TGF-β and MMPs: a complex regulatory loop involved in tumor progression

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Abstract

Transforming growth factor-β (TGF-β) has a dual and contradictory role in cancer. It is a tumor suppressor at early stages of tumor formation by virtue of its growth inhibitory and pro-apoptotic functions. However, at later stages of tumor progression, tumor cells lose their sensitivity to be growth inhibited by this cytokine, and, then, TGF-β facilitates tumor invasion and metastasis by diverse mechanisms, including the induction of an epithelial-mesenchymal transition, the suppression of the immune system and the stimulation of angiogenesis. Matrix metalloproteinases (MMPs) have also been shown to play a pivotal function in tumor cell migration, invasion and angiogenesis. MMPs and TGF-β form an interplay loop that may attenuate or promote tumor progression. On one hand, latent TGF-β, an inactive TGF-β precursor that is sequestered by the extracellular matrix, is proteolytically activated by MMPs; the released active cytokine may, then, suppress or promote tumor cell growth and invasiveness depending on the tumor stage. On the other hand, TGF-β regulates the expression of MMPs and their tissue inhibitors TIMPs in both tumor and stromal cells. MMPs in the tumor microenvironment are involved in the control of tumor cell growth and survival by modulating the bioavailability of growth factors and chemokines, and they also influence inflammation and angiogenesis. Thus, by modulating the net balance of MMPs and TIMPs in both compartments: the tumor and stroma, TGF-β regulates malignant progression.

Introduction

The mammalian transforming growth factor-β (TGF-β) family comprises more than forty structurally related factors including activins, inhibins, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs) and the classical mammalian isoforms: TGF-β1, -β2 and -β3, among others. The three TGF-β isoforms are encoded by different genes and have both overlapping and distinct functions. These growth factors play crucial roles in embryonic development, adult tissue homeostasis and the pathogenesis of a number of diseases including cancer (Gordon and Blobe, 2008; Padua and Massagué, 2009; Santibáñez et al., 2011). In this chapter, we review the current knowledge on the interplay between TGF-β and matrix metalloproteinases (MMPs) in cancer. Tumors exploit the heterotypic reciprocal interactions created between TGF-β and MMPs to fuel tumor progression and metastasis.

The dual role of TGF-β in cancer

TGF-β plays a double and paradoxical role in cancer (Massagué, 2008). It acts as a tumor suppressor during the early phases of epithelial carcinogenesis. This occurs by virtue of its anti-proliferative and pro-apoptotic roles to counter the effects of local mitogenic stimulation in the injured or stressed epithelium. TGF-β is commonly present in tumors. It is secreted by the tumor cells themselves and by a diverse cell population of the stromal compartment, such as fibroblasts, leukocytes, macrophages, bone marrow-derived endothelial and myeloid precursor cells. TGF-β accumulates in the tumor microenvironment due to the infiltration of TGF-β-secreting inflammatory cells in the tumors and because of enhanced production of the cytokine in the mass of tumor cells as a consequence of oncogene activation (Wakefield and Roberts, 2002). Nevertheless, carcinoma cells lose the TGF-β growth inhibitory response. This occurs by a variety of mechanisms, including inactivating mutations in components of the TGF-β signaling system (see below) as well as other alterations not yet fully understood that prevents the TGF-β anti-proliferative cell response. Then, carcinoma cells utilize the ability of TGF-β to modulate processes that stimulate malignant progression and metastasis. The tumor promoting effects of TGF-β may be exerted directly on the tumor cells or indirectly by taking advantage of the interactions between the tumor and stroma (Stover et al., 2007).

Tumor cells persistently exposed to TGF-β may elicit an epithelial-mesenchymal transition (EMT). EMT is a phenotypic conversion by which epithelial cells lose their polarity and cohesiveness (i.e., the hallmark of EMT is the downregulation of the cell-cell adhesion protein E-cadherin) and acquire mesenchymal features, such as invasiveness and resistance to apoptosis. EMTs are crucial in embryonic development and are involved in the pathogenesis of several diseases, such as fibrosis and cancer (Thiery et al., 2009). EMTs are induced by transcription factors belonging to the Snail, ZEB and basic helix-loop-helix (bHLH) families. These factors were formerly identified as transcriptional repressors of E-cadherin, but now are thought to be involved in

additional functions, such as cell survival and angiogenesis, beyond E-cadherin repression and induction of EMT (Peinado et al., 2007). TGF-β has been found to regulate the expression of many of these transcription factors during tumor progression. Interestingly, recent data suggest that EMTs occurring in tumors may lead to cell subpopulations that have acquired embryonic stem traits that confer to them the ability to spread and metastasize (Polyak and Weinberg, 2009). TGF-β can also promote tumor cell growth by inducing the expression of mitogenic growth factors in both the tumor and stroma compartments. Furthermore, TGF-β stimulates malignant progression by stimulating angiogenesis, inhibiting the host immune response, and favouring distal metastasis (Massagué, 2008; Yang et al., 2010).

TGF-β signaling

TGF-β binds cell-surface serine/threonine kinase receptors types I and II, which form heteromeric complexes in the presence of dimerized ligands. Seven type I TGF-β receptors (TβRI), also named activin like-receptor kinase (ALKs), as well as five different type II receptors (TβRII) have been described. Soluble ligands bind first to the constitutively active TβRII, followed by the TβRII-mediated interaction and phosphorylation of a glycine/serine (GS) rich domain of TβRI to produce an activated ligand-receptor complex (Kang et al., 2009). Then, the activated TβRI phosphorylates the downstream effectors Smads. Members of the Smad family are well conserved and can be classified into three groups: i) receptor associated Smads (R-Smad); ii) cooperating Smads (Co-Smad); and iii) inhibitory Smads (I-Smads). In humans, five different R-Smads have been described that are substrates for activated TGF-β receptors (Smad1, 2, 3, 5, and 8). Smad2 and Smad3 are phosphorylated by TβRI activated by TGF-β and activins, whereas Smad1, Smad5 and Smad8 generally mediate signaling by BMPs and other members of the TGF-β superfamily. Upon ligand activation of the TGF-β receptor complex, TβRI phosphorylates R-Smad at a serine rich C-terminal motif, and then the phospho-R-Smad associates with Smad4 (mammalian Co-Smad). The activated Smad complex is shuttled into the nucleus where in collaboration with other transcription factors binds and regulates promoters of different target genes (ten Dijke and Hill, 2004). Two of these genes are I-Smads, Smad6 and Smad7. The induced expression of these inhibitory Smads produces a negative-feedback regulation of TGF-β signaling.

TGF-β ligands may also interact with the co-receptors endoglin and betaglycan (known as type III TGF-β receptors). Endoglin and betaglycan are type I membrane proteins with large extracellular domains and short cytoplasmic tails that lack consensus signaling motifs, but they modulate the TGF-β cell response and have important roles in cancer (Bernabéu et al., 2009; Pérez-Gómez et al., 2010).

In addition to the canonical Smad pathway, TGF-β activates Smad-independent signaling pathways, including several mitogen-activated protein kinase (MAPK) cascades, Rho GTPases and phosphatidylinositol-3-kinase (PI3K). A brief scheme of

the TGF-β signaling pathways through Smad and non-Smad proteins is presented in Fig. 1. These non-canonical TGF-β pathways may not only regulate Smad signaling, but also trigger cell responses that are independent of Smads (Zhang, 2009). It is believed that TGF-β utilizes such a variety of signaling pathways in order to regulate the wide array of cellular functions that are under its control in different cells and tissues, including proliferation, differentiation, apoptosis, adhesion, motility and extracellular matrix (ECM) remodeling. Thus, TGF-β can activate the Ras/Erk MAPK signaling pathway in epithelial cells as rapidly as mitogenic factors independently of Smad proteins. This response seems to be important for the pro-migratory functions of this cytokine (Zavadil et al., 2001). Also, TGF-β stimulates the JNK and p38 MAPK signaling cascades by a mechanism involving activation of TGF-β-activated kinase 1 (TAK1), a MAP kinase kinase kinase acting in stress-activated MAPK cascades. The TAK1-JNK/p38MAPK cascade functions in cooperation with the Smad pathway to induce apoptosis (Liao et al., 2001). TGF-β regulates the dynamics of cytoskeletal organization by activating members of the Rho GTPase family, including RhoA and Cdc42. These activities appear to be essential for TGF-β-induced EMT. In addition, the PI3K/AKT axis is another non-Smad pathway that contributes to TGF-β-induced EMT, although it can be also involved in other TGF-β- cellular responses, such as stimulation of fibroblast proliferation or suppression of apoptosis (Zhang, 2009).

The dual role of MMPs in cancer

Classically, MMPs were thought to be active agents facilitating cancer invasion and metastasis due to its ability to degrade the ECM clearing a path for tumor cells to move through matrix barriers. However, this idea has recently been challenged because of the results of clinical trials in which broad-range MMP inhibitors failed to slow down tumor growth in cancer patients. In fact, MMP inhibition sometimes resulted in a poorer disease outcome. The finding that MMPs have a protective role in cancer has been confirmed using genetically engineered mouse models (reviewed in Egebald and Werb, 2002; López-Otín and Matrisian, 2007; Decock et al., 2011). The tumor suppressor role of MMPs may derive from its ability to produce natural angiogenic inhibitors, such as angiostatin, endostatin and tumstatin, as a result of degrading extracellular components, such as plasminogen, collagen XVIII and collagen IV, respectively. This is the case of MMP12 (macrophage metalloelastase), which produces angiostatin, and of MMP9 (gelatinase B) that can generate the all three inhibitors. In contrast to this protective role of MMP9, an overwhelming number of reports have found this proteinase associated with tumor progression and enhanced angiogenesis. Thus, the balance between its pro- and anti-angiogenic actions appears to be critical for the effect of MMP9 on tumor progression (Decock et al., 2011). In vitro and in vivo studies using MMP8 (collagenase 2)-deficient mice have found a tumor and metastasis suppressor function for this MMP. This finding correlates with the analyses in breast and oral cancer patients suggesting that MMP8 expression is a good prognostic marker (López-Otín et al., 2009). Besides MMP9, other MMPs, such as MMP3 (stromelysin 1), MMP11 stromelysin 3) and MMP19, have been found to play dual roles in cancer and exert pro-tumorigenic or

protective roles depending on the context (López-Otín and Matrisian, 2007). Thus, for example, MMP19 was found to act as a negative regulator of angiogenesis, but the mechanism involved needs to be clarified. Whereas some MMPs appear to protect from malignancy, others such as MMP1 (collagenase 1), MMP2 (gelatinase A) and MMP14 (MT1-MMP), have shown consistently to promote tumor progression (Fingleton, 2006; Gialeli et al., 2011). Several MMPs interact with TGF-β to form a bidirectional regulatory loop associated with cancer. TGF-β needs to be proteolytically activated by MMPs in order to exert its cellular functions, and an important biological activity of TGF-β in tumors is the remodeling of the ECM by regulating the expression of MMPs and its tissue inhibitors TIMPs.

Activation of latent TGF-β by MMPs

Cells secrete TGF-β as an inactive multiprotein complex that is sequestered by the extracellular matrix (ECM). Hence, TGF-β needs to be activated (released from the ECM and from the bound proteins that inhibit its activity) in order to exert its biological effect. Indeed, it is thought that TGF-β is synthesized in excess and, therefore, its activation is the rate-limiting step in TGF-β bioavailability (Annes et al., 2003; Rifkin, 2005; Jenkins, 2008). The three TGF-βs are all synthesized as homodimeric pro-proteins of 75 kDa that are cleaved intracellularly by a furin-type convertase. However, the dimeric pro-peptide, which is denoted as the latency associated protein (LAP), remains non-covalently attached to the TGF-β 25-kDa dimeric active protein forming the small latent complex (SLC). The SLC is bound to a latent TGF-β binding protein (LTBP) via a disulphide bond giving rise to the large latent complex (LLC), which upon secretion may be covalently linked to the ECM (Fig. 2). LTBPs are not only involved in SLC targeting to the ECM but also are important for SLC secretion (Miyazono et al., 1991). LTBPs belong to the superfamily of fibrillin-like ECM proteins. There are four LTBPs encoded by distinct genes that all respond to the same structure: a hinge domain that separates the N-terminal region from the central core of Ca^{2+} binding EGF-like repeats, and four unique 8-cys domains containing eight cystein residues (Annes et al., 2003; Rifkin, 2005). LAP is attached to the third 8-cys domain of LTBP-1, -3 and -4, whereas LTBP-2 appears to be unable to sequester TGF-β (Saharinen and Keski-Oja, 2000). The N-terminal region of LTBP is covalently cross-linked to the ECM by extracellular tissue transglutaminase (Nunes et al., 1997). The hinge domain of LTBP is a protease-sensitive region and, thus, LLC can be released from the ECM by a proteolytic cleavage (Taipale et al., 1994; Yu and Stamenkovic, 2000; Dallas et al., 2002). However, as part of the LLC, TGF-β cannot bind its cell-surface receptors due to the high-affinity non-covalent association of TGF-β with LAP (Lawrence et al., 1984), which prevents further downstream signaling. Therefore, rescue of biologically active TGF-β requires its dissociation from LAP in the SLC and/or the LLC.

The activation of latent TGF-β is a complex and tightly regulated process that involves both proteolytic and non-proteolytic mechanisms. A number of physical and biological cues, including heat, pH extremes, reactive oxygen species (ROS),

thrombospondin 1 (TSP1), integrins and proteinases, have been described to activate latent TGF-β (Hyytiäinen et al., 2004; Rifkin, 2005). Among proteolytic enzymes both serine proteinases and MMPs have been involved in latent TGF-β activation in tumor cells. Members of the MMP superfamily that mediate the activation of latent TGF-β complexes include MMP14, MMP13 (collagenase 3), MMP9 and MMP2 (Jenkins 2008; Wipff and Hinz, 2008). Interestingly, active TGF-β potently induces the expression of these enzymes in tumor and stromal cells (see below), thereby establishing a positive TGF-β autocrine regulatory loop that drives tumor progression.

The role of integrins in latent TGF-β activation

In vivo experiments with mutant mice suggest an important role for αv-containing integrins in activating latent TGF-β. αvβ3, αvβ5, αvβ6 and αvβ8 integrins bind the tripeptide Arg-Gly-Asp (RGD) present in the LAP propeptides of TGF-β1 and TGF-β3, which is absent in the LAP of TGF-β2 (Munger et al., 1998; Munger et al., 1999; Wipff et al., 2007; Yang et al., 2007). Mice knockout for β6, β8 and αv integrin subunits as well as mice bearing a mutation of the integrin-binding site in the LAP complex recapitulate the phenotype produced by TGF-β1 knockout mice (reviewed in Wipff and Hinz, 2008). The binding of LAP to these integrins allow for cells to hold latent TGF-β on their surface, leading to latent TGF-β activation. This activation may be direct by a proteinase-independent mechanism. The direct activation of latent TGF-β involves integrin-mediated cell traction that results in a conformational (deformation) change of the LLC, releasing active TGF-β (Wipff and Hinz, 2008). But more likely, latent TGF-β activation occurs by a mechanism involving proteolysis. Integrins, then, act as a docking platform to bring together latent TGF-β and an activating proteinase (Fig. 3).

αvβ8 integrin binds with high affinity the LAP of TGF-β1, but αvβ8-mediated activation of latent TGF-β1 depends on the proteolytic activity of MMP14 (Mu et al., 2002; Araya et al., 2006; Travis et al., 2007). MMP14 appears to be involved in the liberation of latent TGF-β from the ECM in endothelial cells, a mechanism of regulation that could be important in the activation and resolution phases of angiogenesis (Tatti et al., 2008). αvβ3 acts as a docking site for MMP9 and MMP2 in mammary carcinoma and melanoma cells, respectively (Rolli et al., 2003; Brooks et al., 1996), and it has been shown that both MMPs proteolitically activate TGF-β1 (Fig. 3). Interestingly, in these tumor cells, proteolytic activation of latent TGF-β1 is also dependent on the recruitment of MMP9 to the cell surface by the hyaluronan receptor CD44, after which active TGF-β1 promotes tumor invasion and angiogenesis (Yu and Stamenkovic, 2000). CD44 is known to be involved in the process of cancer metastasis and high CD44 levels on the surface of tumor cells have been correlated with high metastatic ability (Marhaba and Zoller, 2004). MMP2 and MMP9 have also been involved in latent TGF-β activation during progression to an aggressive mammary tumor phenotype in caveolin-1-deficient mice (Sotgia et al., 2006). On the other hand, αvβ3 integrin associates with

TβRII upon stimulation with active TGF-β1 (Scaffidi et al., 2004; Galliher and Schiemann, 2006). Therefore, in addition to clustering latent TGF-β with its activating proteinase, another potential role of integrins as docking platforms may be to facilitate availability of the receptors to locally activated TGF-β.

In breast cancer, it has been suggested a novel anti-tumorigenic and anti-angiogenic mechanism of action for the anti-estrogen tamoxifen by decreasing the levels of active TGF-β1. The reduction of extracellular TGF-β1 levels was likely mediated by MMP9, whose expression and activity are upregulated by tamoxifen (Nilsson et al., 2009). This observation is in contrast to the assigned role of cell-surface associated MMPs as TGF-β activators and highlights the multifunctional role of MMPs in tumor progression, as they may act as tumor suppressors instead of promoting cell invasion and metastasis (see above).

MMPs, TGF-β activation and EMT

MMPs also play an important role in the regulation of EMT. Early studies showed that MMP3 directly degraded the cell-cell adhesion receptor E-cadherin in mammary epithelial cells leading to EMT (Lochter et al., 1997). Also, MMP7 (matrilysin) appears to cleave E-cadherin leading to tracheal epithelial cell scattering and migration (McGuire et al., 2003). MMP2, MMP3 and MMP14 have been involved in developmental EMT occurring during morphogenetic processes, such as neural crest delamination, endocardial cushion invasion and mammary gland branching morphogenesis (Radisky and Radisky, 2010). Many of these MMPs have also been associated with EMT during cancer progression (see below). However, a novel mechanism for MMP-induced EMT involving TGF-β has been reported (Illman et al., 2006). MMP28 (epylisin) is the newest member of the MMP family; when it is expressed in lung adenocarcinoma cells leads to an irreversible EMT associated with loss of E-cadherin, increased cell migration/invasion and upregulation of MMP9 and MMP14. Interestingly, MMP28 is attached to the surface of epithelial cells (the cell-surface molecule responsible for MMP28 recruitment is presently unknown) where it activates the latent TGF-β complex. Active TGF-β seems to be the responsible agent for inducing EMT and upregulating MMP9 and MMP14.

Another novel mechanism for EMT induction in MCF-7 breast cancer cells involving the concerted action of insulin growth factor-1 (IGF-1) and TGF-β has also recently been proposed by Walsh and Damjanovski (Walsh and Damjanovski, 2011). In this model, the extracellular activation of MMPs is dependent on PI3K and MAPK signals triggered by IGF-1. MMPs are then capable of activating latent TGF-β in the ECM, which, in turn, induces an EMT. This is an example of the sophisticated and complex relationships established between different cytokines and signaling pathways that drive tumor progression.

Interestingly, other endogenous TGF-β receptor ligands different from the TGF-βs themselves have been found to modulate EMT. Thus, signal peptide-CUB-EGF-like

domain containing protein 3 (SCUBE3) is a secreted glycoprotein upregulated in lung cancer that behaves as an endogenous ligand for TβRII. MMP2 and MMP9 cleave SCUBE3 in two major fragments: the N-terminal EGF-like repeat and the C-terminal CUB domain. The CUB fragment (and also the full-length SCUBE3 protein) binds TβRII, activates TGF-β signaling and promotes EMT linked to upregulation of MMP2 and MMP9 and increased tumor cell migration/invasion (Wu et al., 2011). This finding constitutes a novel example of a positive TGF-β autocrine regulatory loop linked to tumor progression.

Latent TGF-β activation in bone metastasis

Bone homeostasis is maintained through a balance of bone-depositing osteoblasts and bone-resorbing osteoclasts. Bone is the preferred site for metastasis of breast and prostate cancers. Breast cancer bone metastases are characterized by hyperactivation of osteoclasts and net bone destruction, whereas prostate cancer bone metastases are typically osteoblastic and generally lead to abnormal bone formation (Kingsley et al., 2007). The bone matrix is the major reservoir in the body for TGF-β1, which is secreted by osteoblasts during bone matrix synthesis and stored into the bone as an inactive latent form. TGF-β1 is liberated into the tumor microenvironment by osteolysis (Pfeilschifter and Mundy, 1987). Released TGF-β1, once activated, can influence the bone microenvironment by acting as a chemoattractant for tumor cells. It also can enhance tumor cell growth and stimulate the production of osteoclast-inducing factors, such as the parathyroid hormone-related protein (PTHrP). Thus, TGF-β promotes the so called vicious cycle of osteolytic bone metastasis (Gupta and Massague, 2006; Lynch, 2011). The liberation and activation of latent TGF-β from the bone matrix represents a critical step in bone metastasis. Indeed, increased TGF-β signaling has been found at the tumor-bone interface of breast cancer-induced osteolysis despite no transcriptional upregulation of TGF-β was observed. Furthermore, attenuation of TGF-β signaling using a neutralizing antibody or a TβRI kinase inhibitor reduced mammary tumor-induced osteolysis (Futakuchi et al., 2009). Similarly, it has been found that TGF-β derived from the bone matrix promotes proliferation of prostate carcinoma cells and osteoclast activation-associated osteolysis in the bone microenvironment (Sato et al., 2008).

A major mechanism for sequestering latent TGF-β in the bone matrix is via its association with LTBP-1. In vitro studies using osteoclast cultured cells have found that several proteinases, including elastase, plasmin, MMP2 and MMP9, are able to cleave LTBP-1 and release TGF-β1 from the bone ECM (Dallas et al., 2002; Lynch, 2011). MMP9 has been found to be upregulated at the tumor-bone interface during breast cancer-induced osteolysis, but it is secreted as a zymogen requiring activation by other proteinases, such as cathepsin G (Wilson et al., 2009) or MMP13 (Nannuru et al., 2010). Other authors have proposed the direct cleavage of LTBP by BMP1-like proteinases, resulting in the liberation of LLC from the ECM and subsequent MMP-dependent LAP proteolysis. BMP1-like proteinases are involved in the activation of

BMPs and processing of matrix precursors necessary for ECM formation during morphogenesis (Ge and Greenspan, 2006).

Regulation of MMP expression and activity by TGF-β

The proteolytic activities of MMPs are regulated at different levels: gene expression, compartmentalization, conversion from inactive zymogen to active enzyme, and, finally, the presence of specific inhibitors. The most important MMP inhibitors are TIMPs (Kessenbrock et al., 2010). Under normal physiological conditions, the expression of MMPs is regulated at the transcriptional level. MMPs are synthesized as inactive zymogen precursors that can be proteolitically activated intracellularly by furin-like serine proteinases; but most of MMPs are activated after secretion by serine proteinases, such as plasmin, or by other activated MMPs. Also, endogenous TIMPs provide a balancing mechanism to prevent excessive degradation of ECM by MMPs.

The regulation of MMP expression by TGF-β is found to be complex and controversial because multiple signaling pathways and transactivators may be involved. Also, the effect of TGF-β on MMP gene transcription may be cell type and context-dependent.

Regulation of MMP gene transcription

Yan and Boyd have proposed three categories of MMP promoters based on the composition of cis-elements (Yan and Boyd, 2007). The first group (MMP1, MMP3, MMP7, MMP9, MMP10, MMP12, MMP13, MMP19, MMP20 and MMP26) contains a TATA box and a proximal AP-1 binding site. Most of these MMP promoters also contain an upstream PEA-3 binding site that is adjacent to and cooperates with an AP-1 binding site. The second group (MMP8, MMP11, MMP15, MMP21 and MMP27) contains a TATA box but lacks a proximal AP-1 binding site. The third group (MMP2, MMP14, MMP16, MMP17, MMP23, MMP24, MMP25 and MMP28) does not harbor a TATA box and expression of MMPs in this group is mainly determined by Sp1 transcription factors (Yan and Boyd, 2007; Clark et al 2008). A schematic representation of these categories is presented in Fig. 4.

The regulation of MMP expression by TGF-β is even more complex because TGF-β can both stimulate and inhibit MMP gene transcription. The molecular mechanism underlying inhibition of MMP expression by TGF-β is the presence of cis-acting elements in the MMP promoters that repress MMP gene transcription. Kerr and coworkers were the first to report the presence of a TGF-β inhibitory element (TIE) in the promoter of rat MMP3 (Kerr et al., 1990). TIE contains the consensus sequence GNNTTGGtGa where N denotes any nucleotide, and lower-case letters mark preferred nucleotides. TIEs have also been found in the promoters of MMP1, MMP7, MMP12, MMP13 and MMP14 (Gaire et al., 1994; White et al., 2000; Lohi et al., 2000) (Fig. 4). However, the biological significance of many of these sequences for MMP regulation is

still uncertain. Other elements have also been involved in the repression of MMP promoters by TGF-β. Thus, an AP-1 site was found to be crucial for inhibition of MMP1 expression in dermal fibroblasts and of MMP12 expression in macrophages by TGF-β (Yuan and Varga, 2001; Feinberg et al., 2000). Also, an NFkB site appears to be indispensable for TGF-β-mediated suppression of MMP9 transcription in macrophages and monocytes (Ogawa et al., 2004).

MMPs in cancer cells

The expression and activity of MMPs are enhanced in most human cancers associated with advanced stages of tumor progression, increased invasion and metastasis as well as shortened patient survival. Interestingly, clinical studies suggest that increased levels of TIMP-1 and -2 also correlate with a bad prognosis, likely reflecting a net balance between higher MMP and TIMP activities in favor of MMPs in order to accomplish the increased remodeling of the ECM occurring during tumor progression (Egeblad and Werb, 2002). The upregulation of MMPs in cancer cells is likely due to transcriptional activation rather than genetic alterations, and this might be triggered by cytokines that are upregulated during tumor progression, such as TGF-β (Yan C & Boyd, 2007). TGF-β is overexpressed in many human cancers (Derynck et al., 2001). Thus, patients with more advanced stages of breast cancer and melanoma were found to have higher serum/plasma TGF-β1 levels (Sheen-Chen et al., 2001; Krasagakis et al., 1998). TGF-β1 was shown to induce the expression of a variety of MMPs in mammary carcinoma, squamous cell carcinoma (SCC), melanoma and other types of cancer cells.

TGF-β1 enhances the expression of MMP2, MMP9, MMP13 and MMP14 in breast carcinoma cells concomitantly to stimulation of their migratory, invasive and metastatic abilities (Safina et al., 2008; Matsuura et al., 2010; Kuo et al., 2009; Wiercinska et al., 2011; Kim et al., 2007; Kwok et al., 2009). The signaling pathways involved in these cellular responses are highly variable depending on the particular MMP and/or the cellular model used. Thus, while the Smad pathway was proposed to mediate the TGF-β1 induction of MMP2 and MMP9 in a spheroid model of Ras-transformed normal breast epithelial MCF10A1 cells (Wiercinska et al., 2011), the p38 MAPK-mediated phosphorylation of ATF2, a member of the CREB/ATF family of transcription factors, has been involved in the TGF-β-induced transcriptional activation of MMP2, but not MMP9, in the same cells (Kim et al., 2007). Moreover, Smad3 may cooperate with S100A4 (metastatin-1), which belongs to the S100 family calcium-binding proteins, for TGF-β1-induced expression of MMP9 in a MCF10-derived breast cancer cell line (Matsuura et al., 2010). Other authors have found that the CBP/p300 co-activator Cited2 cooperates with Smad3 for TGF-β1-mediated upregulation of MMP9 and TGF-β-mediated cell invasion of the highly metastatic MDA-MB-231 cell line (Chou et al., 2006). In these cells, however, the induction of MMP9 by TGF-β1 was reported to critically depend on activation of the NFkB pathway by TAK1 (Safina et al., 2008), which mediates TGF-β signaling to p38 and JNK MAPK as mentioned above.

TGF-β1 also stimulates the expression of MMP13 in MDA-MB-231 cells, which depends on the activation of ATF3 transcription factor (Kwok et al 2009). Interestingly, in normal epithelial cells, ATF3 is a common target for TGF-β/Smad3 signals and p38 MAPK-mediated stress signals that is involved in growth inhibition (Kang et al., 2003).

A sophisticated mechanism for TGF-β1 stimulation of cell migration involving CD44 and the membrane metalloproteinase MMP14 has been proposed in cancer cells. TGF-β1 upregulates the expression of MMP14 in MDA-MB-435 cells, which in turn cleaves the extracellular domain of CD44, a process (CD44 deadhesion) that plays a decisive role in tumor cell migration (Kuo et al., 2009). There are controversial reports on whether MDA-MB-435 represents a melanoma cell line (Christgen and Lehmann, 2007) or a breast carcinoma cell line that has undergone an aberrant differentiation program or lineage infidelity (Montel et al., 2009). Elevated expression levels of MMP2 and TGF-β were found in tumor tissue and plasma in patients with metastatic melanoma with respect to healthy donors or patients with primary tumors, suggesting that induction of MMP2 by TGF-β in tumor cells is associated with melanoma progression (Malaponte et al., 2010). TGF-β may regulate the expression of MMP2 and MMP9 in melanoma cells by an indirect mechanism involving the induction of Gli2 and Gli1 transcription factors, the end point of the hedgehog signal transduction pathway. In melanoma cells, Gli expression in response to TGF-β is, however, independent of hedgehog signaling and mediated by the Smad pathway (Alexaki et al., 2010). Thus, autocrine TGF-β production by melanoma cells contribute directly to tumor cell aggressiveness in part by upregulating MMP2 and MMP9.

In oral SCCs, TGF-β1 promotes MMP-dependent cell scattering and collagen invasion. It increases the expression of MMP2 and MMP14, and enhances proMMP2 activation (Munshi et al., 2004). Efficient activation of proMMP2 by MMP14 on the cell surface requires the interaction of the inhibitor TIMP-2 with both MMP14 and proMMP2. Thus, TIMP-2 is unique because it acts both as an MMP inhibitor and an activator (Bourboulia and Stetler-Stevenson, 2010). TGF-β1 induces the concomitant activation of Erk1,2 and p38 MAPKs in these cells, and the control of MMP14 catalytic activity involves the reciprocal modulation of TIMP-2 expression by Erk1,2 and p38 MAPKs (Munshi et al., 2004). TGF-β1 has been shown to induce an EMT in transformed epidermal keratinocytes linked to upregulation of MMP9 expession and progression from well differentiated SCC to a highly invasive and metastatic spindle tumor phenotype (SpCC) (Caulín et al., 1995; Cui et al., 1996; Frontelo et al., 1998; Santibáñez et al., 2002). TGF-β1-induced SCC-SpCC transition and MMP9 production was Smad-independent, required the cooperation of Erk1,2 MAPK and Rac1 signaling activities, and was associated with enhanced expression of Snai1 (Snail) transciption factor (Santibáñez et al., 2002; Santibáñez et al., 2010). Likewise, TGF-β1 induction of MMP2 and MMP9 in oral SCC cells was mediated by upregulated expression of Snai1 and Snai2 (Slug) members of Snail family of transcription factors (Sun et al., 2008; Joseph et al., 2009; Qiao et al., 2010). TGF-β upregulation of Slug in these cells was dependent on Erk1,2 MAPK signaling activity and independent of the Smad and PI3K

pathways (Joseph et al., 2009). On the contrary, it has been reported that the Smad pathway regulates TGF-β1-induced MMP13, MMP1 and MMP9 expression in head and neck SCC cells (Leivonen et al., 2006; Sinpitaksakul et al., 2008). In addition, a number of laboratories have reported TGF-β induction of MMPs and/or TIMPs in other types of cancer cells, such as prostate, pancreatic, endometrial, hepatocarcinoma and chronic myeloid leukemia (Sehgal and Thomson, 1999; Binker et al., 2011; Van Themsche et al., 2007; Wang et al., 2010; Zhu et al., 2011).

Most of those studies were performed in vitro by treating cultured cells with TGF-β, and a question remains about the real significance of these results in vivo. However, these studies highlight that cancer cells may respond to TGF-β by producing MMPs that, in turn, promote tumor cell migration, invasion and metastasis. MMPs exert these effects not only by degrading components of the ECM but also by cleaving cell adhesion molecules, growth factor precursors, receptor tyrosine kinases and other proteinases (Egebald and Werb, 2002).

MMPs in the tumor microenvironment

Although cancer cells are able to produce and secrete MMPs, they are predominantly synthesized by stromal cells infiltrating the tumors (Egeblad and Werb, 2002; Kessenbrock et al., 2010). Cancer cells are thought to be primarily involved in the regulation of stromal MMP expression (Mook et al., 2004). The tumor microenvironment includes activated fibroblasts (the so called cancer-associated fibroblasts or CAFs), immune cells and blood vessels scattered throughout the ECM. Both compartments, tumor cells and the local tumor microenvironment, act synergistically to push malignant progression. Signals derived from the tumor compartment alter the initially non-transformed stroma thereby leading to a tumor reactive stroma that, in turn, respond sending out signals that further contribute to malignant progression (Figure 5). Cells within the reactive stroma communicate among themselves as well as with cancer cells. They do that directly through cell contact and indirectly through paracrine/exocrine signals (cytokines, chemokines) and proteinases. MMPs released by stromal cells, besides cleaving ECM components mediate a wide range of biological effects on the surrounding tissue. Thus, MMPs can modulate the bioavailability of factors that regulate cancer cell growth/survival and tumor angiogenesis/lymphangiogenesis. MMPs not only activate latent TGF-β (see above), but also mediate the release of other factors, such as the pro-angiogenic vascular endothelial growth factor (VEGF), the pro-apoptotic FAS ligand, and cleave and activate a diverse array of cytokines and chemokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-8, and monocyte chemoattractant proteins (MCPs), that have the effect of either potentiating or inhibiting inflammation (Egebald and Werb, 2002; Nghia et al., 2007; Kessenbrock et al., 2010).

TGF-β is a key factor regulating cell interactions within the tumor microenvironment (Bierie and Moses, 2006; Stover et al., 2007; Yang et al., 2010). Tumor-derived TGF-β is involved in the recruitment and activation of stromal

fibroblasts. CAFs produce cytokines, chemokines and MMPs that promote tumor cell proliferation, invasion and angiogenesis (Kalluri and Zeisberg, 2006). CAF-derived MMP1 and MMP3 affect breast cancer cell motility and invasion. We have already mentioned that MMP3 can directly cleave the extracellular domain of E-cadherin thereby promoting a cascade of events leading to EMT (Lochter et al., 1997). Recently, Giannoni and coworkers have reported that prostate PC3 cancer cells activate a MMP-dependent pro-inflammatory route in response to CAF contact. This route is crucial for EMT and metastatic dissemination of prostate cancer cells (Giannoni et al., 2010; Giannoni et al., 2011).

MMPs in inflammation

The concept that inflammation is a critical component of tumor progression derives from observations linking cancer development to sites of chronic inflammation. For example, colon carcinogenesis arises in individuals with chronic ulcerative colitis and Crohn's disease, and individuals with either hereditary or chronic pancreatitis can develop pancreatic carcinomas (Coussens and Werb, 2002). The emerging evidence suggests that chronic inflammation can transform a normal tissue into a neoplastic tissue. The continuous release of pro- and anti-inflammatory cytokines and ROS can activate a cascade of events, including DNA damage, enhanced proliferation, inhibition of apoptosis and angiogenesis, leading to cancer.

MMPs, which are secreted by inflammatory cells responding to cytokines (i.e., TGF-β) and chemokines present in the tumor microenvironment, are both effectors and regulators of inflammation (Nghia et al., 2007). MMPs mediate different events related to inflammation: i) degradation of the vascular basement membrane during transendothelial migration of leukocytes from blood vessels to the site of inflammation; ii) inactivation of inhibitors (serpins) of serine proteinases associated with tissue destruction and remodeling during inflammation; iii) activation of pro-inflammatory cytokines (TNF-α and IL-1β); iv) activation of anti-inflammatory cytokines (TGF-β); v) cleavage of chemokines (IL-8) to potentiate inflammation; and vi) cleavage of chemokines (MCP-3) to inhibit inflammation. The most important MMPs secreted by inflammatory cells in cancer seem to be MMP2 and MMP9, and they have pro-inflammatory and anti-inflammatory functions (Nghia et al., 2007). As mentioned above, MMP activity is also dependent on the stage of tumor development.

TGF- β has an important immunosuppressive role in tumors by inhibiting both the innate and the adaptive immune responses (Yang et al., 2010). MMPs are involved in the mechanism of escape immune surveillance of tumors not only by activating TGF-β, but also by means of disrupting cytokine signaling in T lymphocytes through degradation of interleukin receptors or by modulating natural killer cytotoxicity (Egeblad and Zerb, 2002). TGF-β also has a profound impact on the recruitment of myeloid cells into the tumor microenvironment. This is demonstrated by studies in which TGF-β signaling has been disrupted in the epithelial tumor compartment of genetically engineered mice by deleting TβRII. The deletion of *Tgfbr2* in mammary

epithelial cells results in chemokine-induced recruitment of a population of immature myeloid cells (Gr-1+CD11b+) that produce high levels of MMPs (particularly MMP2, MMP13, MMP14) and TGF-β (Yang et al., 2008). These cells are also called myeloid immune suppressor cells (MISCs) or myeloid derived suppressor cells (MDSCs) as they have a profound immune suppressive effect. MISC cells are also overproduced in cancer patients with a variety of tumors (Gabrilovich and Nagaraj, 2009). MMPs secreted by MISC cells are essential for tumor invasion, since inhibition of MMP activity abolishes MISC mediated tumor cell invasiveness (Yang et al., 2008). Moreover, they (in particular MMP9) contribute to tumor angiogenesis and vasculogenesis (Yang et al., 2004). Similarly to blocking the TGF-β pathway in the tumor compartment, the disruption of TGF-β signaling in stromal cells (i.e., fibroblasts and T lymphocytes), also promotes inflammatory cell infiltration and tumor progression (Yang et al., 2010). These results demonstrate that TGF-β1 acts as a potent anti-inflammatory agent what in principle should be beneficial for tumor suppression. However, excessive inflammation produced by a defective TFG-β response in inflammatory cells may favor tumor progression. Also, the strong immunosuppressive effects of TGF-β overcome the tumor-suppressive benefits of its anti-inflammatory function (Massagué, 2008).

TGF-β, MMPs and the tumor vasculature

The tumor vasculature develops from circulating endothelial progenitor cells derived from the bone marrow (vasculogenesis) and sprouting of preexisting capillaries (angiogenesis). TGF-β is crucial in regulating vascular development, as demonstrated by the fact that dysregulation of TGF-β signaling in vascular cells is associated with a number of vascular pathologies (ten Dijke and Arthur, 2007). TGF-β modulates the proliferation and migration of endothelial cells, and several studies have pointed out the importance of regulating the bioavailability of active TGF-β, since low and high concentration of the cytokine may have opposite effects associated with the activation and maturation phases of angiogenesis (Lebrin et al., 2004; Blanco et al., 2005). TGF-β has also long been implicated as a regulator of vascular integrity. Thus, paracrine TGF-β signaling from endothelial cells to neighbouring mesenchymal cells is necessary to promote smooth muscle cell (SMC) or pericyte differentiation to cover and "muscularize" blood vessels (ten Dijke and Arthur, 2007). In addition, in fibroblasts and vascular SMCs, TGF-β can induce ECM synthesis and promote contractility that increases the tension on ECM leading to enhanced interstitial fluid pressure. This represents an obstacle in the delivery of drugs in cancer therapy due to restricted capillary outflow (Dumont and Arteaga, 2003; Heldin et al., 2004).

We have already discussed above the interplay between TGF-β and MMPs by which MMPs activate latent TGF-β sequestered within the ECM, and TGF-β, in turn, upregulates MMP production in both cancer and stromal cells (Figure 5). Then, MMPs contribute to angiogenesis by many ways (Fingleton, 2006). They may simply facilitate angiogenesis by degrading the ECM to allow endothelial cells to invade the tumor

stroma. But, also, MMP-mediated proteolysis, as mentioned above, can release bound pro-angiogenic factors or generate cryptic matrix bioactive fragments, such as endostatin, angiostatin and tumstatin, that inhibit angiogenesis.

The main MMPs involved in tumor angiogenesis are MMP2, MMP9 and MMP14. MMP14 appears to be essential for the generation of active PDGF-B, which is critical for vascular SMC and pericyte "muscularization" of blood vessels (Lehti et al., 2005). MMP14 is also critical for regulation of vascular stability and permeability. Sounni and coworkers identified a post-translational pathway whereby type I collagen fibrils regulate perivascular MMP14 activity and TGF-β bioavailability, which, in turn, regulate vascular homeostasis by altering vessel stability and leakage (Sounni et al., 2010). Thus, pericellular type I collagen fibrils represents a sensor-type molecule that regulate vasodilation and extravasation of plasma proteins through MMP14 proteolytic activation of TGF-β. MMP14 is also involved in the activation of proMMP2, which is thought to facilitate endothelial cell migration and invasion by its association with αvβ3 integrin (Brooks et al., 1996; Silletti et al., 2001).

Nevertheless, the most important MMP related to the tumor vasculature appears to be MMP9 (Fingleton, 2006). MMP9 secreted by inflammatory cells regulates the bioavailability of the pro-angiogenic factor VEGF in different models of carcinogenesis (Bergers et al., 2000; Lee et al., 2005). Stromal MMP9 is also involved in vessel investment by pericytes, since nascent vessels in neuroblastoma tumors from MMP9-deficient mice were unable to maturate because of a failure in the recruitment of pericytes (Chantrain et al., 2004). In this neuroblastoma model, MMP9 was also involved in the recruitments of bone marrow-derived hematopoietic progenitor cells to the tumor microenvironment (Jodele et al., 2005). It also has been found that MMP9 plays a critical role in the mobilization of endothelial progenitor cells from the bone marrow, a process that depends on the generation of a soluble Kit-ligand (Heissig et al., 2002). MMP9 has also been implicated in the endothelial differentiation of bone marrow-derived Gr+CD11b+ immature myeloid cells that become CD31-positive and associate with the tumor vasculature (Yang et al., 2004). Moreover, Ahn and Brown, using a model of tumor transplantation in an irradiated normal tissue to prevent angiogenesis, found that tumors were unable to growth in MMP9-deficient mice. Nevertheless, tumor growth was restored by transplanting wild-type CD11b+ myeloid cells expressing MMP9 that promoted the development of immature blood vessels (Ahn and Brown, 2008). Therefore, these results strongly implicate MMP9 in vasculogenesis in addition to angiogenesis.

TGF-β, MMPs and the pre-metastatic niche

The ability of MMP9 to release soluble Kit-ligand to recruit endothelial progenitor cells as well as its role in releasing VEGF from the ECM to support angiogenesis have been associated with the involvement of MMP9 in the formation of the pre-metastatic niche; i.e., a receptive environment for the formation of metastasis in certain tissues that are distant from the primary tumor (Kessenbrock et al., 2010;

Peinado et al., 2011). Kaplan and coworkers demonstrated that bone marrow-derived hematopoietic progenitor cells expressing the VEGF receptor 1 (VEGFR1) and c-Kit precede the arrival of metastatic tumor cells (Kaplan et al., 2005). In this scenario, MMP9 expressed in the pre-metastatic lung by macrophages and endothelial cells promoted the invasion of tumor cells into the lung tissue by a mechanism that was dependent on VEGF-A secreted from the primary tumor (Hiratsuka et al., 2002). The primary tumor secrete growth factors, such as VEGF-A, TNF-α and TGF-β, that trigger the expression of S100 chemokines by the lung endothelium, which, in turn, attracts CD11b+ immature myeloid cells to the pre-metastatic milieu. These bone marrow-derived cells efficiently allow tumor cell migration to the lung (Hiratsuka et al., 2006).

Conclusions and perspectives

TGF-β and MMPs are mutually regulated in normal and cancer tissues. TGF-β overproduced in the tumor microenvironment is a potent inducer of MMP expression. MMPs, on the other hand, mediate the release and activation of latent TGF-β as well as other growth factors sequestered by the ECM that influence tumor development. Both TGF-β and MMPs contribute to tumor progression by promoting tumor cell growth and survival, facilitating migration/invasion, inducing EMT, stimulating angiogenesis, modulating the inflammatory response and favoring the formation of metastasis in certain niches distant from the primary tumor.

Initially it was thought that blocking MMP activity by small-molecule inhibitors of broad specificity would hamper tumor metastasis and angiogenesis. However, the effects of these inhibitors in clinical trials were disappointing due to the advanced stage of cancer disease of selected patients, which hampered any benefit in survival, and, also, because of the tumor suppressor function of some MMPs (Lopez-Otín and Matrisian, 2007; Kessenbrock et al., 2010). Novel approaches to target MMP for cancer treatment, such as the localized gene transfer delivery of TIMPs instead of systemic drug administration, are under examination. This has the advantage of bring about an elevated local concentration of the molecule avoiding side effects in other tissues and, also, to supply a sustained and prolonged production of the inhibitor at the tumor site (Gialeli et al, 2011). However, these strategies should take into account that some MMPs have biological functions other than proteolytic degradation of the ECM. Therefore, strategies aimed to develop site-directed inhibitors or antibodies to single MMPs; for example, MMP9 and MMP14 that have crucial roles in vasculogenesis/angiogenesis and in tumor cell migration/invasion, respectively, are promising therapeutic tools for the future (Cuniasse et al., 2005; Kessenbrock et al., 2010). These strategies could be combined with improved activity-based imaging probes specific for MMPs and minimal invasive imaging techniques in order to assess the efficacy of the compounds in vivo.

It has been proposed that a better approach may be to block MMP expression rather than MMP activity (Kessenbrock et al., 2010). At this respect, to target the TGF-β pathway in tumors may have the double benefit of blocking an important source of MMP production and to inhibit the wide array of pro-oncogenic actions of TGF-β. However, there are potential problems as well. First, in addition to TGF-β, there are other factors that stimulate MMP expression in tumors, such as the hepatocyte growth factor (HGF), stromal cell-derived factor-1 (SDF-1), VEGF, IL-6 and TNF-α. Second, inhibition of TGF-β signaling may have undesirable side effects, such as chronic inflammatory and autoimmune reactions, and may enhance the progression of premalignant lesions because of the anti-oncogenic effects of this cytokine (Massagué, 2008). A number of TGF-β inhibitors have been developed to date that have yield promising results in preclinical studies. These include antisense oligonucleotides, small molecules competitors of ligand-receptor interactions, anti-TGF-β receptor antibodies and inhibitors of TGF-β receptor kinases (Santibanez et al., 2011). Clinical trials are ongoing for many of them. It could be interesting to ascertain whether the potential favorable effects of these compounds in cancer treatment are mediated by changes in MMP expression and activity levels. To this end, a closer integration between preclinical and clinical studies would be necessary.

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Legends to Figures

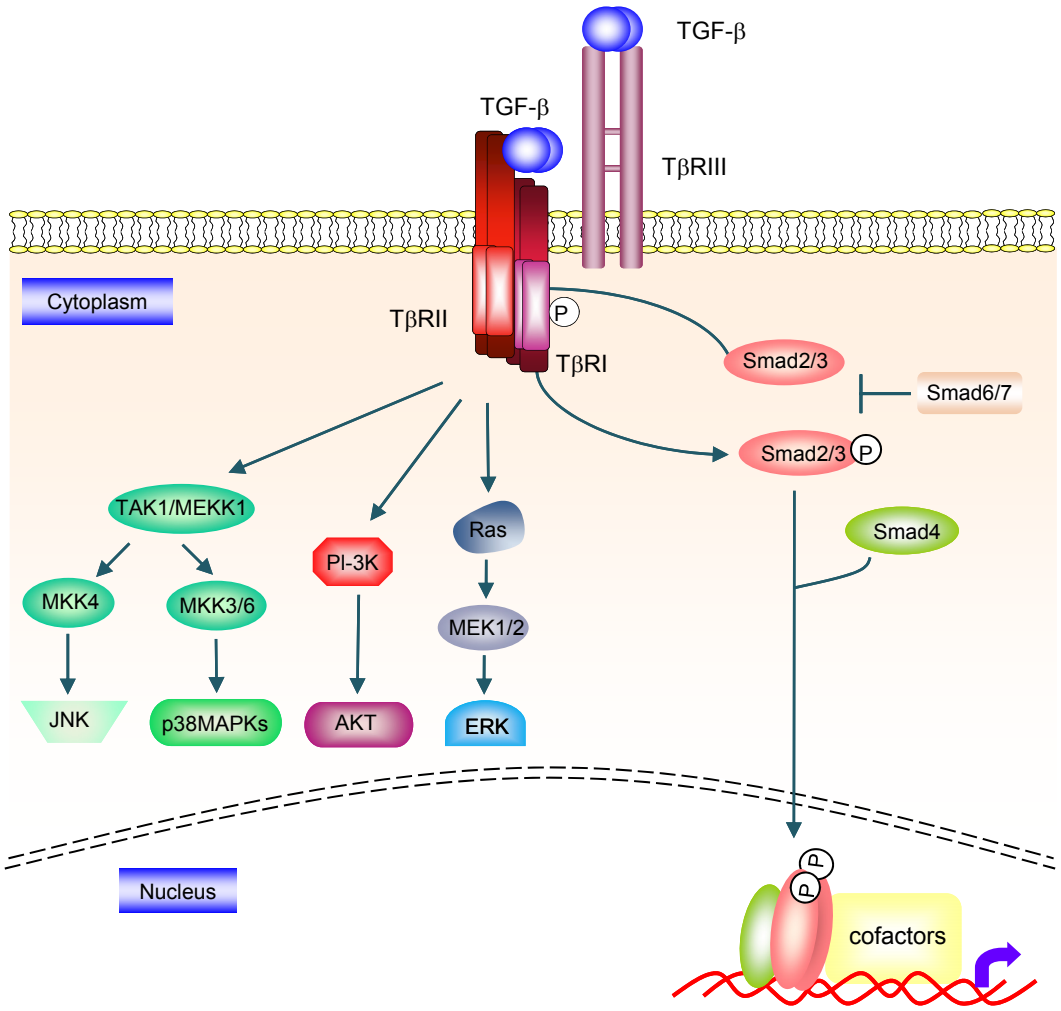
Figure 1. Schematic diagram of TGF-β Smad-dependent and –independent signaling pathways. TGF-β binds TβRII inducing the heterodimerization of TβRI and its activation by TβRII phosphorylation. The activated complex phosphorylates R-Smads (Smad2/3), which associate with Smad4 and translocate to the nucleus, where they interact with both DNA-binding cofactors and co-activators or co-repressors to modulate transcription of TGF-β target genes. The inhibitory Smad6/7 are under the control of the Smad pathway, which represents a negative feedback autoregulatory mechanism. TGF-β also triggers non Smad signaling pathways, such as Ras/MAPK, PI3K/AKT and TAK1/MEKK1, by different mechanisms.

Figure 2. Secretion of TGF-β occurs in a large latent complex. TGF-β is synthesized as a precursor molecule formed by the C-terminal mature growth factor covalently linked to its latency-associated protein (LAP). This complex is proteolytically cleaved to form a small latent complex (SLC), which often associate with a latent binding protein (LTBP) to be secreted as a large latent complex (LLC). The highly conserved structural domains of LTBPs are indicated. LTBPs bind to the ECM by their N-terminal and C-terminal binding sites. The flexible hinge region is sensitive to proteinase cleavage.

Figure 3. Latent TGF-β activation. The LLC crosslinked to the ECM is released after proteolytic cleavage. MMPs are recruited to the latent complex by binding to integrins that act as a docking site for both the latent complex and MMPs. This close proximity enables MMPs to cleave LAP and release active TGF-β permitting it to bind to its receptors.

Figure 4. Categories of MMP promoters based on the presence of cis-elements. AP-1, activator protein 1; AP-2, activator protein 2; CCAAT (C/EBP-β), enhancer binding protein-β; GC, SP-1 binding site; NFkB, nuclear factor-kB; PAE-3, polyoma enhancer A binding protein-3; TATA, TATA box; TIE, TGF-β inhibitory element.

Figure 5. TGF-β and MMP interplay at the tumor microenvironment. Although both tumor and stromal cells contribute to the secretion of latent TGF-β and MMPs into the tumor microenvironment, these proteins are mostly produced by cancer-associated fibroblasts (CAFs) and inflammatory cells. MMPs besides degrading and remodeling the extracellular matrix (ECM), activate latent TGF-β as wells as a variety of cytokines and chemokines that regulate many processes, including angiogenesis, inflammation as well as tumor cell survival, proliferation and migration/invasion. The diagram shows in a simplistic manner the reciprocal heterotypic signaling interactions involving TGF-β and MMPs.


TGF-β
TGF-β
TβRIII
Cytoplasm
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P
TβRI
Smad2/3
Smad6/7
Smad2/3 P
TAK1/MEKK1
MKK4
MKK3/6
JNK
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AKT
Ras
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ERK
Smad4
Nucleus
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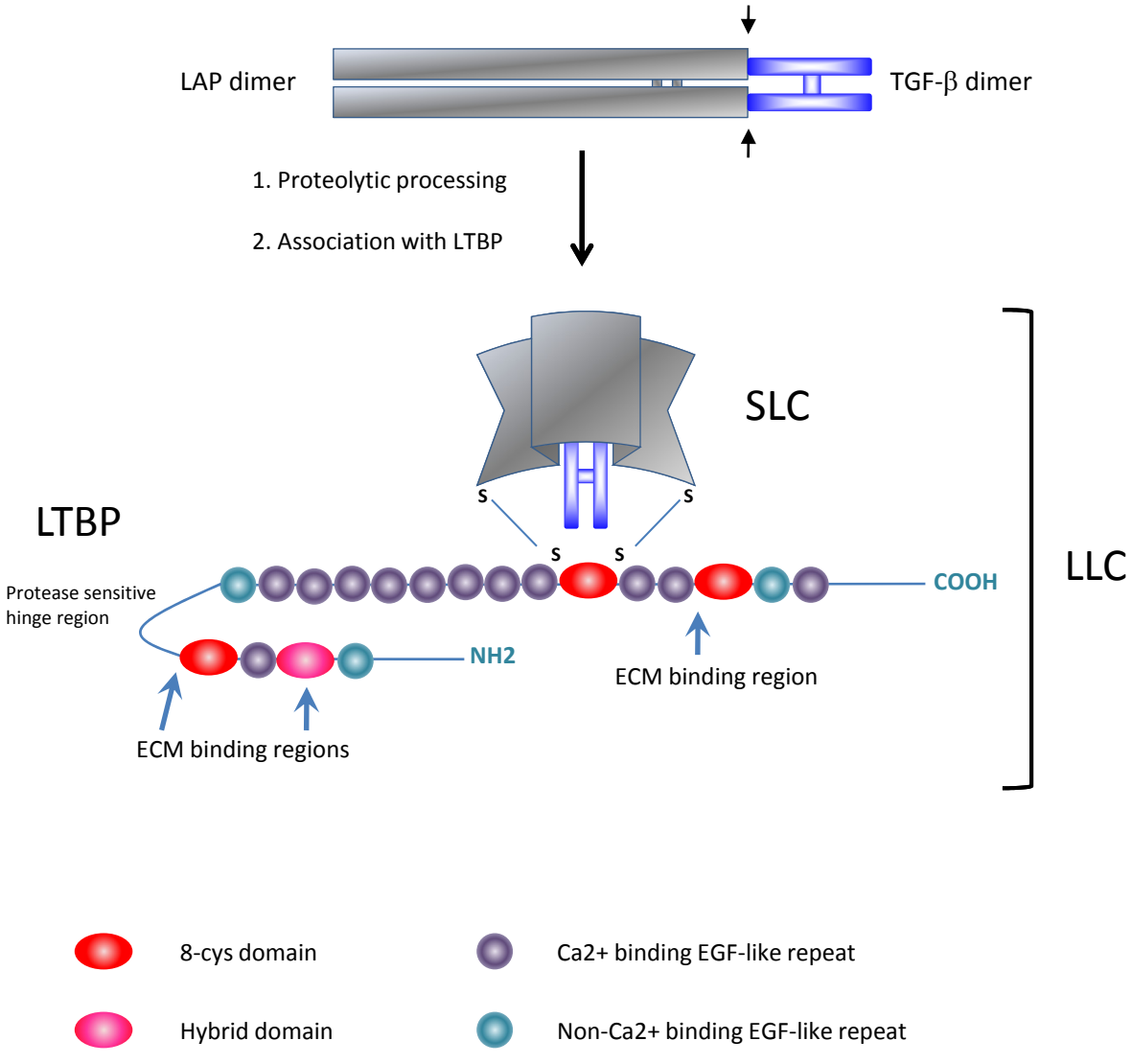
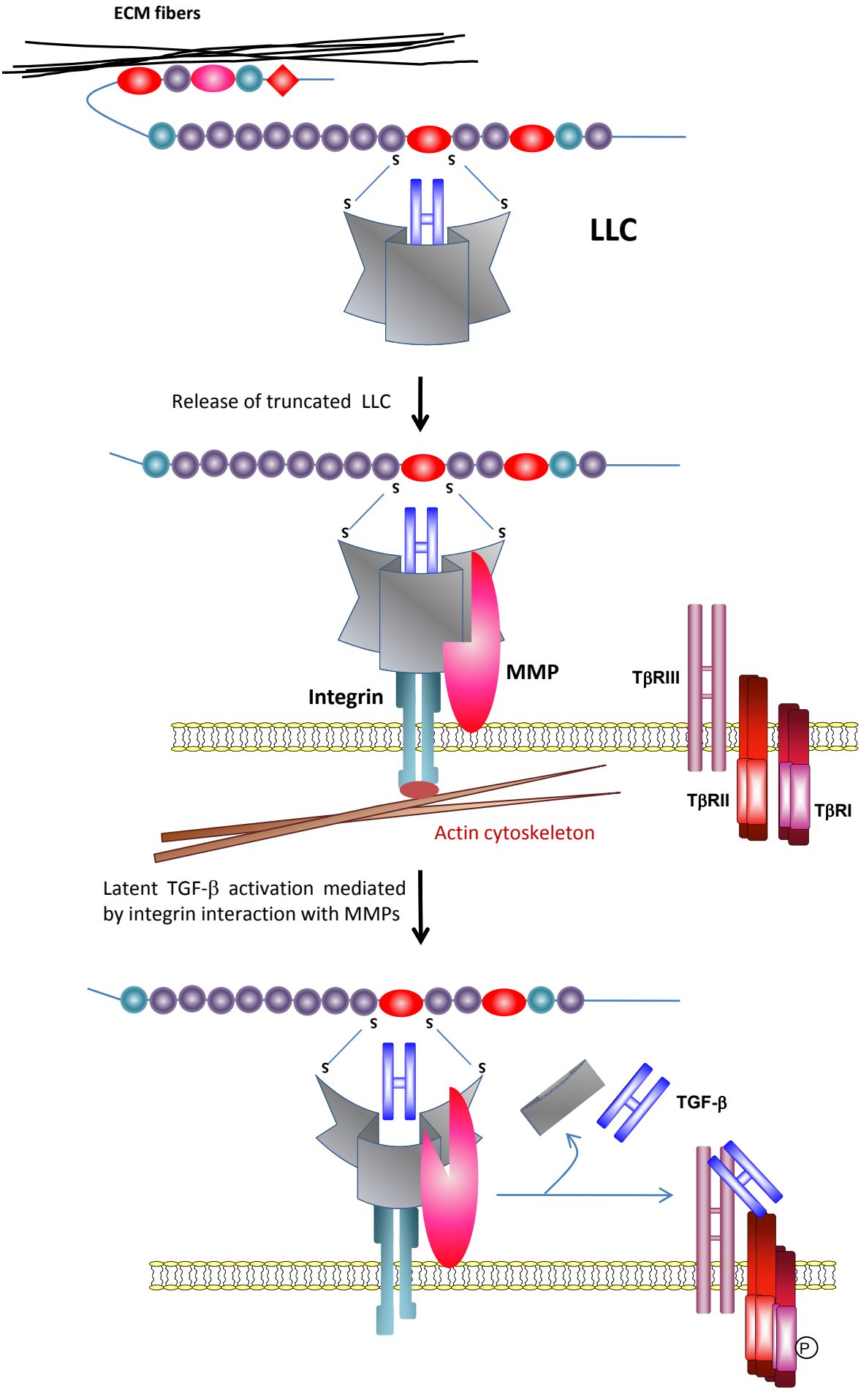

LAP dimer
TGF-β dimer
1. Proteolytic processing
2. Association with LTBP
SLC
LTBP
LLC
S
S
S
S
Protease sensitive
hinge region
COOH
NH2
ECM binding region
ECM binding regions
8-cys domain
Ca2+ binding EGF-like repeat
Hybrid domain
Non-Ca2+ binding EGF-like repeat


ECM fibers
S
S
S
S
LLC
Release of truncated LLC
S
S
S
S
MMP
Integrin
TβRIII
TβRII
TβRI
Actin cytoskeleton
Latent TGF-β activation mediated by integrin interaction with MMPs
S
S
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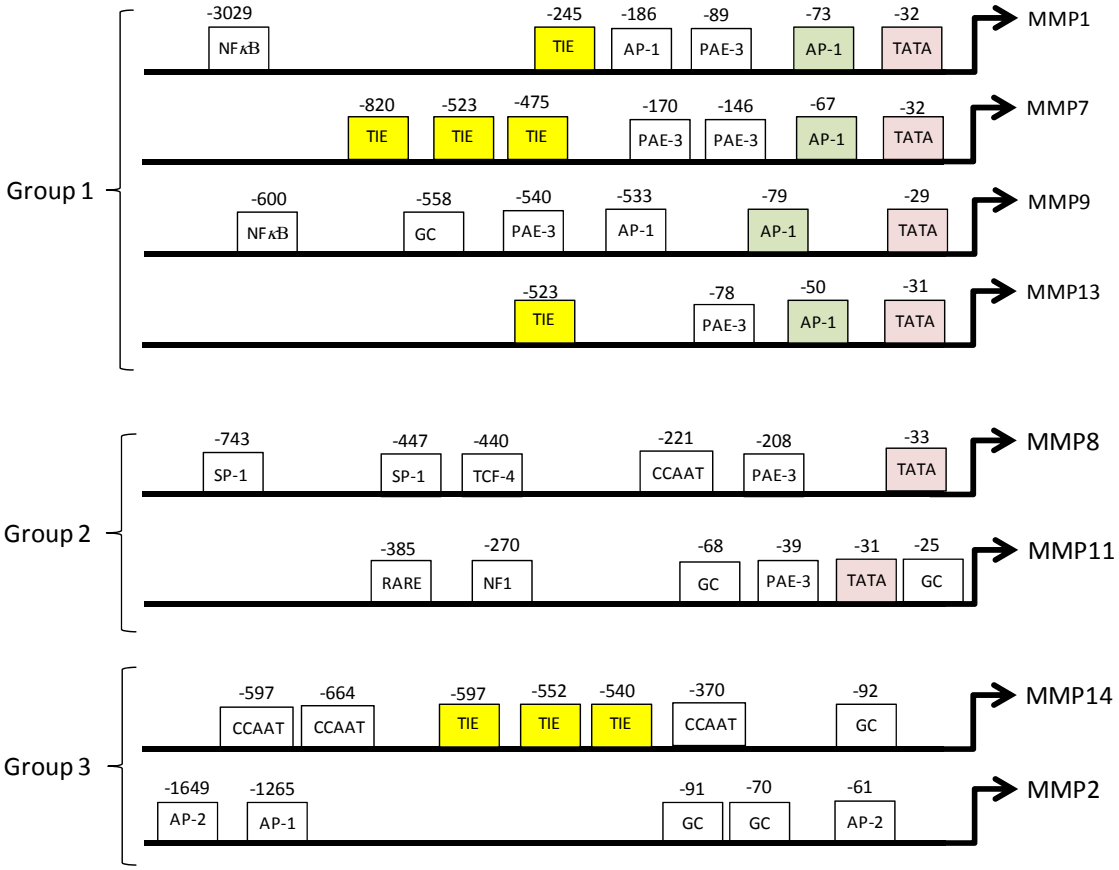
S
TGF-β
P


Group 1
-3029
NFκB
-245
TIE
-186
AP-1
-89
PAE-3
-73
AP-1
-32
TATA
MMP1
-820
TIE
-523
TIE
-475
TIE
-170
PAE-3
-146
PAE-3
-67
AP-1
-32
TATA
MMP7
-600
NFκB
-558
GC
-540
PAE-3
-533
AP-1
-79
AP-1
-29
TATA
MMP9
-523
TIE
-78
PAE-3
-50
AP-1
-31
TATA
MMP13
Group 2
-743
SP-1
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SP-1
-440
TCF-4
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CCAAT
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PAE-3
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MMP8
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RARE
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NF1
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GC
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PAE-3
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TATA
-25
GC
MMP11
Group 3
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CCAAT
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TIE
-552
TIE
-540
TIE
-370
CCAAT
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GC
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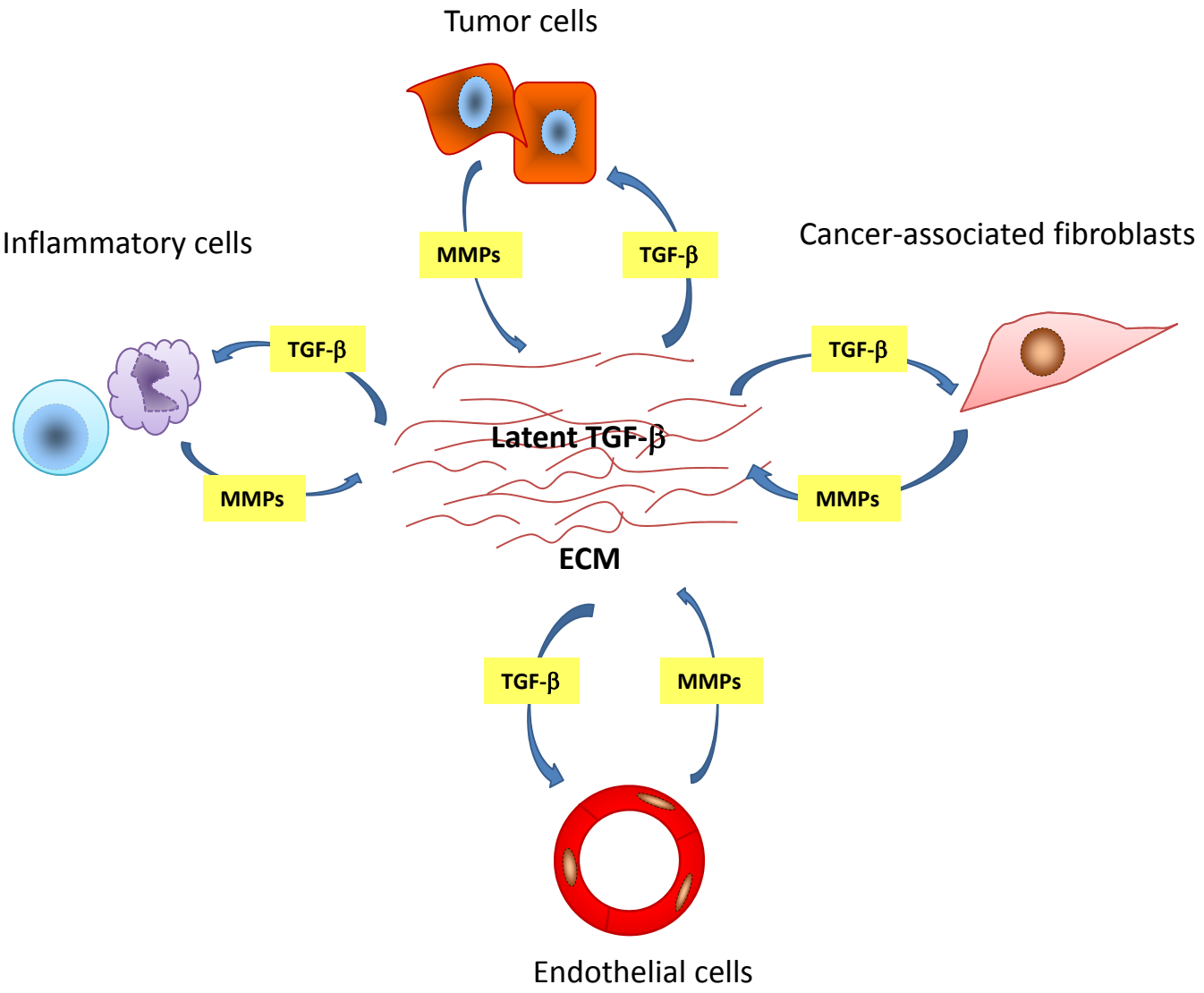
MMP2


Tumor cells
Inflammatory cells
Cancer-associated fibroblasts
MMPs
TGF-β
TGF-β
TGF-β
Latent TGF-β
MMPs
MMPs
ECM
TGF-β
MMPs
Endothelial cells