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Review

Mechanisms of pericyte recruitment in tumour angiogenesis: A new role for metalloproteinases

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α-SMA, α-smooth muscle actin
ALK, activin-like kinase
Ang1, angiopoietin 1
AngII, angiotensin II
bFGF, basic fibroblast growth factor
EC, endothelial cell

ABSTRACT

Pericytes occur in tumour blood vessels and are critical for the development of a functional vascular network. Targeting tumour pericytes is a promising anti-angiogenic therapy but requires identifying the mechanisms of their recruitment in tumour and addressing whether these mechanisms can be selectively harnessed. Among the pathways involved in pericyte recruitment during embryonic development, the contribution of platelet-derived growth factor B and sphingosine 1-phosphate is confirmed in tumour angiogenesis. The effect of angiopoietin 1 depends on the tumour model. Transforming growth factor-β1 enhances tumour vascularization and microvessel maturation. Recent reports suggest a participation of matrix metalloproteinases (MMP) in tumour pericyte recruitment that is consistent with the effect of certain MMPs in the development of microvasculature in embryonic development and in in vitro models of vascular remodelling. Here, we discuss the possibility for MMPs to contribute to pericyte recruitment at six levels: (1) direct promotion of pericyte invasion by extracellular matrix degradation; (2) stimulation of pericyte proliferation and protection against apoptosis by modification of the ECM; (3) activation of pericytes through the release of growth factor bound to the ECM; (4) transactivation of angiogenic cell surface receptor; (5) propagation of angiogenic signalling as cofactor; and (6) recruitment of bone marrow-derived stem cells.

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ECM, extracellular matrix
EDG-1, endothelial differentiation gene-1
EGFR, epidermal growth factor receptor
GPCR, G-protein coupled receptor
HB-EGF, heparin-binding epidermal growth factor-like growth factor
MMP, matrix metalloproteinase
MT1-MMP, membrane-type 1 matrix metalloproteinase
PAI-1, plasminogen activator inhibitor-1
PDGFB, platelet-derived growth factor B
PDGFR-β, PDGF receptor-β
RTK, receptor tyrosine kinase
S1P, sphingosine 1-phosphate
SphK, sphingosine kinase
TGF-β1, transforming growth factor-β1
TGFβR, transforming growth factor-β receptor
Tie2, receptor tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2
VEGF, vascular endothelial growth factor
VSMC, vascular smooth muscle cell

1. Introduction

The development of a functional microvasculature requires the tubular organization of endothelial cells (EC) and their maturation as structurally stable and functionally adjustable vessels. Blood vessel maturation involves the recruitment of mural cells and the deposition of a perivascular extracellular matrix (ECM). Among mural cells, pericytes constitute a heterogeneous population of cells in close contact with EC. The definition and the ontogeny of pericytes remain controversial. Pericytes contain myofilaments and express markers such as α-smooth muscle actin (α-SMA) and desmin suggesting a common origin for pericytes and vascular smooth muscle cells (VSMC) [1]. However, the expression of these markers by pericytes is dynamic and varies according to the species, the tissue and the differentiation state. Pericyte plasticity is demonstrated by their capacity to transdifferentiate into other mesenchymal cell types such as smooth muscle cells, fibroblasts, osteoblasts and adipocytes [2]. The most reliable criterion for the identification of pericytes is that they are surrounded by a basement membrane shared with EC, as demonstrated by electron microscopy [1]. Pericytes are in contact with EC through discontinuities in the shared basement membrane. The pericyte–EC interface is rich in fibronectin deposition and contains tight and gap junctions as well as N-cadherin, β-catenin-based adherens junctions [1,3]. Recruitment of pericytes along EC is promoted by platelet-derived growth factor B (PDGFB), sphingosine 1-phosphate (S1P), angiopoietin 1 (Ang1) and transforming growth factor-β1 (TGF-β1) [4]. Pericytes stabilize the newly formed endothelial tubes, modulate blood flow and vascular permeability and regulate EC proliferation, survival, migration, differentiation and branching [5]. They are therefore important actors in the development, maintenance and regulation of the microvasculature.

It has long been thought that tumour blood vessels fail to mature, based on their disorganized appearance, with uneven diameter and excessive branching, and their leakiness [6]. However, there is growing evidence that pericytes are present along the EC tubes in human tumour tissues [7,8] and that interfering with pericytes may inhibit tumour angiogenesis as developed in this review [9]. Targeting tumour pericytes as a potential anticancer approach will require determining the mechanisms of pericyte recruitment in tumour angiogenesis and addressing whether these mechanisms can be selectively harnessed.

This review summarizes the variable occurrence and possible functions of pericytes in tumour microvasculature. We discuss the contribution of PDGFB, S1P, Ang1, TGF-β1 and their corresponding receptors in the recruitment of tumour pericytes. We then go on to examine the contribution of matrix metalloproteinases (MMPs) in this recruitment process [10,11]. We finally propose several mechanisms to account

for the involvement of MMPs in pericyte recruitment at the tumour microvasculature.

2. The variable occurrence and possible functions of pericytes in tumour blood vessels

Until recently, the apparent disorganization of tumour blood vessels was attributed to their failure to mature and to become quiescent, two processes in which pericytes have been implicated [12]. Pericytes occur in the microvasculature of several human cancers and in animal tumour models. However, the microvessel pericyte coverage index, measured by quantifying the percentage of microvessels with colocalization of EC marker and α-SMA positive pericytes varied considerably from 10% to 20% in human glioblastoma and renal cell carcinomas, 30–40% in prostate and lung carcinomas, to 70% in mammary and colon carcinomas [7]. It reached 97% in spontaneous or transplanted tumours developed in mice [13]. Furthermore, in human neuroblastoma tumour samples, the extent of pericyte coverage surprisingly correlated with histological criterion for unfavourable prognosis [14].

In physiological angiogenesis, pericytes have multiple functions that seem to be relevant in the development and the maintenance of tumour microvasculature. A first potential role is a modulatory function on EC proliferation and survival. Indeed, the number of proliferating EC is reduced by half when tumour vessels are covered by pericytes [15]. In addition, pericytes may promote EC survival through secretion of diffusible angiogenic factors such as vascular endothelial growth factor (VEGF) and Ang1 [16]. Indeed, in several tumour models, pericytes protect EC from apoptosis induced by withdrawal of tumour cell-secreted VEGF [17] or by addition of recombinant interleukin-12 [15]. Accordingly, the combination of a VEGFR inhibitor with a PDGFR inhibitor, the latter blocking pericyte recruitment, showed superior anti-angiogenic efficacy than these two agents alone and allowed the regression of late-stage tumours [9,18]. A second potential role for tumour pericytes is the stabilization of nascent cancer microvessels. It is generally admitted that multiple EC sprouts form small tumour vessels initially lacking pericytes; subsequently, pericyte recruitment around these sprouts reduces EC proliferation and sprouting and leads to the formation of larger perfused microvessels [15]. In neuroblastoma and melanoma models, reduction of pericyte recruitment resulting from MMP inhibition is associated with decreased tumour vessel perfusion [10,11]. A third potential role for tumour pericytes is the local control of blood flow due to their contractile activity. Pericytes and VSMC are indeed known to constitute a reactive framework that modulates the blood flow into normal blood microvessels and could thereby regulate oxygen, metabolites and drug delivery into the tumour tissue [12]. However, tumour pericytes display several features that question whether, in tumours, they still function as normal pericytes. For instance, pericytes in tumour microvessels are loosely associated with EC and sometimes overlay other pericytes and extend processes far away from the vessel wall or beyond EC. The presence of pericytes beyond endothelial sprouts and even pericyte tubes without EC in some tumours suggests that pericytes could also be involved earlier, in sprout growth and retraction [13,19].

2.1. *Mechanisms of pericyte recruitment during embryonic development and tumour angiogenesis*

Under physiological conditions during embryonic development, pericytes are recruited around vascular EC by four different pathways namely PDGFB/PDGF receptor-β (PDGFR-β), S1P/endothelial differentiation gene-1 (EDG-1), Ang1/Tie2 (a short surname of a receptor tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2) and TGF-β1/activin-like kinase receptor (ALK5) (Fig. 1) [4]. Binding of EC-produced PDGFB to PDGFR-β expressed on VSMC and pericytes leads to pericyte proliferation and migration during developmental microvessel formation. Genetic ablation of PDGFB pathway in PDGFB and PDGFR-β deficient mice is associated with a lack of pericytes that leads to microvascular aneurysms and lethal microhaemorrhages [20]. Shingosine 1-phosphate (S1P), the signalling lipid, is generated by phosphorylation of sphingosine by sphingosine kinase (SphK) and degraded by S1P phosphatases and S1P lyases [21]. Beside intracellular functions, S1P is secreted and interacts with its G-protein coupled receptor (GPCR) called EDG-1 or $S1P_1$. Most of S1P present in serum is secreted by mast cells, monocytes and activated platelets [21]. EDG-1 is expressed by VSMC and EC during embryonic angiogenesis. Its genetic ablation in mice results in a decrease of vascular maturation comparable to that observed in PDGFB or PDGFR-β deficient mice [4]. Activation of EDG-1 on EC enhances the production of ECM proteins that promotes the recruitment of pericytes [4,21]. In addition, activation of EDG-1 on pericytes facilitates their migration towards microvessels [21]. Ang1, produced by VSMC and pericytes, binds to the receptor Tie2 expressed at the EC surface. Ang1/Tie2 engagement maintains and stabilizes mature vessels by promoting interactions between EC and pericytes and by mediating cell–matrix interactions in vessel morphogenesis but how this pathway acts is still not fully understood [22]. Ang1/Tie2 engagement also induces the expression by EC of the mitogen and chemotactic heparin-binding epidermal growth factor-like growth factor (HB-EGF) that promotes VSMC migration upon binding to the epidermal growth factor receptors (EGFRs) ErbB1 and ErbB2 [23]. TGF-β1 is expressed by a number of cell types, including EC and pericytes. Depending on the context and the concentration, TGF-β1 inhibits or promotes angiogenesis [4]. TGF-β1 is secreted in a latent form that needs to be activated, either by proteolytic cleavage mediated by proteases or by conformational change mediated by thrombospondins, for binding to TGF-type II receptors [24]. These receptors then recruit and phosphorylate type I receptors, such as ALK receptors, that transduce the signal to the nucleus via a phosphorylation cascade involving Smad proteins. The angiogenic effects of TGF-β1 are mediated by two type I receptors, ALK1 and ALK5. ALK1 is mainly expressed by EC where the TGF-β1/ALK1 signalling pathway stimulates EC proliferation and migration. ALK5 is not expressed by EC but by pericytes, where TGF-β1/ALK5 signalling inhibits cell proliferation and migration, stimulates the differentiation of

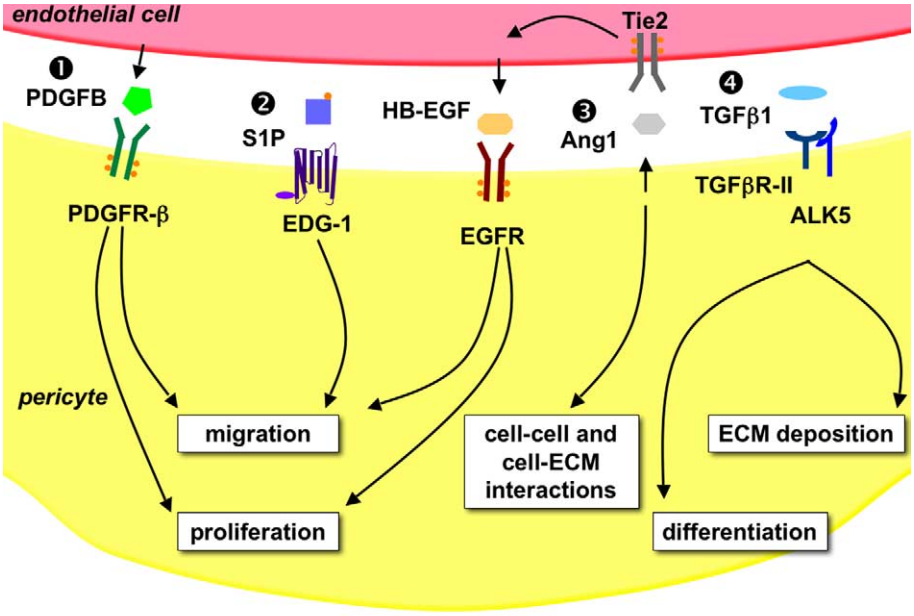

Fig. 1 – Regulation of pericyte recruitment by four major pathways: (1) platelet-derived growth factor B (PDGFB) released by endothelial cells binds to platelet-derived growth factor receptor-β (PDGFR-β) on pericytes and stimulates their migration and proliferation; (2) sphingosine 1-phosphate (S1P) binding to endothelial differentiation gene-1 (EDG-1) promotes cell migration; (3) angiopoietin 1 (Ang1)/receptor tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2 (Tie2) enhances cell–cell and cell–extracellular matrix (ECM) interactions and activates the expression and release of heparin-binding epidermal growth factor like growth factor (HB-EGF), that also promotes cell migration and proliferation; and (4) transforming growth factor-β1 (TGF-β1) binding to transforming growth factor-β receptor II (TGFβR-II) leading to activin-like kinase-5 (ALK5), stimulates pericyte differentiation and ECM deposition.

pericyte and promotes the expression of fibronectin and plasminogen activator inhibitor 1 (PAI-1).

It is still unclear to what extent the mechanisms mentioned above are involved in tumour angiogenesis. The retention motif that allows EC-derived PDGFB to bind within the cell or to proteoglycans at the cell surface and in the ECM is critical for the recruitment of tumour pericytes and their integration into the tumour vascular wall [25]. PDGFB expressed by tumour cells increased pericyte recruitment in several in vivo tumour models but failed to correct their detachment in PDGFB retention motif deficient mice [25,26]. Alternatively, genetic abolition of PDGFR-β expressed by embryonic pericytes decreased their recruitment in tumour [25]. EDG-1 expression is induced in EC and pericytes during tumour angiogenesis. In Lewis lung carcinoma tumours implanted in mice, inhibition by RNA interference of EDG-1 expression in EC strongly reduced pericyte coverage [27]. The role of Ang1 in tumour angiogenesis is unclear. Overexpression of Ang1 has been documented in various types of human tumours including glioblastoma, neuroblastoma and lung cancer but others studies have suggested a selective loss of Ang1 expression during tumour progression [28]. Overexpression of Ang1 had also variable effects on tumour angiogenesis. In a human glioma model developed in rat, Ang1 led to enhanced pericyte recruitment and increased tumour growth, presumably by favouring angiogenesis [29]. Alternatively, in a colon cancer model, overexpression of Ang1 led to smaller tumours with fewer blood vessels and higher degree of pericyte coverage resulting in decreased vascular permeability and reduced hepatic metastasis [30,31]. Thus, depending on the tumour model, stabilization of blood vessels by Ang1 may either promote tumour angiogenesis or reduce tumour growth, possibly by making EC unresponsive to further angiogenic factors [28]. TGF-β1 expression has been associated with increased tumour vascularization in several human tumours, such as breast and liver cancer, where its effect on vessel maturation has not been investigated so far [32]. In a xenograft model of human prostate cancer, inhibition of TGF-β1 activity led to fewer but larger and immature vessels [33].

2.2. *MMPs promote pericyte recruitment along tumour blood vessels*

Recent observations have suggested that MMPs could play a role in tumour vessel maturation, including pericyte recruitment. In human glioma and breast cancer, MMP-9 is expressed by VSMC and in particular by pericytes at the proliferating tumour borders [8,34]. In a human neuroblastoma xenotransplanted model, pericyte coverage along tumour microvessels is decreased by half in tumours grafted to MMP-9 deficient mice and transplantation with MMP-9-expressing bone marrow cells restores the formation of mature tumour vessels [10,14]. In addition, overexpression of TIMP-3, a natural inhibitor of MMPs, results in decreased pericyte recruitment in neuroblastoma and melanoma tumour models [11].

Whether the contribution of MMPs in pericyte recruitment is specific to tumour angiogenesis remains to be elucidated. Until recently, no deficit in pericyte coverage was reported in MMP deficient mice [4]. Our own studies failed to show abnormal pericyte coverage of endothelial cells in the normal liver, kidney and muscle of MMP-9 −/− as compared with wild-type mice (Jodele and colleagues, unpublished data). However, several limitations need to be considered when evaluating the validity of this approach. First, as in multiple other systems, MMP substrate redundancy might explain the absence of abnormal phenotype. Second, the effect of MMP deficiency during development might be transitional. Third, reduction of up to 90% of the pericyte coverage in mice, while causing structural and functional abnormalities in the microvasculature, is compatible with embryonic and postnatal survival [25]. Thus MMP −/− mice might have decreased number of pericytes that might not be enough to result in obvious abnormal phenotype. Likewise, an unexpected role of membrane-type 1 matrix metalloproteinase (MT1-MMP) in mural cells has been reported in a recent study that revealed a marked reduction in mural cell density as well as abnormal vessel wall morphology in brain tissues of MT1-MMP deficient mice [35]. Active MMP-2 has been detected by immunolocalization in pericytes of telencephalic vessels of human embryos while a necessary role of this MMP in brain microvessel maturation has not been demonstrated [36]. These observations are consistent with observations performed in other models of pathological neovascularization and vascular remodelling. For example, expression of MMP-1, -3 and -9 by VSMC was induced by vascular stimulation such as arterial injury and exposure to basic fibroblast growth factor (bFGF) or PDGFB [37,38]. In inflammatory bowel diseases, pericytes expressed MMP-1, MMP-9 and TIMP-1 [39].

Although we admit that it is still not demonstrated if pericytes and VSMC come from the same lineage, we have reviewed the role of MMPs in the recruitment of both cell types in many in vitro and in vivo models of neovascularization. Here, we discuss the possibility for MMPs to contribute to pericyte recruitment at six levels (Fig. 2): (1) direct promotion of pericyte invasion by ECM degradation; (2) stimulation of pericyte proliferation and protection against apoptosis by modification of the ECM; (3) activation of pericytes through the release of growth factor bound to the ECM; (4) transactivation of angiogenic cell surface receptor; (5) propagation of angiogenic signalling as cofactor; and (6) recruitment of bone marrow-derived stem cells.

2.3. *ECM degradation may promote pericyte invasion*

The observation that pericytes express MMPs in many human tumours in vivo [8,34] and in various in vitro models of

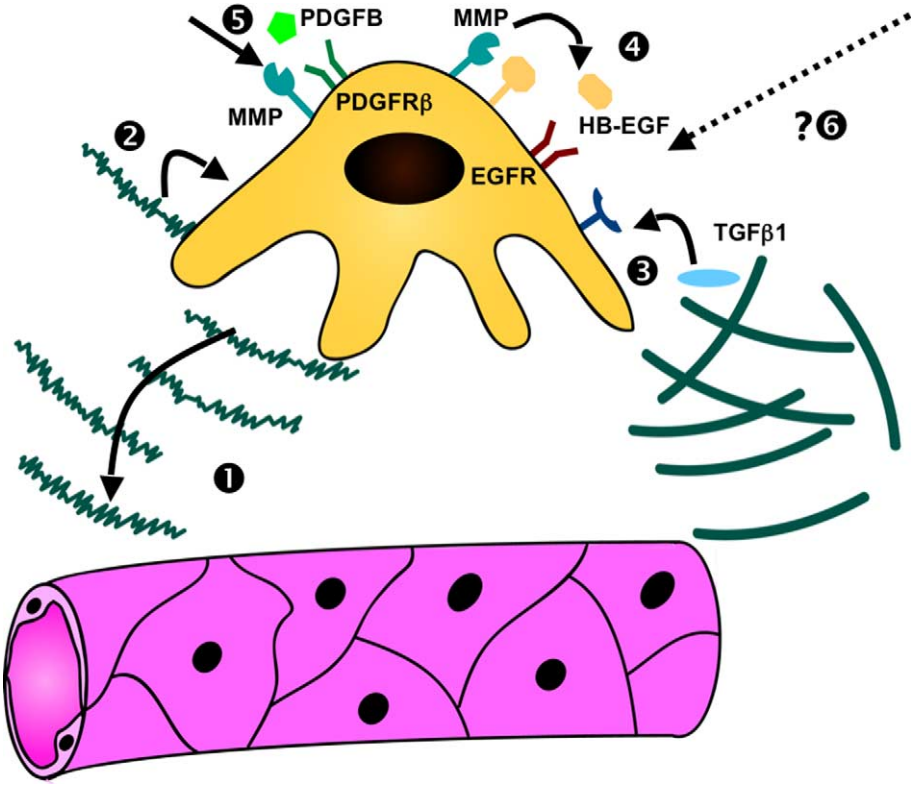

Fig. 2 – MMPs may contribute to pericyte recruitment at six levels: (1) ECM degradation mediated by MMPs promotes pericyte invasion; (2) proteolytic modification of ECM by MMP stimulates pericyte proliferation and/or decreases pericyte apoptosis; (3) MMPs release angiogenic growth factor such as ECM-bound TGFβ1; (4) MMPs contribute to G protein-coupled receptor (GPCR)-mediated transactivation of cell surface receptor, i.e. epidermal growth factor receptor (EGFR); (5) MMPs, i.e. MT1-MMP act as cofactor for PDGFB/PDGFRβ signalling; and (6) evidence lacks for a role of MMPs in the recruitment and differentiation of bone marrow-derived stem cells into tumor pericytes.

vascular remodelling [37,38] suggests that pericyte invasion requires the proteolytic degradation of ECM by proteases including MMPs [40]. As expected, synthetic inhibitors of MMPs reduce VSMC invasion through a filter coated with reconstituted ECM in vitro but do not modify VSMC intrinsic motility, as assessed by their ability to migrate through an uncoated filter [41,42]. A similar decrease in VSMC invasion has been observed in VSMC from MMP-9 deficient mice or VSMC overexpressing TIMP-1, -2, -3, or -4 [38,43,44]. Thus, it is conceivable that, as demonstrated for various cell types [40], MMPs increase pericyte coverage of tumour blood vessel by promoting, at least in part, cell invasion.

2.4. *Proteolytically modified ECM may modulate pericyte proliferation and apoptosis*

Cell–ECM and cell–cell interactions influence cell proliferation and survival. By their ability to modify the nature and structure of ECM proteins, MMPs modulate ECM–cell interactions and subsequently cell proliferation. For example, denaturation of fibrillar collagen was found to stimulate the proliferation of melanoma tumour cells through down-regulation of $p27^{kip1}$ [45]. Conversely, whereas mitogens caused isolated VSCM to proliferate by inducing proteasomal degradation of $p27^{Kip1}$, they had no effect on VSMC when connected to their native ECM in rat aorta [46]. Fibronectin is concentrated at the pericyte–EC interstitium and its degradation by proteolytic enzymes such as MMPs, gives rise to biologically active fragments [3]. Among these, a 45 kDa fibronectin fragment inhibits EC proliferation and stimulates pericyte and VSMC proliferation, suggesting a role for this fragment in vessel maturation [47]. Although contact with denatured collagen and expression of MMPs have been reported to protect tumour cells from apoptosis [48,49], the effect of MMPs on VSMC and pericyte apoptosis is less clear since overexpression of TIMP-3 or -4 induces VSMC apoptosis through a mechanism not related to its protease inhibitory activity [43,44,46]. Thus, MMPs may alternatively promote pericyte recruitment in tumour by the ECM denaturation and/or release of ECM components that are able to stimulate pericyte proliferation.

MMPs also modify cell–cell interaction by degrading adhesion proteins such as cadherins. Cadherins are transmembrane glycoproteins that associate with α, β and γ catenins. A decrease of cell surface cadherin releases β-catenin that translocates to the nucleus where it acts as a transcription factor for several genes involved in the control of cell cycle. VSMC proliferation induced by PDGFB was found to be associated with proteolytic shedding of N-cadherin. Conversely, synthetic MMP inhibitors or overexpression of TIMP-1 or -2 prevented N-cadherin shedding, decreased nuclear translocation of β-catenin and inhibited VSMC proliferation [50]. Likewise, VSMC deficient for MMP-9 displayed an increased association of β-catenin with E-cadherin and impaired proliferation in response to FGF [38]. However, in TIMP-3 overexpressing tumours which disclose immature blood vessels and lack of pericyte recruitment, VE-cadherin expression is decreased at the surface of EC [11]. Thus, it is also possible that MMPs promote pericyte proliferation through the proteolytic cleavage of adhesion proteins such as cadherins.

2.5. *Release of angiogenic factors bound to the ECM may promote pericyte recruitment*

We have reported that MMP-9 expressed by bone marrow-derived leucocytes enhances pericyte recruitment in a neuroblastoma model [14]. In several tumour models in vivo, inflammatory cells-derived MMP-9 has been shown to promote tumour angiogenesis by releasing ECM-bound angiogenic factors such as VEGF [51,52]. Similarly, in vitro MMP-2, -3, -7 release TGFβ1 from decorin, a proteoglycan that acts as ECM reservoir of TGFβ1 [53]. However, since pericyte recruitment and integration into the vessel wall are impaired when PDGFB losses its retention motif and is freely diffusible, it is uncertain that MMPs favour pericyte coverage by the release of ECM-bound PDGFB [25]. Thus, MMPs might contribute to pericyte recruitment through their "sheddase activity", by increasing release and bioavailability of angiogenic factors.

2.6. *Transactivation of cell surface receptor mediated by MMPs may promote pericyte recruitment*

RTKs are activated upon binding to their specific ligands or via other additional ligands that trigger GPCR-mediated transactivation. Transactivation of EGFR by angiotensin II (ANGII), the major bioactive peptide of the renine–angiotensin system, as well as transactivation of EGFR and PDGFR-β by S1P have been reported to contribute to VSMC proliferation in vitro [54,55]. Whereas PDGFR-β transactivation did not depend on MMPs, synthetic MMP inhibitors blocked EGFR transactivation [54]. The effect of these inhibitors could be explained by a "triple membrane-passing" signalling model (Fig. 3) [54,56]. The first transmembrane signalling depends on the binding of ligands such as ANGII or S1P to their GPCR. The resulting intracellular signal induces the expression or the activation of a metalloproteinase at the cell surface (second inside-out signalling). This metalloproteinase proteolytically cleaves proHB-EGF that, in term, activates EGFR (third outside-in signalling). The identity of the involved metalloproteinases and the exact mechanisms of their induction or activation remain

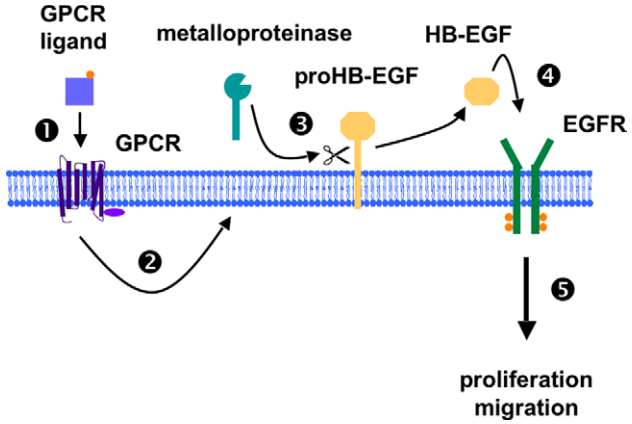

Fig. 3 – Triple membrane-passing signalling. Upon binding to its ligand (1), GPCR signalling induces a metalloproteinase activity (2) leading to the proteolytic cleavage of proHB-EGF (3). Released HB-EGF activates EGFR (4) that promotes cell proliferation and migration (5).

unknown. Several proteases of the ADAM (a disintegrin and metalloprotease) family have been found to mediate shedding of HB-EGF [55]. In addition, MT1-MMP was reported to cooperate with S1P to induce EC migration and morphogenic differentiation [57]. The intracytoplasmic domains of ADAM and MT-MMP provide a conceptual mechanism to inside-out signals whereby extracellular proteolytic activity is achieved. However, other MMPs without intracytoplasmic domain have been implicated in EGFR transactivation. For example, MMP-3 cleaves proHB-EGF at a specific juxtamembrane site, to release active HB-EGF in vitro [58]. CD44 heparan sulfate proteoglycan recruits active MMP-7 that complexes proHB-EGF and secondarily activates HB-EGF [59]. MMP-2 and MMP-9 proteolytic activities participate in EGFR transactivation in pressure-induced myogenic tone in mouse mesenteric resistance arteries [60]. Gonadotropin-releasing hormone transactivates EGFR through the secretion of soluble MMP-2 and MMP-9 [61]. Thus, MMP-induced shedding of HB-EGF bound to the cell membrane to transactivate RTK is a third mechanism that may account for the promotion of pericyte recruitment by MMPs.

2.7. *MMPs may act as necessary cofactors in propagating signalling through the angiogenic factor/receptor axis*

An original role for MT1-MMP has been identified in the PDGFB/PDGFR-β signal transduction. MT1-MMP-deficient VSMC cultured in vitro, displayed PDGFB-selective defects in chemotaxis, proliferation and intracellular signalling response such as revealed by an absence of ERK1/2 and Akt activation. This was consistent with the abnormalities of vessel wall morphology observed in vivo in MT1-MMP deficient mice and the absence of pericyte recruitment in MT1-MMP deficient explants in an ex vivo model of neovascularization. The catalytically active domain, the transmembrane domain but not the intracytoplasmic tail of MT1-MMP, was required to rescue VSCM response to PDGFB stimulation. Lehti and colleagues reported the co-precipitation of MT1-MMP with PDGFR-β in lysates of VSCM, and proposed a model wherein MT1-MMP proteolytically processes either PDGFR-β itself or a near neighbour accessory molecule. Hence, optimal signalling not only requires an intact PDGFB/PDGFR-β couplet but also membrane-tethered and catalytically active MT1-MMP [35].

2.8. *Evidence lacks for a role of MMPs in promoting bone marrow-derived stem cells differentiation into tumour pericyte*

Consistent with the presence of bone marrow precursors-derived EC in tumour microvasculature and the positive role of MMP-9 in the recruitment and mobilization of these precursors, it has been investigated whether bone marrow stem cells participate in the maturation of tumour microvasculature by differentiating into pericytes [62]. Flk-1 positive stem cells can differentiate into both EC and mural cells upon VEGF and PDGF-B stimulation, respectively [63]. In a subcutaneous melanoma model and in VEGF-induced angiogenesis, bone-marrow-derived stem cells did not differentiate into vascular EC but rather into vascular mural periendothelial cells that are morphologically indistinguishable from pericytes. While these periendothelial cells expressed the NG2 proteoglycan, a marker for developing pericytes, they did not express detectable levels of desmin or α-SMA [64]. However, in a human neuroblastoma model, we demonstrated that bone marrow-derived stem cells give raise to 14% of CD31-positive EC but do not differentiate into α-SMA-positive pericytes. In this model, the presence or the absence of MMP-9 expression had no effect on the recruitment of bone-marrow-derived pericytes in tumour microvasculature [14].

3. Conclusion

Over the last 30 years, tumour angiogenesis has become a field of considerable interest in cancer therapy. Based on their chaotic features suggesting perpetual remodelling and absence of maturation, it has been proposed that tumour blood vessels could be selectively targeted without affecting the quiescent normal microvasculature. There is now growing evidence that pericytes may occur in tumour blood vessels and are critical for the development and the maintenance of a functional vascular network. This new perspective does not rule out the possibility to interfere selectively with cancer angiogenesis. To the contrary, it becomes evident that tumour pericytes represent an additional therapeutic target to synergize with other anti-angiogenic therapies. Morphologic and molecular alterations of tumour pericytes suggest that they differ from pericytes in normal tissues [13,65]. Further investigations are thus warranted to define the characteristics of tumour pericytes and to identify their mechanisms of recruitment. A better knowledge of these aspects will, no doubt, indicate new directions not only to hamper the development of tumour blood vessels but also to interfere with established tumour vessels that have often been incriminated in the limited clinical efficacy of anti-angiogenic therapy.

Conflict of interest statement

None declared.

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