumacher, E. Groth, J.K. Boehm, L.K. Reiss, S. Uhlig, A. Ludwig, Smooth muscle cells relay acute pulmonary inflammation via distinct ADAM17/ErbB axes, J. Immunol. 192 (2014) 722–731.
[123] S.P. Bagby, E.A. Kirk, L.H. Mitchell, M.M. O'Reilly, W.E. Holden, P.E. Stenberg, A. C. Bakke, Proliferative synergy of ANG II and EGF in porcine aortic vascular smooth muscle cells, American Journal of Physiology-Renal Physiology 265 (1993) F239–F249.
[124] J. Norman, B. Badie-Dezfooly, E.P. Nord, I. Kurtz, J. Schlosser, A. Chaudhari, L. G. Fine, EGF-induced mitogenesis in proximal tubular cells: potentiation by angiotensin II, Am. J. Phys. Anthropol. 253 (1987) F299–F309.
[125] P.E. Rippetoe, J.W. Olson, R.J. Altiere, T.H. Pauly, M.N. Gillespie, Epidermal growth factor augments reactivity to angiotensin II in the rat pulmonary circulation, Am. Rev. Respir. Dis. 140 (1989) 821–824.
[126] R. Natarajan, J. Nadler, Angiotensin II-induced aldosterone synthesis is potentiated by epidermal growth factor, Endocrinology 128 (1991) 2285–2290.
[127] B. Schreier, M. Dohler, S. Rabe, B. Schneider, G. Schwerdt, S. Ruhs, M. Sibilia, M. Gotthardt, M. Gekle, C. Grossmann, Consequences of epidermal growth factor receptor (ErbB1) loss for vascular smooth muscle cells from mice with targeted deletion of ErbB1, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 1643–1652.
[128] B. Schreier, G. Schwerdt, C. Heise, D. Bethmann, S. Rabe, S. Mildenberger, M. Gekle, Substance-specific importance of EGFR for vascular smooth muscle cells motility in primary culture, Biochim. Biophys. Acta 1863 (2016) 1519–1533.
[129] S. Meinel, S. Ruhs, K. Schumann, N. Strätz, K. Trenkmann, B. Schreier, I. Grosse, J. Keilwagen, M. Gekle, C. Grossmann, Mineralocorticoid receptor interaction with SP1 generates a new response element for pathophysiologically relevant gene expression, Nucl Acids Res 41 (2013) 8045–8060.
[130] W. Cai, M. Torreggiani, L. Zhu, X. Chen, J.C. He, G.E. Striker, H. Vlassara, AGER1 regulates endothelial cell NADPH oxidase-dependent oxidant stress via PKC-delta: implications for vascular disease, Am. J. Physiol. Cell Physiol. 298 (2010) C624–C634.
[131] N. Makki, K.W. Thiel, F.J. Miller Jr., The epidermal growth factor receptor and its ligands in cardiovascular disease, Int. J. Mol. Sci. 14 (2013) 20597–20613.
[132] C.K. Buharalioglu, C.Y. Song, F.A. Yaghini, H.U.B. Ghafoor, M. Motiwala, T. Adris, A.M. Estes, K.U. Malik, Angiotensin II-induced process of angiogenesis is mediated by spleen tyrosine kinase via VEGF receptor-1 phosphorylation, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H1043–H1055.
[133] S.C. Tai, G.B. Robb, P.A. Marsden, Endothelial nitric oxide synthase: a new paradigm for gene regulation in the injured blood vessel, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 405–412.
[134] B. Schreier, M. Hünerberg, S. Mildenberger, S. Rabe, D. Bethmann, C. Wickenhauser, M. Gekle, Deletion of the EGF receptor in vascular smooth muscle cells prevents chronic angiotensin-II-induced arterial wall stiffening and media thickening, Acta Physiol. 222 (2017) e12996.
[135] A.C. Dreux, D.J. Lamb, H. Modjtahedi, G.A.A. Ferns, The epidermal growth factor receptors and their family of ligands: their putative role in atherogenesis, Atherosclerosis 186 (2006) 38–53.
[136] R. Iwamoto, E. Mekada, ErbB and HB-EGF signaling in heart development and function, Cell Struct. Funct. 31 (2006) 1–14.
[137] H. Zhang, S.W. Sunnarborg, K.K. McNaughton, T.G. Johns, D.C. Lee, J.E. Faber, Heparin-binding epidermal growth factor-like growth factor signaling in flow-induced arterial remodeling, Circ. Res. 102 (2008) 1275–1285.
[138] A.K. Chan, A. Kalmes, S. Hawkins, G. Daum, A.W. Clowes, Blockade of the epidermal growth factor receptor decreases intimal hyperplasia in balloon-injured rat carotid artery, J. Vasc. Surg. 37 (2003) 644–649.
[139] F. Helle, C. Jouzel, C. Chadjichristos, S. Placier, M. Flamant, D. Guerrot, FranÃ§ois H, Dussaule JC, Chatziantoniou C, Improvement of renal hemodynamics during hypertension-induced chronic renal disease: role of EGF receptor antagonism, Am. J. Physiol. Renal Physiol. 297 (2009) F191–F199.
[140] I.F. Benter, M. Benboubetra, A.J. Hollins, M.H.M. Yousif, H. Canatan, S. Akhtar, Early inhibition of EGFR signaling prevents diabetes-induced up-regulation of multiple gene pathways in the mesenteric vasculature, Vasc. Pharmacol. 51 (2009) 236–245.
[141] N. Ulu, G.M. Mulder, P. Vavrinec, S.W. Landheer, B. Duman-Dalkilic, H. Gurdal, M. Goris, M. Duin, R.P.E. van Dokkum, H. Buikema, H. van Goor, R.H. Henning, Epidermal growth factor receptor inhibitor PKI-166 governs cardiovascular protection without beneficial effects on the kidney in hypertensive 5/6 nephrectomized rats, J. Pharmacol. Exp. Ther. 345 (2013) 393–403.
[142] S. Kagiyama, K. Qian, T. Kagiyama, M.I. Phillips, Antisense to epidermal growth factor receptor prevents the development of left ventricular hypertrophy, Hypertension 41 (2003) 824–829.
[143] S. Kagiyama, S. Eguchi, G.D. Frank, T. Inagami, Y.C. Zhang, M.I. Phillips, Angiotensin II-induced cardiac hypertrophy and hypertension are attenuated by epidermal growth factor receptor antisense, Circulation 106 (2002) 909–912.
[144] M.P. Sambhi, N. Swaminathan, H. Wang, H. Rong, Increased EGF binding and EGFR mRNA expression in rat aorta with chronic administration of pressor angiotensin II, Biochem. Med. Metab. Biol. 48 (1992) 8–18.
[145] P. Beaucage, P. Moreau, EGF receptor transactivation in angiotensin II and endothelin control of vascular protein synthesis in vivo, J. Cardiovasc. Pharmacol. 44 (Suppl 1) (2004) S20–S23.
[146] C.J. Barrick, R.B. Roberts, M. Rojas, N.M. Rajamannan, C.B. Suitt, K.D. O'Brien, S. S. Smyth, D.W. Threadgill, Reduced EGFR causes abnormal valvular differentiation leading to calcific aortic stenosis and left ventricular hypertrophy in C57BL/6J but not 129S1/SvImJ mice, AJP - Heart and Circulatory Physiology (2009) 00866.
[147] P.J. Fuller, M.J. Young, Mechanisms of mineralocorticoid action, Hypertension 46 (2005) 1227–1235.
[148] C.C. Lin, I.T. Lee, C.H. Hsu, C.K. Hsu, P.L. Chi, L.D. Hsiao, C.M. Yang, Sphingosine-1-phosphate mediates ICAM-1-dependent monocyte adhesion through p38 MAPK and p42/p44 MAPK-dependent Akt activation, PLoS One 10 (2015) e0118473.
[149] M. Saifeddine, M. El-Daly, K. Mihara, N.W. Bunnett, P. McIntyre, C. Altier, M. D. Hollenberg, R. Ramachandran, GPCR mediated EGFR transactivation regulates TRPV4 action in the vasculature, Br. J. Pharmacol. 172 (2015) 2493–2506.
[150] B. Schreier, S. Rabe, B. Schneider, M. Bretschneider, S. Rupp, S. Ruhs, J. Neumann, U. Rueckschloss, M. Sibilia, M. Gotthardt, C. Grossmann, M. Gekle, Loss of epidermal growth factor receptor in vascular smooth muscle cells and cardiomyocytes causes arterial hypotension and cardiac hypertrophy, Hypertension 61 (2013) 333–340.
[151] B. Schreier, S. Rabe, S. Winter, S. Ruhs, S. Mildenberger, B. Schneider, M. Sibilia, M. Gotthardt, S. Kempe, K. Mader, C. Grossmann, M. Gekle, Moderate inappropriately high aldosterone/NaCl constellation in mice: cardiovascular effects and the role of cardiovascular epidermal growth factor receptor, Sci. Rep. 4 (2014) 7430.
[152] S. Belmadani, D.I. Palen, R.A. Gonzalez-Villalobos, H.A. Boulares, K. Matrougui, Elevated epidermal growth factor receptor phosphorylation induces resistance artery dysfunction in diabetic db/db mice, Diabetes 57 (2008) 1629–1637.
[153] I.F. Benter, M.H.M. Yousif, S.M. Griffiths, M. Benboubetra, S. Akhtar, Epidermal growth factor receptor tyrosine kinase-mediated signalling contributes to diabetes-induced vascular dysfunction in the mesenteric bed, Br. J. Pharmacol. 145 (2005) 829–836.
[154] C. Stern, B. Schreier, A. Nolze, S. Rabe, S. Mildenberger, M. Gekle, Knockout of vascular smooth muscle EGF receptor in a mouse model prevents obesity-induced vascular dysfunction and renal damage in vivo, Diabetologia 63 (2020) 2218–2234.
[155] X.P. Yang, J.Y. Fu, R.C. Yang, W.T. Liu, T. Zhang, B. Yang, L. Miao, B.B. Dou, C. Tan, H.C. Chen, X.R. Wang, EGFR transactivation contributes to neuroinflammation in streptococcus suis meningitis, J. Neuroinflammation 13 (2016) 274.
[156] F. Zeng, L.A. Kloepfer, C. Finney, A. Diedrich, R.C. Harris, Specific endothelial heparin-binding EGF-like growth factor deletion ameliorates renal injury induced by chronic angiotensin II infusion, Am. J. Physiol. Ren. Physiol. 311 (2016) F695–F707.
[157] L. Wang, Z. Huang, W. Huang, X. Chen, P. Shan, P. Zhong, Z. Khan, J. Wang, Q. Fang, G. Liang, Y. Wang, Inhibition of epidermal growth factor receptor attenuates atherosclerosis via decreasing inflammation and oxidative stress, Sci. Rep. 7 (2017) 45917.
[158] M. Villmow, U. Klöckner, C. Heymes, M. Gekle, U. Rueckschloss, NOS1 induces NADPH oxidases and impairs contraction kinetics in aged murine ventricular myocytes, Basic Res. Cardiol. 110 (2015) 1–15.
[159] B. Schreier, M. Hünerberg, S. Rabe, S. Mildenberger, D. Bethmann, C. Heise, M. Sibilia, S. Offermanns, M. Gekle, Consequences of postnatal vascular smooth muscle EGF-receptor deletion on acute angiotensin II action, Clin. Sci. 130 (2016) 19–33.
[160] B. Schreier, M. Hünerberg, S. Rabe, S. Mildenberger, D. Bethmann, C. Heise, M. Sibilia, S. Offermanns, M. Gekle, Consequences of postnatal vascular smooth muscle EGFR deletion on acute angiotensin II action, Clin. Sci. (Lond.) 130 (2016) 19–33.
[161] W.W. Batenburg, P.M. Jansen, A.J. van den Bogaerdt, J. Danser, Angiotensin II-aldosterone interaction in human coronary microarteries involves GPR30, EGFR, and endothelial NO synthase, Cardiovasc. Res. 94 (2012) 136–143.
[162] G.Z. Kalil, W.G. Haynes, Sympathetic nervous system in obesity-related hypertension: mechanisms and clinical implications, Hypertens. Res. 35 (2012) 4–16.
[163] Y. Marcus, G. Shefer, N. Stern, Adipose tissue renin-angiotensin-aldosterone system (RAAS) and progression of insulin resistance, Mol. Cell. Endocrinol. 378 (2012) 1–14.
[164] B. Schreier, C. Stern, V. Dubourg, A. Nolze, S. Rabe, S. Mildenberger, C. Wickenhauser, M. Gekle, Endothelial epidermal growth factor receptor is of minor importance for vascular and renal function and obesity-induced dysfunction in mice, Sci. Rep. 11 (2021) 7269.