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Transforming Growth Factor-β, Matrix Metalloproteinases, and Urokinase-Type Plasminogen Activator Interaction in the Cancer Epithelial to Mesenchymal Transition

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Transforming growth factor-β (TGF-β) is a pleiotropic factor that acts as a tumor suppressor in the early stages, while it exerts tumor promoting activities in advanced stages of cancer development. One of the hallmarks of cancer progression is the capacity of cancer cells to migrate and invade surrounding tissues with subsequent metastasis to different organs. Matrix metalloproteinases (MMPs) together with urokinase-type plasminogen activator (uPA) and its receptor (uPAR), whose main original function described is the proteolytic degradation of the extracellular matrix, play key cellular roles in the enhancement of cell malignancy during cancer progression. TGF-β tightly regulates the expression of several MMPs and uPA/uPAR in cancer cells, which in return can participate in TGF-β activation, thus contributing to tumor malignancy. TGF-β is one of the master factors in the induction of cancer-associated epithelial to mesenchymal transition (EMT), and recently both MMPs and uPA/uPAR have also been shown to be implicated in the cancer-associated EMT process. In this review, we analyze the main molecular mechanisms underlying MMPs and uPA/uPAR regulation by TGF-β, as well as their mutual implication in the development of EMT in cancer cells. *Developmental Dynamics 247:382–395, 2018*. © 2017 Wiley Periodicals, Inc.

Key words: EMT; tumor progression; invasion; stroma; extracellular matrix proteinases; metastasis; TGF-beta; MMP; uPA

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Introduction

Transforming growth factor-β (TGF-β) plays key roles in many biological functions, such as embryonic stem cell self-renewal and differentiation, homeostasis of differentiated cells, suppression of the immune system, and promotion of cancer development (Massague, 2012). During the advance of malignancy, one of the main characteristics of a cancer cell is its enhanced capacity for migration, which allows invasion of surrounding tissues and metastasis to different organs (de Groot et al., 2017; Lambert et al., 2017).

Matrix metalloproteinases (MMPs) are a group of mainly extracellular matrix (ECM) proteolytic enzymes which enable cells to migrate and invade surrounding tissue (Kessenbrock et al., 2010). Due to their importance, many MMPs are tightly regulated at transcriptional level during normal development but are deregulated in cancer, when their activity and expression are related to the worsening in the development of cancer (Yan and Boyd, 2007). Both urokinase-type plasminogen activator (uPA) and its cell surface receptor uPAR have profound effects on the increased capacity of cancer cells to migrate and invade surrounding tissues (Duffy, 2002; Dellas and Loskutoff, 2005; Santibanez, 2013). Consistent with its role in cancer dissemination, the high level of uPA correlates with the adverse patient outcome (Seetoo et al., 2003; Harbeck et al., 2004). TGF-β regulates both MMP and uPA expression in cancer cells, while MMPs and uPA may activate the latent secreted TGF-β (Annes et al., 2003), thus producing a pernicious cycle that contributes to cancer progression. Importantly, TGF-β, MMPs, and uPA/uPAR induce epithelial to mesenchymal transition (EMT) in cancer cells. These cells can exploit the heterotypic reciprocal interactions established between TGF-β, MMPs, and uPA/uPAR to induce EMT and to strengthen cancer progression and metastasis. In this review, we aim to describe the molecular mechanisms involved in TGF-β, MMPs, and uPA interplay in cancer EMT, and the complex cascades that culminate in the clinical manifestation of metastasis.

The TGF-β

TGF-β was discovered as a potent inductor of growth in normal rat kidney fibroblast in the soft agar assay (Anzano et al., 1982).

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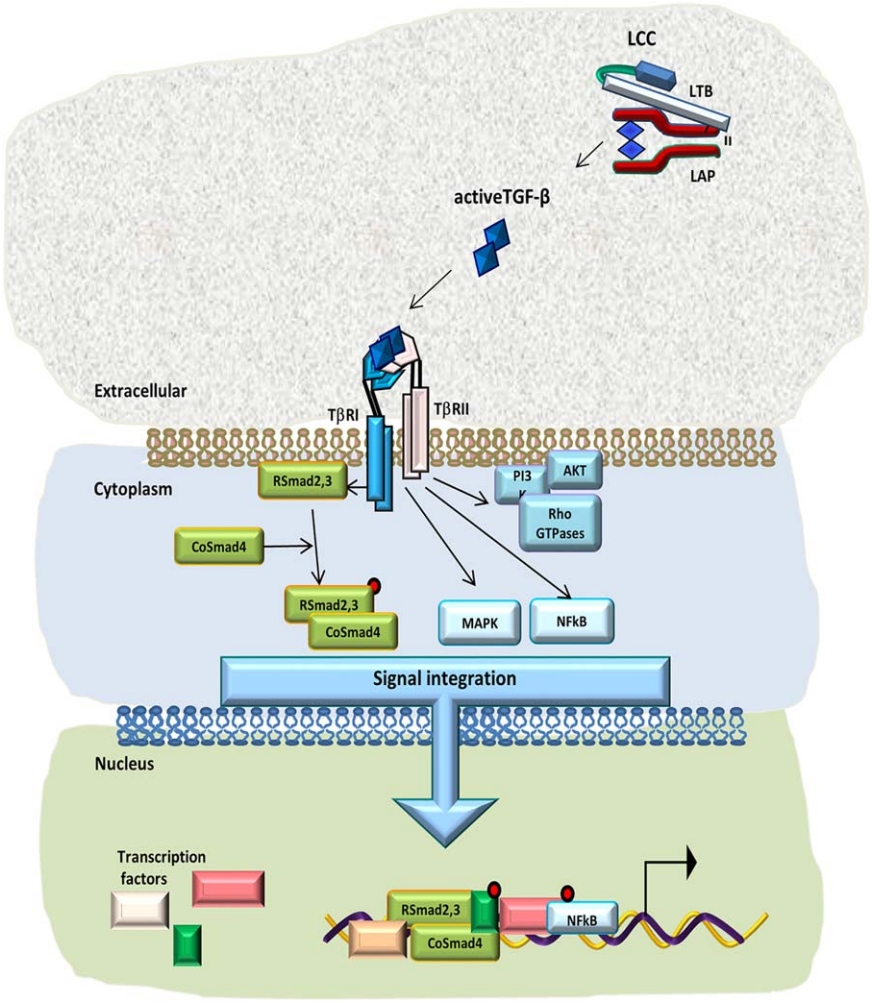

Fig. 1. TGF-β signaling. Activated TGF-β1 from the latent complex binds to its cell surface type II receptor (TβRII) inducing the activation of TGF-β type I receptor (TβRI). Then activated TβRI phosphorylates Smad2,3 which in turn promotes the Smads release from the inner face of the plasmatic membrane. Phosphorylated Smads interact with co-Smad4 forming a heteromeric complex to be translocated into the cell nucleus. Activated TGF-β-receptor complexes may also activate MAPK, NFκB, and PI3K signals, among others. These signals can activate or interact with other transcription factors through an integration pathway to regulate target genes (Attisano and Wrana, 2002; Shi and Massagué, 2003; Kubiczkova et al., 2012; Luo, 2017).

Following its discovery, TGF-β was found to be a strong inhibitor of epithelial cell proliferation (Roberts et al., 1985), thus suggesting, for the first time, its dual role in cell growth. Mammals express three genetically distinct isoforms of TGF-β (TGF-β1, -2, and -3) with high protein sequence homology. The corresponding human genes are located on chromosomes 19q13, 1q41, and 14q24, respectively (Meulmeester and Ten Dijke, 2011; Santibanez et al., 2011).

TGF-β or TGF-β1, belongs to the large family of structurally related regulatory proteins that comprises more than 40 proteins. The TGF-β superfamily is composed of activins, inhibins, bone morphogenetic proteins (BMP) and growth and differentiation factors, among others (Wakefield and Hill, 2013). TGF-βs have been involved in a plethora of distinct biological processes, which include cell growth, differentiation, and development, as well as tumorigenesis (Santibanez et al., 2011; Weiss and Attisano, 2013).

Bioactive TGF-β is a dimer that initiates intracellular signaling by binding to its cell-surface serine/threonine kinase receptors type I and II (TβRI and TβRII) (Fig. 1). Binding of TGF-β to TβRII leads to the phosphorylation of TβRI, thus activating its kinase domain (Attisano and Wrana, 2002). Then, the activated ligand/receptor complex phosphorylates and stimulates the release of the signaling mediators on the inner face of the cytoplasmic membrane, Smad2 and Smad3. The phosphorylated Smad2,3 then forms a heterotrimeric complex with the common Smad4. The activated Smad complex is further translocated into the nucleus where it binds and regulates the promoters of different target genes (Shi and Massagué, 2003). In the nucleus, the Smad complex can associate with different cofactors, such as CREB/ATF, RUNX, and activator protein 1 (AP1), further enabling Smads to target genes in a collaborative manner (Kubiczkova et al., 2012).

TGF-β signal transduction is negatively regulated by the expression of other Smad components, the inhibitory Smad-6 and Smad-7 (I-Smads). Of interest, TGF-β signaling induces I-Smads expression, which creates a negative feedback loop. In general, Smad7 antagonizes TGF-β by interacting with TβRI and

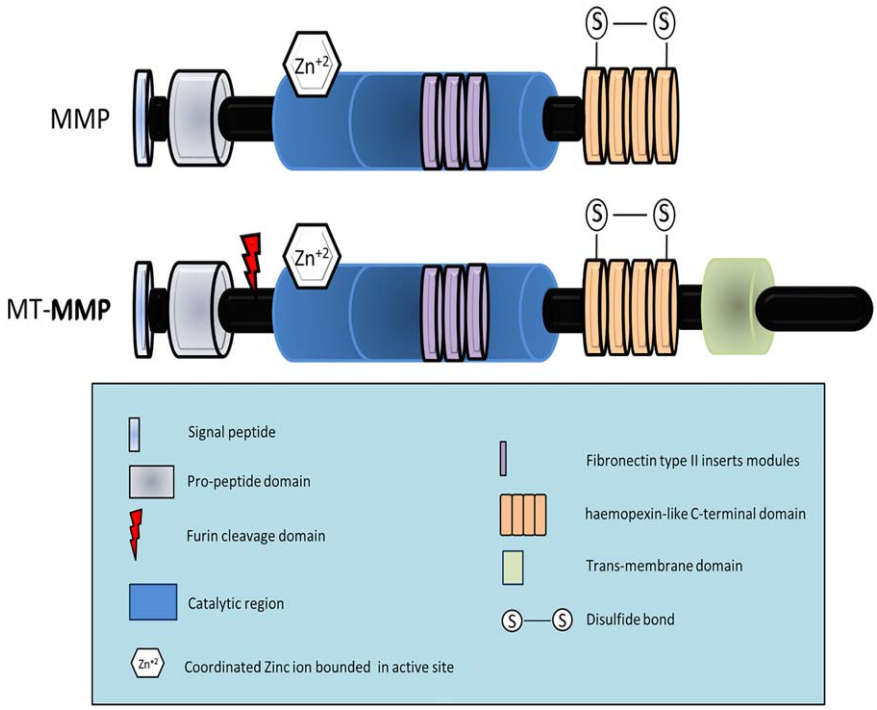

Fig. 2. Basic structure of MMPs and MT-MMPs. For further details refer to the text (Löffek et al., 2011; Radisky and Radisky, 2015).

leading to its degradation. Smad6 preferentially inhibits BMP signalling by disrupting the Smad1–Co-Smad interaction and forming an inactive Smad1–Smad6 complex (Moustakas and Heldin, 2016).

In addition, TGF-β/receptors/Smad proteins are subjected to posttranslational modifications, including phosphorylation/dephosphorylation, sumoylation, and ubiquitination which reversibly regulate their stability and availability. Moreover, ligand-receptor complexes are subjected to internalization and recycling by means of either lipid rafts/caveolae or clathrin-coated vesicles, which can modulate signaling as well as protein degradation in the proteasome. Thus, all these mechanisms finely regulate TGF-β signaling (Kang et al., 2009).

In addition to the Smad2,3 pathway, TGF-β can activate numerous other signal transduction pathways, commonly referred to as non-Smad pathways (Fig. 1), which increase the capacity of TGF-β to participate in a diversity of cellular functions. These signals include the mitogen-activated protein kinases (MAPKs): ERK1/2, JNK, and p38; phosphoinositide 3-kinase (PI3K); AKT1/2 and mTOR, known cell survival mediators; nuclear factor kappa B (NFκB); Cyclooxygenase-2 and prostaglandins; the small GTPase proteins: Ras and Rho family (Rho, Rac1, and Cdc42), among others (Luo, 2017).

The Role of TGF-β in Cancer

In the early steps of epithelial carcinogenesis, TGF-β operates as a tumor suppressor factor, due to its anti-proliferative and pro-apoptotic roles which counter the effects of local mitogenic stimulation in the injured or stressed epithelium (Padua and Massagué, 2009). Conversely, in advanced stages, TGF-β operates as tumor promoter, as cancer cells become refractory to its growth inhibitory effects by different mechanisms, including modifications in the components of TGF-β signaling, such as inactivating mutations in TβRII and Smad4, and other not fully elucidated alterations (Bierie and Moses, 2006; Padua and Massagué, 2009). This contradictory function of TGF-β in cancer is also reflected in human patients, since its level is positively related to a favorable prognosis in early stages of cancer, while in advance stages, the level of TGF-β within tumor stroma is associated with poor prognosis. This makes TGF-β a useful biomarker for cancer prognosis and also a predictor of cancer recurrence (Principe et al., 2014). Moreover, cancer cells produce and secrete high levels of TGF-β that can act as a potent immunosuppressor factor, and can influence the tumor-infiltrating immune cells, thus creating an environment of immune tolerance, which finally allows metastatic cancer cells to escape immune surveillance (Teicher, 2007; Park et al., 2009). Importantly, TGF-β produced by the tumor and local stromal cells contributes to the progression and metastatic potential of cancer through autocrine and paracrine signals (Teicher, 2007).

The MMPs

MMPs are grouped in a family of approximately 23 zinc-dependent endopeptidase enzymes that share a similar structure. MMPs are capable of degrading almost all protein components in the ECM and participate in tissue remodeling, cell migration and invasion, proliferation and angiogenesis in both normal and physiological and pathological conditions (Radisky and Radisky, 2015; Mittal et al., 2016). Based on their domain, structure and substrate preference, MMPs may be classified as follows: (i) collagenases, that include MMP1, MMP8 and MMP13; (ii) gelatinases, that include MMP2 and MMP9; (iii) membrane-type MMPs (MT-MMPs); (iv) stromelysins, that include MMP3, MMP10 and

MMP11; and (v) matrilysins, including MMP7, MMP26, and others (Löffek et al., 2011).

All MMPs are synthesized as inactive zymogens and, with the exception of the membrane-bound MT-MMPs, are secreted into the extracellular microenvironment. At least four well-conserved domains have been described in almost all MMPs: The N-terminal pro-domain of approximately 80 amino-acids, a hinge region, the catalytic domain, and finally a C-terminal hemopexin-like domain (Fig. 2). Pro-MMPs can be activated by the cleavage of the propeptide that enables the dissociation of the pro-domain from the catalytic site. This cleavage occurs intracellularly by furin or in the extracellular milieu by other MMPs or serine proteinases, and allows MMPs to be active and associate with substrates (Löffek et al., 2011). The proteolytic activity of MMPs is mainly regulated by tissue inhibitors of MMPs (TIMPs). There are four different TIMPs (TIMP1, -2, -3, and -4). TIMPs can inhibit all active MMPs, however, not with the same efficacy (Murphy, 2011; Arpino et al., 2015).

Of interest, some MMPs have a protective role in cancer. MMPs release 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 (Quintanilla et al., 2012). For instance, in breast and oral cancer patients, MMP8 expression is a good prognostic marker (López-Otín et al., 2009). Conversely, elevated MMP8 level, in cooperation with TGF-β, is a useful tool for poor prognosis prediction of hepatocellular carcinoma (HCC) patients (Qin et al., 2016). In addition to MMP8, other MMPs, such as MMP3 (stromelysin 1), MMP11 (stromelysin 3), and MMP19 play dual roles in cancer and exert pro-tumorigenic or protective roles, depending on cell context (López-Otín et al., 2009; Quintanilla et al., 2012).

Regulation of MMPs Expression by TGF-β

MMP promoters hold several *cis*-elements which in turn can either activate or repress MMP gene expression, and allow fine-tuned regulation of MMPs. MMP promoters possess several transactivator domains, including AP-1, PEA3, Sp-1, β-catenin/Tcf-4-lef-1, RARE, and NF-κB, among others (Yan and Boyd, 2007; Clark et al., 2008). According to the *cis*-element composition within their promoters, MMPs can be classified as follows: (1) TATA and AP-1 group contain TATA boxes at around −30 bp with AP-1 sites around −70 bp. This group is composed of MMP1, MMP3, MMP7, MMP9, MMP10, MMP12, MMP13, MMP19, and MMP26; (2) the TATA no AP-1 group contains a TATA box lacking a proximal AP-1 site, such as MMP8, MMP11, and MMP21; finally, (3) the no TATA no AP-1 group of promoters, which neither harbor TATA boxes nor proximal AP-1 site; this causes the transcription of these MMPs to start at multiple sites at the promoters, and includes MMP2, MMP14, and MMP28 (Yan and Boyd, 2007). Furthermore, the expression of MMPs in the third group is mainly regulated by the specificity protein 1 (SP1) transcription factors which makes expression of these MMPs partly constitutive, with low modulation by growth factors (Chakraborti et al., 2003). According to bioinformatic analysis, MMP20 has been included in the first group; MMP15 and MMP27 into the second group; and finally MMP16, MMP17, MMP23, MMP24, and MMP25 as members of the third group (Clark et al., 2008) (Table 1).

TABLE 1. MMPs by *cis*-Element Classification

1) TATA and AP-1	2) TATA no AP-1	3) no TATA no AP-1
MMP1, MMP3, MMP7, MMP9, MMP10, MMP12, MMP13, MMP19, MMP20 and MMP26	MMP8, MMP11,MMP15, MMP21 and MMP27	MMP2,MMP14, MMP16, MMP17, MMP23, MMP24, MMP25 and-MMP28

Classification of MMPs based on their basic *cis*-elements of promoter composition (Chakraborti et al., 2003; Yan and Boyd, 2007; Clark et al., 2008)

Two main regulatory binding sites within gene promoters for TGF-β have been discovered; TGF-β inhibitory element (TIE), represented by the consensus sequence 5′-GNNTTGGtGa-3′ that was first characterized in MMP3 promoter (Kerr et al., 1990; Zawel et al., 1998; Narayan et al., 2005); and the Smad binding elements (SBE) whose sequence contains 5′-GTCTG-3′ and its palindrome CAGAC within particular promoters, both are recognized by Smad3 through its N-terminal Mad homology 1 (MH1) domain (Zawel et al., 1998). Meanwhile, Smad4 recognizes the nonconsensus GC-rich motifs in gene promoters. Of interest, because Smad2 contains a 30-amino-acid insert in the MH1 domain its direct binding to gene promoters is disabled (Shi and Masague, 2003).

The MMP1, MMP3, MMP7, MMP9, MMP13, and MMP14 are harboring TIE sites. The analyses of the role of TIE in these MMPs revealed that TGF-β represses the expression of MMP1, MMP3, and MMP7 (Kerr et al, 1990; Gaire et al., 1994; White et al., 2000; Yuan and Varga, 2001; Kitamura et al., 2009; Tian et al., 2009). Meanwhile, the TIE sequence in MMP9, MMP13 and MMP14 promoters seems not to be implicated in TGF-β-regulated MMP expression (Lohi et al., 2000; Tardif et al., 2001; Ogawa et al., 2004).

The capacity of TGF-β to regulate MMPs expression can also be mediated through the interaction of Smads with other transcription factors, which enormously increase the possible interactions within the MMP promoters. For instance, TGF-β regulates MMP13 gene expression by means of the AP1 site and through interaction between Smad3 and JunB and Runx-2 (Uría et al., 1998; Selvamurugan et al., 2004).

Beyond the capacity for Smad activation, TGF-β, by inducing other intracellular signals, transactivates AP1, PEA3, NF-κB or SP1 transcription factors to regulate MMP expression (Fini et al., 1998; Yan and Boyd, 2007). For example, AP1/JunB complex mediates TGF-β repression of MMP1 in dermal fibroblasts. Conversely, in epidermal keratinocytes, TGF-β stimulates MMP1 expression by means of c-Jun/AP1 complexes (Mauviel et al., 1996). Furthermore, in the breast cancer MCF10A cells, TGF-β induces MMP2 promoter transactivation by means of activation of ATF2/AP1 and SP1 (Kim et al., 2007). In addition, TGF-β induces MMP10 expression through the myocyte enhancer factor (MEF)-2 and in Smad3-independent manner. MMP10 has a key function through its capacity to activate several pro-MMPs such

as MMP1, MMP7, MMP8, MMP9, and MMP13 (Ishikawa et al., 2010).

Beyond Smads, TGF-β activates several other intracellular signal transduction pathways, converging in the transactivity of different transcription factors necessary for the regulation of MMPs expression.

The MMP2 expression is induced by both Smads and TAK1-p38 MAPK-ATF2 pathway (Sano et al., 1999; Kim et al., 2007). Moreover, p38 signaling also contributes to the induction of MMP2 by TGF-β in breast epithelial cells and in pleural malignant mesothelioma (Zhong et al., 2006). On the other hand, in squamous cell carcinoma from the oral cavity, TGF-β enhances MMP2 and MMP14 expression independent of p38, but p38 participates in TGF-β1-mediated MMP2 activation (Munshi et al., 2004). Furthermore, Binker at al. (2011) reported that TGF-β stimulates MMP2 production and activation by means of Rac1/reactive oxygen species (ROS)/NFκB/IL-6 pathways in the human pancreatic cancer cell line SW1990.

In HCC cells, elevated expression of MMP8 was mediated by Rac1/PI3K/Akt pathway in response to TGF-β treatment (Qin et al., 2016). Of interest, MMP9 seems to be an important target of TGF-β in cancer cells, as it was shown in transformed mouse keratinocytes that TGF-β is able to stimulate this MMP through the activation of both ERK1,2 and Rac1/ROS/NFκB dependent mechanisms (Santibanez et al., 2002a; Tobar et al., 2010). Also, in breast cancer cells, ERK1,2 mediates TGF-β-induced MMPs, while in both breast and HCC cells, ERK1,2 and TAK1/NFκB pathways are essential for TGF-β-stimulated MMP9 expression (Arsura et al., 2003; Safina et al., 2007, 2008). Furthermore, TGF-β activates PI3K/Akt/NFκB signaling in myeloid leukaemia pathogenesis to increase MMP9 expression (Zhu et al., 2011), thus suggesting NFκB proteins as key pathways for the induction of MMP9 by TGF-β in cancer cells. Conversely, in nontransformed cells, NFκB participates in the inhibition of MMP9 expression by TGF-β (Ogawa et al., 2004). MMP9 and MMP13 are also induced by means of p38 in human keratinocytes and cutaneous SCC cells (Johansson et al., 2000). Furthermore, MMP14 collaborates in the capacity of TGF-β to activate JNK MAPK and expression of MMP9 in human keratinocytes (Soemun et al., 2008), thus suggesting a novel interplay between MMP14 and the TGF-β-dependent activation of JNK and expression of MMP9.

One of the hallmarks of cancer is dysregulation of the epigenome, i.e. tumor progression is associated with global epigenetic modifications that alter the expression of tumor-promoting as well as of tumor suppressing proteins (Dawson, 2017). These epigenetic alterations include DNA methylation, histone modifications and microRNAs (miRs) expression (Perri et al., 2017). TGF-β is able to induce global changes in DNA methylation in cancer cells by either inducing expression or activity of DNA methyltransferases (DNMT-1, -3A, and -3B) (Cardenas et al., 2014). Moreover, TGF-β also contributes to the regulation of biogenesis of miRs by either inhibiting or enhancing miRNA maturation (Hata and Davis, 2009; Guo et al., 2016).

Furthermore, MMPs are highly subjected to multiple epigenetic regulations in cancer cells; MMPs expression is susceptible to be regulated by DNA methylation, histone acetylation and by miRs (Chernov and Strongin, 2011; Li and Li, 2013 and references therein). Although TGF-β can severally induce cancer cell epigenome dysregulation, contribution of these alterations in the capacity of TGF-β to regulate MMPs expression is not well elucidated. Due to TGF-β functions in the epigenome and the susceptibility of MMPs to be regulated by epigenetic mechanisms, further investigations should be able to define the specific contribution of epigenetic mechanisms in the capacity of TGF-β to regulate MMPs expression in cancer cells.

The uPA System

The uPA system has a broad range of targets and controls many aspects of cell biology and physiology in cancer that are critical for the progress of initiated malignant cells in tumor progression (Smith and Marshall, 2010; Gonias and Hu, 2015). The uPA system plays key role in cell migration, invasion and tumorigenesis by regulating cell-associated proteolysis and both cell–cell and cell–ECM interactions (Blasi and Carmeliet, 2002; Danø et al., 2005). Four main members compose the uPA system: uPA, its cell surface receptor uPAR, the main substrate plasminogen (Plg), and the plasminogen activator inhibitor-1 (PAI1), also known as the serine protease inhibitor 1 (SERPINE1) (Mekkawy et al., 2014) (Fig. 3). uPA is synthesized as a 54 kDa latent single chain protein (sc-uPA), which comprises three different functional regions: the amino terminal domain that is harboring the EGF-like growth factor sequence (GFD) by which uPA binds to uPAR; one kringle domain implicated in protein interaction; and the carboxy-terminal catalytic domain containing the serine protease that converts the latent plasminogen into the active form of plasmin (Plm).

The first two domains comprise the amino-terminal fragment ATF (Poliakov et al., 2001; Blasi and Carmeliet, 2002; Smith and Marshall, 2010). Meanwhile, uPAR is produced as 50–60 kDa protein highly glycosylated and anchored to the plasma membrane by a glycosylphosphatidylinositol moiety. Mainly, uPAR possesses three structural domains, D1, D2, and D3, which generate a central cavity for the GFD binding (Barinka et al., 2006; Rijken and Lijnen, 2009) (Fig. 3). The uPAR shedding, by phosphatidylinositol-specific phospholipase D, releases the full protein moiety from cell surface membrane generating the soluble form of uPAR (suPAR). The suPAR maintains intact uPA binding ability and may act as an uPA-scavenger and it can interfere with the activity of the cell surface uPAR by means of integrin interaction (Thunø et al., 2009; Blasi and Sidenius, 2010).

Plg is a 92 kDa single-chain glycoprotein consisting of 791 amino acids. The uPA converts plasminogen to plasmin by cleaving a single Arg561–Val562 peptide bond (Rijken and Lijne, 2009). Plasminogen, similarly to uPA, can bind to specific cell surface receptors to form a highly localized proteolytic microdomain (Smith and Marshall, 2010). Binding of uPA to uPAR strongly enhances its capacity to generate plasmin. Furthermore, a positive feedback loop is produced between uPA and plasmin. Plasmin, by proteolytic cleavage at Lys158–Ile159 peptide bond in turn converts sc-uPA to active uPA (Rijken and Lijne, 2009; Smith and Marshall, 2010).

The 50 kDa glycoprotein PAI1 is the primary physiological inhibitor of uPA in vivo, and functions as a suicide inhibitor in stoichiometry 1:1 with uPA (Dellas and Loskutoff, 2005; Hagelgans et al., 2013). Also, when PAI1 is bound to uPA/uPAR complex at the cell surface, an interaction with the low-density lipoprotein related protein-1 (LPR1) is triggered to form a clathrin-dependent endocytosis complex. Then, uPAR and LPR1 are recycled to the plasma membrane, while uPA and PAI1 are subjected to lysosomal degradation (Czekay et al., 2001). Interestingly, the recycling of cell surface uPAR facilitates its

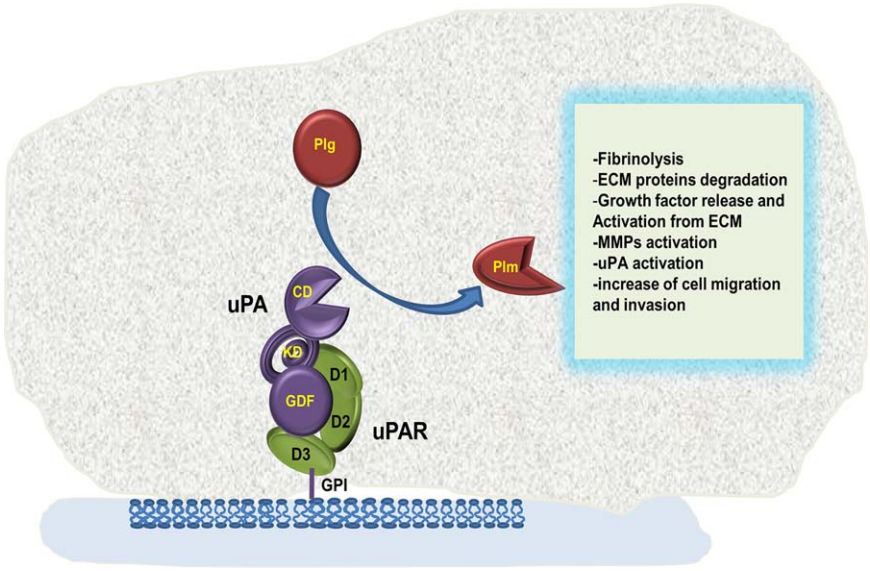

Fig. 3. Basics of the uPA system. The 54 kDa uPA, that comprises the EGF-like growth factor sequence/GDF and the kringle domain (KD) at the N-terminal region and the catalytic domain (CD) at the C-terminal of the protein, is secreted as latent single chain polypeptide. Then, uPA by means of its GDF can bind to its cell surface receptor uPAR composed of three structural domains (D1, D2, and D3) that generate a central cavity for the uPA binding. In turn, uPAR is anchored to the plasma membrane by a glycosyl phosphatidylinositol (GPI) moiety. Bound uPA is proteolytically activated and is able to proteolytically cleave the inactive Plg to its active form Plm. UPA/uPAR/Plm system contributes to the enhancement of cell malignancy by participating in several molecular and cellular events as indicated. For further details see the text (Mekkawy et al., 2014; Blasi and Carmeliet, 2002; Danø et al., 2005; Rijken and Lijnen, 2009; Smith and Marshall, 2010).

redistribution, as a ligand-free receptor, to the invasive front of cancer cells, which further enhances cell migration and invasion. Moreover, in addition to their implication in cancer cellular events and behavior, uPA, uPAR, and PAI1 are poor prognosis and shortened survival biomarkers in cancer (Smith and Marshall, 2010; Santibanez, 2013).

TGF-β Regulates uPA Expression

TGF-β is able to regulate uPA expression in several types of transformed cells, and many studies contributed in the elucidation of the underlying molecular mechanisms. In mouse transformed keratinocytes, TGF-β induces uPA expression through Ha-Ras/ERK1,2 MAPK (Santibanez et al., 2002a). This is also confirmed by the ectopic expression of Sprouty-related EVH1 domain-containing protein 2 (Spred2) that interacts and inhibits Ha-Ras, thereby inhibiting the capacity of TGF-β to stimulate uPA expression (Villar et al., 2010a). Of MAPKs, JNK was also shown to collaborate in the expression of uPA in transformed cells (Santibanez, 2006). Furthermore, Rac1/ROS/NFκB axis contributes to the elevated expression of uPA in cancer cells under TGF-β treatment (Tobar et al., 2010). In addition, Smad3 and its co-activator Sky interacting protein (SKIP) mediate TGF-β-induced uPA expression (Villar et al., 2010b; Kocic et al., 2013).

Contradictory studies have been published about the contribution of Smad4 on uPA expression in cancer cells. In colon cancer cells, overexpression of Smad4 reduces uPA expression, while it mediates TGF-β–stimulated uPA expression in breast cancer cells (Schwarte-Waldhoff et al., 1999; Shiou et al., 2006). Moreover, ectopic expression of Smad4 in spindle carcinoma cells does not affect uPA expression, whereas inactivation of Smad4 in transformed mouse keratinocytes increases uPA expression by leading hyperactivation of Ha-Ras (Iglesias et al., 2000; Santibanez et al., 2002b).

Although TGF-β induces uPAR expression (Nagamine et al., 2005), the underlying molecular mechanisms have not been elucidated. Nevertheless, similar to the regulation of uPA, the transcription factors that regulate uPAR expression are able to respond to TGF-β (Santibanez, 2013). Also, it has been recently determined that uPA/uPAR system participates in the expression of TGF-β1 in breast cancer cell line MDA-MB 468 (Hu et al., 2014). This is a very interesting point because it suggests positive feedback loop between uPA and TGF-β, which can, in a synergistic pathway, contribute to the enhancement of cancer cell malignancy.

The MMPs and uPA Activate TGF-β

TGF-β is ubiquitously expressed in all cells and tissues within the body, and is synthesized as a latent protein that exerts its cellular functions after being activated (Annes et al., 2003). TGF-β is synthesized and secreted as a 75 kDa precursor complex that comprises a signal peptide, latency-associated peptide (LAP) domain and the mature TGF-β protein. TGF-β precursor is intracellularly cleaved in the Golgi apparatus, by the furine-like convertase. This produces the small latent complex (SLC) between LAP and mature TGF-β that interact by noncovalent bonds. Usually the SLC binds to the latent TGF-β binding protein (LTBP) to form the large latent complex (LLC). The secreted LLC remains covalently associated to the ECM (Annes et al., 2003; Janssens et al., 2005). Furthermore, this complex needs to be activated and released from ECM to be bioavailable to exert its cellular effects (Fig. 4) (Annes et al., 2003).

Furthermore, TGF-β is synthesized in excess and the activation of TGF-β acts as a rate-limiting step in its bioavailability

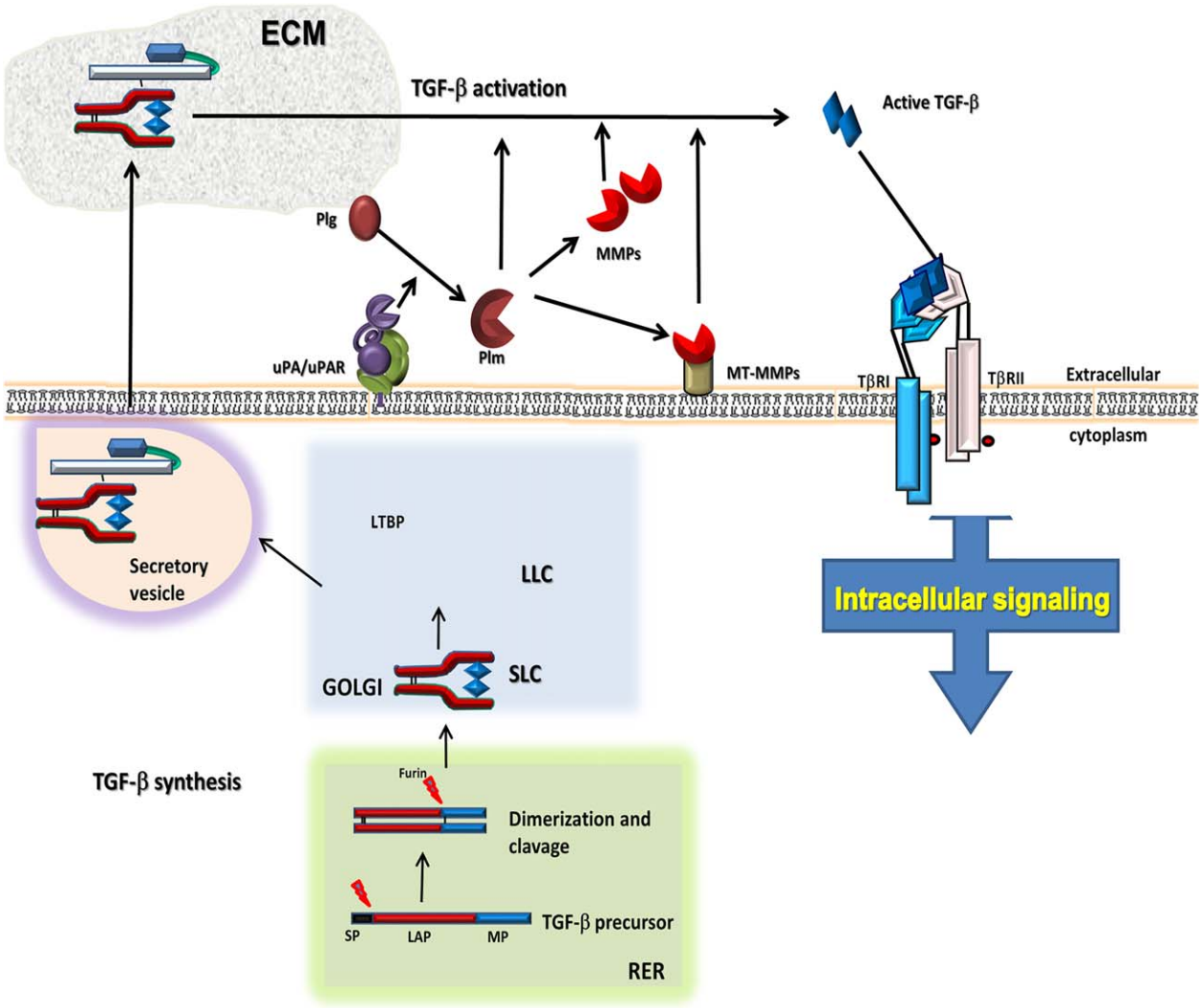

Fig. 4. TGF-β processing and uPA- and MMPs-dependent activation. TGF-β is synthesized as precursor protein. The signal peptide (SP) targeting TGF-β precursor protein to the secretory pathway, which is cleaved during the transit through the rough endoplasmic reticulum (RER); a homodimer of protein is formed and then is cleaved by furin convertase to produce the SLC formed by the mature TGF-β noncovalently bound to the LAP; Furthermore, SLC is covalently bound to the LTBP producing the LLC; finally, LLC is secreted and stored in the ECM for subsequent activation. When uPA is bound to its receptor (uPAR), it activates Plg to the active form Plm, Plm can promote the activation of latent TGF-β by proteolytic cleavage within the N-terminal region of the LAP. Similarly, membrane-bound MMPs or soluble MMPs can degrade ECM and/or activate the latent TGF-β by proteolytic cleavage within the N-terminal region of the LAP. In addition, pre-MMPs can be activated directly by plasmin. Activated TGF-β, by binding to its cell surface receptors TβRI-TβRII, triggers the activation of intracellular signal transduction to exert its cellular effects (Lyons et al., 1990; Janssens et al., 2005; Annes et al., 2003; Jenkins, 2008; Santibanez, 2013).

(Jenkins, 2008). Importantly, activation of the latent TGF-β is a key biological checkpoint and it is a highly regulated process to ensure the precise levels of active TGF-β (Jenkins, 2008). Moreover, latent TGF-β does not bind to receptors and needs to be released from the complexes with LAP and LTBP. The release of TGF-β from the latent complex can be performed by both proteolytic and nonproteolytic mechanisms, such as heat, local acidification, reactive oxygen species (ROS), thrombospondin-1 (TSP1), integrins, and proteases (Hyytiäinen et al., 2004; Rifkin, 2005; Jobling et al., 2006). Activation of TGF-β is involved in the proteolytic cleavage of LAP, and TGF-β can be activated by MMPs (Annes et al., 2003; Jinkins, 2008). Soluble or cell-surface MMP9 bound to CD44, MMP2, MMP13 and MMP14 activate TGF-β by cleaving the LTBP complex, solubilizing ECM-bound TGF-β and increasing its bioavailability (Fig. 4) (Dallas et al., 2002; Tatti et al., 2008).

The uPA/plasmin can activate TGF-β in tumor cells in vitro. Plasmin proteolytically cleaves within the N-terminal region of the LAP disrupting the noncovalent bonds resulting in TGF-β release (Fig. 4) (Lyons et al., 1990). Also, the conversion from the latent form of TGF-β to the active TGF-β is inhibited by using both anti-uPA neutralizing antibodies or by preventing uPA binding to uPAR (Odekon et al., 1994). Nevertheless, it is not clear if the TGF-β activation by uPA/plasmin operates in vivo, because *Plg*-null mice show normal embryonic development and do not display significant changes such as severe immune dysregulation and massive inflammatory responses observed in *tgfb*-null mice (Bugge et al., 1995; Böttinger et al., 1997). However, TGF-β can

be activated by several mechanisms and a redundancy can occur during mice development, whereas ECM-TGF-β releasing and activation by uPA/plasmin within tumor stroma may be an important step in the enhancement of cancer malignancy (Santibanez, 2013).

The EMT

The discovery that, through the EMT, cancer cells acquire the capacity of self-renewing stem-like cells opens the promise to fully understand and resolve the metastatic process, a major problem in cancer biology. EMT is a key step in providing the stationary tumor cells with the capability to migrate and invade surrounding tissues, a process that is critical for cancer cell dissemination and metastasis (Yeung and Yang, 2017). This is a multi-step process characterized by strong remodeling of the epithelial cytoarchitecture and changes in the functional capacity of cancer cells (Diepenbruck and Christofori, 2016). In the course of EMT epithelial cells lose their differentiated phenotype, switching their marker expression toward mesenchymal cell type. EMT was originally described as an integral program displayed during the morphogenesis in embryonic development, now defined as type 1 EMT; later it was described in pathogenesis including wound healing and fibrosis processes defined as type 2 EMT; and finally in cancer progression and metastasis as type 3 EMT (Thiery, 2003; Thiery et al., 2009; Kalluri and Weinberg, 2009; Yeung and Yang, 2017).

Early phenotypic changes of EMT include loss of cell–cell contact due to the downregulation of the junction protein complexes that included E-cadherin, claudin-1, and ZO-1. Moreover, the down-regulation of E-cadherin, important for epithelial homeostasis, leads to the decrease of desmosomal proteins such as plakoglobin, desmogleins, and desmoplakins (Thiery et al., 2009; Moreno-Bueno et al., 2009). Moreover, during the display of EMT program epithelial cells lose their apical/basal polarity and intercellular adhesions and express a characteristic set of mesenchymal genes and phenotype, their cytoskeleton is subjected to profound rearrangement, and the expression of cytokeratins is down-regulated while the expression of mesenchymal vimentin network is upregulated. These events work together with cells motile behavior, which is in part induced by the increased expression of ECM-degrading enzymes, such as serine-proteinases (e.g. uPA) and MMPs, to finally increase tumor cell invasive and metastatic phenotype (Moustakas and Heldin 2009; Juárez and Guise, 2011; Heldin et al., 2012; Macara and McCaffrey, 2013).

The Role of TGF-β in EMT

Currently, TGF-β receives substantial attention as a master regulator of EMT, as it participates in all types of EMT. TGF-β interacts with several other oncogenic signal transduction pathways to stimulate and sustain the mesenchymal phenotype of metastatic cells (Derynck et al., 2014).

The Smad3/Smad4 complex participates in the induction of EMT (Deckers et al., 2006; Roberts et al., 2006; Kocic et al., 2013; Heldin et al., 2012). Smad2 was in turn shown to have a paradoxical role in EMT, because Smad2 ablation stimulates EMT in mouse skin carcinogenesis, while, on the other hand, mediates the EMT in carcinoma cells (Oft et al., 2002; Hoot et al., 2008).

Furthermore, non-Smad signaling elicited by TGF-β contributes to cancer EMT, including Ras and Rho GTPases; MAPKs; Wnts and NFκB (Santibanez, 2006; Xu et al., 2009; Tobar et al., 2010; Santibanez et al., 2010). Through the convergence between Smads and non-Smad signaling, TGF-β is able to regulate the activation of several EMT-related transcription factors, including SNAI1 and SNAI2 (also named as Snail and Slug) or Twist (Heldin et al., 2012; Diepenbruck and Christofori, 2016). For instance, SNAI1 and 2 participate in EMT by repressing E-cadherin promoter transactivation and by stimulating the expression of mesenchymal markers such as vimentin and α smooth muscle actin (SMA) (Sánchez-Tilló et al., 2012; Yu et al., 2013; Lamouille et al., 2014). Moreover, by up-regulating the expression of pro-inflammatory interleukins IL-1, -6, and -8, SNAI1 creates an inflammatory microenvironment that supports the acquisition of cancer EMT (Cano et al., 2000; Juarez et al., 2006; Lyons et al., 2008; Juárez and Guise, 2011). Importantly, one vital feature during TGF-β-induced EMT is the acquisition of migratory and invasive properties of cancer cells. The up-regulation of serine proteinases such as uPA and MMPs allows cells to degrade ECM proteins and provides tumor cells with the capacity to invade surrounding tissues and colonize distant organs (Quintanilla et al., 2012; Santibanez, 2013; Obenauf and Massagué, 2015).

The TGF-β and MMP Interaction in EMT

Novel evidence indicates that, beyond their role in the facilitation of cancer cell invasion and ECM degradation, MMPs can stimulate EMT. MMPs have been shown to induce EMT in several types of epithelial cells, including kidney, ovary, lung, pancreas, and prostate (Radisky and Radisky, 2015). Three different mechanisms have been postulated to explain the role of MMPs in the induction of the EMT program: (i) elevated levels of MMPs directly induce EMT; (ii) during the EMT advance , cancer cells produce MMPs that further enhance EMT and facilitate cell invasion and metastasis; (iii) the conversion of cancer cells and other cells within tumor stroma to activated mesenchymal/stromal-like cells by EMT, drives additional cancer cell progression by means of MMPs production by these activated cells within the cancer (Orlichenko and Radisky, 2008; Radisky and Radisky, 2010).

One of the first evidence implicating MMPs in the induction of EMT comes from studies of MMP3 (Lochter et al. 1997). MMP3 directly degrades E-cadherin in mammary epithelial cells, which leads cells to EMT. MMP3 treatment of the mammary epithelial SCp2 cell line provokes the loss of cell–cell interaction, which parallels to cytokeratins down-regulation and vimentin upregulation. The display of EMT was accompanied with in vivo production of highly invasive tumors with mesenchymal cell phenotype in the tumor edge. On the contrary, bitransgenic mice that co-express both MMP3 and TIMP1 do not develop tumors, demonstrating that active MMP3 is necessary for the display of EMT in mammary neoplasias (Sternlicht et al., 1999). These effects of MMP3 are mediated by inducing the expression of Rac1b concomitantly with the increase in intracellular levels of ROS (Radisky and Radisky, 2015). Although MMP2 seems to be necessary and sufficient for the induction of EMT, in melanoma cells the treatment with the purified and activated MMP2 does not modulate the EMT (Liu et al., 2012). Recently, and conversely to the protective role of MMP8 in cancer (López-Otín et al., 2009), a positive mutual interplay between TGF-β and MMP8 in the induction of EMT in HCC cells has been reported (Qin et al., 2016).

MMP9 induces EMT by cleaving the E-cadherin ectodomain, which induces the dissociation of β-catenin from cell–cell adhesion complexes. Furthermore, the disruption of E-cadherin-β-catenin complexes by either MMP9 or TGF-β1 increases the expression of SNAI2 that strongly represses E-cadherin expression at the transcriptional level (Symowicz et al., 2007; Zheng et al., 2009; Yu et al., 2013). Moreover, MMP9 strongly and sustainably stimulate cancer cell transition into a mesenchymal phenotype (Lin et al., 2011). The MMP7 also mediates the E-cadherin ectodomain shedding, leading to tracheal epithelial cell scattering and migration (McGuire et al., 2003). Moreover, MMP14 overexpression also provokes a cleavage of E-cadherin in breast cancer cells (Rozanov et al., 2004), oral squamous cell carcinoma SCC9 (Yang et al., 2013) and in nasopharyngeal carcinoma cells (Zhao et al., 2015). Furthermore, in prostate cancer cells, the EMT induced by MMP14 overexpression shows activation of the extracellular latent TGF-β, which in turn stimulates the secretion of Wnt5a. This induction was accompanied with cell scattering and acquisition of migratory mesenchymal-like phenotype, whereas these changes were disabled by either MMP14 activity inhibition or targeting of the hemopexin domain of MMP14 (Nguyen et al., 2016). Conversely, the expression of MMP14 did not correlate with EMT-like changes in ovarian cancer patients (Vos et al., 2016).

One interesting and indirect mechanism has been described for the induction of EMT in MCF-7 cancer cell line by insulin-like growth factor-1 (IGF-1) (Walsh and Damjanovski, 2011). IGF-1, by a mechanism dependent of PI3K and MAPK signals, induces activation of MMP9 that stimulates EMT by means of TGF-β activation (Walsh and Damjanovski, 2011). Also, MMP9 mediates EGF-induced EMT in head and neck squamous carcinoma SCC10A cell line by E-cadherin shedding, and cell migration and invasion as well (Zuo et al., 2011). These are good examples staging the sophisticated and complex interaction between MMPs and different cytokines/growth factors that participate in driving EMT and tumor progression (Quintanilla et al., 2012).

In addition, a positive feedback loop can operate between MMPs and TGF-β during the promotion of EMT program in tumor progression. The signal peptide-CUB-EGF-like domain containing protein 3 (SCUBE3), which is a secreted glycoprotein up-regulated in lung cancer, acts as an endogenous ligand for TβRII and MMPs. In fact, MMP2 and MMP9 can cleave SCUBE3, thus producing an N-terminal EGF-like repeat and a C-terminal CUB domain fragment. In turn, these CUB fragments activate TGF-β signaling, stimulate MMP2 and MMP9 expression and promote EMT concomitantly with the enhancement of migration and invasion of cancer cells (Wu et al., 2011). MMP15, also named as MT2-MMP, has been involved in the induction of EMT: the overexpression of MMP15 in HCT116 human colorectal and A549 lung cancer cell lines leads to EMT by intracellular cleavage of E-cadherin and ZO-1 (Liu et al., 2016).

Furthermore, the overexpression of MMP28 (Epilysin), one of the last MMP members discovered, induces irreversible EMT in A549 lung adenocarcinoma cells. MMP28 proteolytically activates the latent TGF-β and increases bioavailable TGF-β, which in turn stimulates MMP9 and MMP14 expression and the EMT program (Illman et al., 2006).

Finally, these data indicate that TGF-β potently regulates MMPs expression and EMT in tumor cells. As shown in many cell types, the interaction between TGF-β and MMPs to maintain the mesenchymal cell features during cancer EMT is of high importance (Fig. 5).

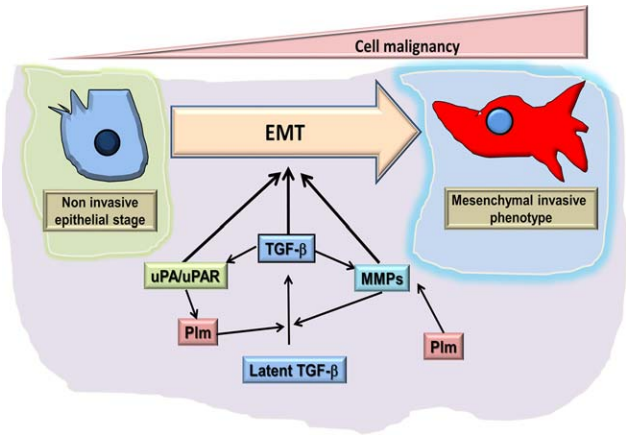

Fig. 5. TGF-β, MMPs, and uPA interplay in cancer EMT. TGF-β, MMPs, and uPA/uPAR are involved in the induction of EMT, with a mutual interplay. TGF-β stimulates the expression of MMPs which through either the activation of latent TGF-β or direct interaction with cancer cells may contribute to the induction of cancer EMT. Similarly, TGF-β stimulates uPA/uPAR expression which increases the amount of active Plm. Plm, in turn, may participate directly in TGF-β activation or indirectly by means of activation of MMPs. Meanwhile, uPA/uPAR expression participates in EMT induction by mediating TGF-β effect, as well as may by direct contribution to the induction of the epithelial conversion to the mesenchymal phenotype, thus strengthening cancer cell malignancy (Orlichenko and Radisky, 2008; Radisky and Radisky, 2010; Heldin et al., 2012; Santibanez, 2013; Krstic and Santibanez, 2014).

The TGF-β and uPA Interplay in EMT

Although the most studied function of uPA system is its proteolytic ability, it is now widely acknowledged that this system participates in several other activities supporting cancer invasion and metastasis. It has recently been suggested that uPA/uPAR complex can stimulate EMT to promote cancer progression. UPA/uPAR induces cancer cell EMT by means of several intracellular signaling pathways, such as Ras-ERK1,2 MAPK, Rac1, and PI3K-AKT (Lester et al., 2007; Jo et al., 2009a). Also, uPAR plays a key role in EMT program by inducing the expression of the Forkhead Box M1 transcription factor (Huang et al., 2014). Moreover, uPAR plays an important role in hypoxia-induced EMT, where uPAR expression is increased, and the silencing of uPA or uPAR strongly diminishes EMT (Gupta et al., 2011). Moreover, the down-regulation of uPA by shRNA, in the EMT-associated uPAR overexpression in breast cancer cells, is sufficient to inhibit this phenomenon, indicating that without adequate endogenous uPA level uPAR is not enough to induce EMT. In addition, uPAR overexpression enhances uPA expression in a positive feedback manner and probably by stimulating ERK1,2 signaling (Jo et al., 2009b). Furthermore, uPAR can induce cancer stem cell properties concomitantly with EMT in breast cancer cell lines (Jo et al., 2010). In addition, an interesting connection between MMP-9 and uPA has been reported, because their simultaneous knockdown by siRNA in breast cancer cells greatly inhibited EMT as is observed by the increase in the expression of E–cadherin, and in the reduction of vimentin and SNAI1 expression in MDA-MB-231 cells (Moirangthem et al., 2016).

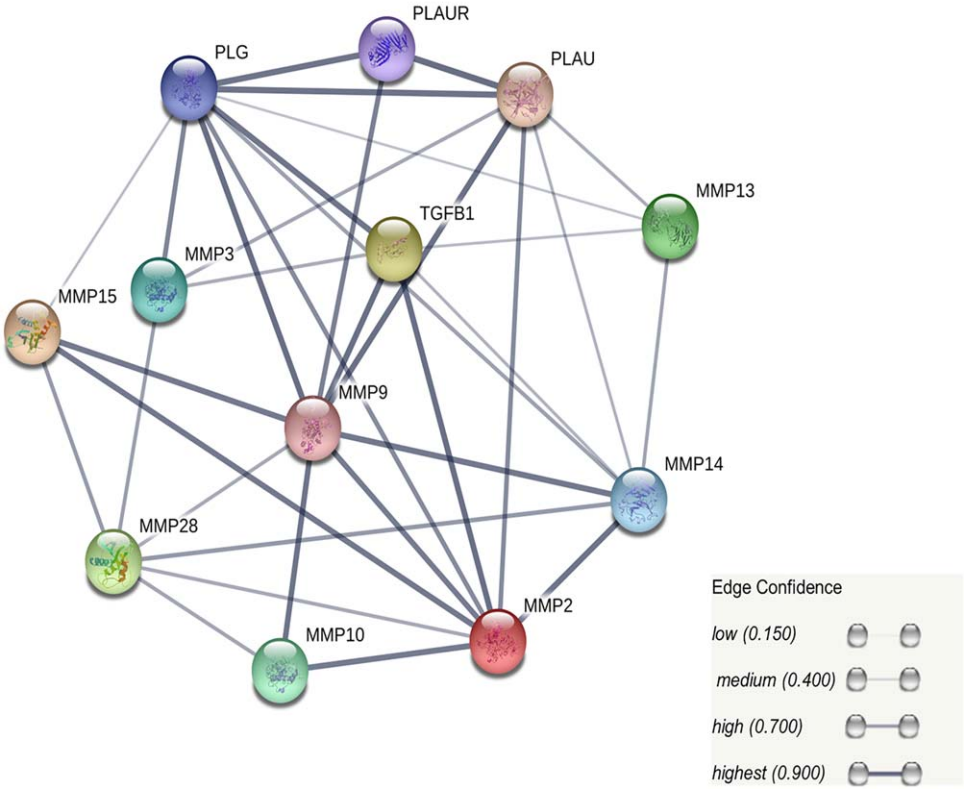

Fig. 6. Protein–protein interaction network of TGF-β, uPA, and MMPs. The STRING search program was used to determine the network interaction of TGF-b, uPA system, and the indicated MMPs. Line thickness indicates the strength of data support (http://string-db.org).

As aforementioned, TGF-β is a potent stimulator of the expression of uPA and its binding sites on the cell surface during tumor progression in the model of mouse skin carcinogenesis (Santibanez et al., 1999). The increased uPA expression level occurs by the same intracellular signal transduction associated to TGF-β1-induced EMT (Santibanez, 2006; Tobar et al., 2010; Villar et al., 2010a; Kocic et al., 2013), however, it is not well known whether uPA and/or uPAR may play a direct role in TGF-β−induced EMT and vice versa. Recently, Laurenzana et al. (2015) demonstrated that uPA mediates TGF-β-induced EMT, because the down-regulation of uPAR, by antisense oligonucleotide, reduces the capacity of this factor present in mesenchymal stem/stromal cells conditioned medium, to stimulate the transition to a mesenchymal phenotype. Nevertheless, it is clear that both TGF-β and uPA/uPAR collaborate in the induction of cancer-associated EMT (Fig. 5), and it is important to elucidate the molecular mechanisms underlying the TGF-β and uPA/uPAR interaction in tumor progression.

Concluding Remarks

Increasing evidence support the important role of the TGF-β, MMPs and uPA/uPAR system in tumor progression and metastasis. Due to their importance in tumorigenesis, combinatory targets for cancer chemotherapy may be developed. In this review, we described the TGF-β, MMPs uPA/uPAR pathway interplay and the amplification loop operated between them that enhances cancer progression and metastasis. Moreover, TGF-β, MMPs, and uPA/uPAR contribute to cancer EMT induction, which enhances tumor cells migration and invasion, and at the same time may increase the population of cancer associated fibroblasts (Glaire et al., 2012). Within the tumor, all three types of proteins dynamically interact to facilitate cancer cell migration and invasion by producing a positive stroma/microenvironment-driven cancer progression (Fig. 6).

Although collaboration between uPA and MMP9 has been demonstrated (Moirangthem et al., 2016), it is necessary to clearly define the interaction between other MMPs, such as MMP2 and MMP14, and the uPA/uPAR system in cancer EMT. Nevertheless, the simultaneous regulation of TGF-β, MMPs and uPA/uPAR could be used to control the positive tumor microenvironment and cancer cells–stromal cells interaction. The key future challenge is to develop highly specific MMP and uPA/uPAR inhibitors for both activity and/or expression usable in cancer treatment. Nonetheless, elucidating the complexity of TGF-β, MMPs and uPA/uPAR protein interactions in cancer is critical for understanding their participation in the initiation, progression, and tumor metastasis, and could eventually reveal potential combinations with current oncotherapies. We hope future clinical trials using combined therapies targeting simultaneously TGF-β, MMPs, and uPA/uPAR could increase the success of cancer treatment.

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References

Annes JP, Munger JS, Rifkin DB. 2003. Making sense of latent TGFβ activation. J Cell Sci 116:217–224.

Anzano MA, Roberts AB, Meyers CA, Komoriya A, Lamb LC, Smith JM, Sporn MB. 1982. Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells. Cancer Res 42:4776–4778.

Arpino V, Brock M, Gill SE. 2015. The role of TIMPs in regulation of extracellular matrix proteolysis. Matrix Biol 44-46:247–254.

Arsura M, Panta GR, Bilyeu JD, Cavin LG, Sovak MA, Oliver AA, Factor V, Heuchel R, Mercurio F, Thorgeirsson SS, Sonenshein GE. 2003. Transient activation of NF-kappaB through a TAK1/IKK kinase pathway by TGF-beta1 inhibits AP-1/SMAD signaling and apoptosis: implications in liver tumor formation. Oncogene 22: 412–425.

Attisano L, Wrana JL. 2002. Signal transduction by the TGF-beta superfamily. Science 296:1646–1647.

Barinka C, Parry G, Callahan J, Shaw DE, Kuo A, Bdeir K, Cines DB, Mazar A, Lubkowski J. 2006. Structural basis of interaction between urokinase-type plasminogen activator and its receptor. J Mol Biol 63:482–495.

Bierie B, Moses HL. 2006. TGF-b and cancer. Cytokine Growth Factor Rev 17:29–40.

Binker MG, Binker-Cosen AA, Gaisano HY, de Cosen RH, Cosen-Binker LI. 2011. TGF-b1 increases invasiveness of SW1990 cells through Rac1/ROS/NF-kB/IL-6/MMP-2. Biochem Biophys Res Commun 405:140–145.

Blasi F, Carmeliet P. 2002. uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 3:932–943.

Blasi F, Sidenius N. 2010. The urokinase receptor: focused cell surface proteolysis, cell adhesion and signaling. FEBS Lett 584: 1923–1930.

Böttinger EP, Letterio JJ, Roberts AB. 1997. Biology of TGF-beta in knockout and transgenic mouse models. Kidney Int 51:1355–1360.

Bugge TH, Flick MJ, Daugherty CC, Degen JL. 1995. Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. Genes Dev 9:794–807.

Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA. 2000. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-Cadherin expression. Nat Cell Biol 2:76–83.

Cardenas H, Vieth E, Lee J, Segar M, Liu Y, Nephew KP, Matei D. 2014. TGF-β induces global changes in DNA methylation during the epithelial-to-mesenchymal transition in ovarian cancer cells. Epigenetics 9:1461–1472.

Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. 2003. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 253:269–285.

Chernov AV, Strongin AY. 2011. Epigenetic regulation of matrix metalloproteinases and their collagen substrates in cancer. Biomol Concepts 2:135–147.

Clark IM, Swingler TE, Sampieri CL, Edwards DR. 2008. The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell Biol 40:1362–1378.

Czekay RP, Kuemmel TA, Orlando RA, Farquhar MG. 2001. Direct binding of occupied urokinase receptor (uPAR) to LDL receptor-related protein is required for endocytosis of uPAR and regulation of cell surface urokinase activity. Mol Biol Cell 12:1467–1479.

Dallas SL, Rosser JL, Mundy GR, Bonewald LF. 2002. Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. J Biol Chem 277:21352–21360.

Danø K, Behrendt N, Høyer-Hansen G, Johnsen M, Lund LR, Ploug M, Rømer J. 2005. Plasminogen activation and cancer. Thromb Haemost 93:676–681.

Dawson MA. 2017. The cancer epigenome: concepts, challenges, and therapeutic opportunities. Science 355:1147–1152.

Deckers M, van Dinther M, Buijs J, Que I, Lowik C, van der Pluijm G, ten Dijke P. 2006. The tumor suppressor Smad4 is required for transforming growth factor β-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. Cancer Res 66:2202–2209.

de Groot AE, Roy S, Brown JS, Pienta KJ, Amend SR. 2017. Revisiting seed and soil: examining the primary tumor and cancer cell foraging in metastasis. Mol Cancer Res 15:361–370.

Dellas C, Loskutoff DJ. 2005. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. Thromb Haemost 93:631–640.

Derynck R, Muthusamy BP, Saeteurn KY. 2014. Signaling pathway cooperation in TGF-β-induced epithelial-mesenchymal transition. Curr Opin Cell Biol 31:56–66.

Diepenbruck M, Christofori G. 2016. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr Opin Cell Biol 43:7–13.

Duffy MJ. 2002. Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers. Biochem Soc Trans 30:207–210.

Fini ME, Cook JR, Mohan R, Brinckerhoff CE. 1998. Regulation of matrix metalloproteinase gene expression. In: Parks WC, Mecham RP, editors. Matrix metalloproteinases. Amsterdam: Elsevier Inc. p 299–359.

Gaire M, Magbanua Z, McDonnell S, McNeil L, Lovett DH, Matrisian LM. 1994. Structure and expression of the human gene for the matrix metalloproteinase matrilysin. J Biol Chem 269:2032–2040.

Glaire MA, El-Omar EM, Wang TC, Worthley DL. 2012. The mesenchyme in malignancy: a partner in the initiation, progression and dissemination of cancer. Pharmacol Ther 136:131–141.

Gonias SL, Hu J. 2015. Urokinase receptor and resistance to targeted anticancer agents. Front Pharmacol 6:154.

Guo L, Zhang Y, Zhang L, Huang F, Li J, Wang S. 2016. MicroRNAs, TGF-β signaling, and the inflammatory microenvironment in cancer. Tumour Biol 37:115–125.

Gupta R, Chetty C, Bhoopathi P, Lakka S, Mohanam S, Rao JS, Dinh DE. 2011. Downregulation of uPA/uPAR inhibits intermittent hypoxia-induced epithelial-mesenchymal transition (EMT) in DAOY and D283 medulloblastoma cells. Int J Oncol 38:733–744.

Hagelgans A, Menschikowski M, Fuessel S, Nacke B, Arneth BM, Wirth MP, Siegert G. 2013. Deregulated expression of urokinase and its inhibitor type 1 in prostate cancer cells: role of epigenetic mechanisms. Exp Mol Pathol 94:458–465.

Harbeck N, Kates RE, Schmitt M, Gauger K, Kiechle M, Janicke F, Thomassen C, Look MP, Foekens JA. 2004. Urokinase-type plasminogen activator and its inhibitor type 1 predict disease outcome and therapy response in primary breast cancer. Clin Breast Cancer 5:348–352.

Hata A, Davis BN. 2009. Control of microRNA biogenesis by TGFbeta signaling pathway--a novel role of Smads in the nucleus. Cytokine Growth Factor Rev 20:517–521.

Heldin CH, Vanlandewijck M, Moustakas A. 2012. Regulation of EMT by TGFβ in cancer. FEBS Lett 586:1959–1970.

Hoot KE, Lighthall J, Han G, Lu SL, Li A, Ju W, Kulesz-Martin M, Bottinger E, Wang XJ. 2008. Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. J Clin Invest 118:2722–2732.

Hu J, Jo M, Eastman BM, Gilder AS, Bui JD, Gonias SL. 2014. uPAR induces expression of transforming growth factor β and interleukin-4 in cancer cells to promote tumor-permissive conditioning of macrophages. Am J Pathol 184:3384–3393.

Huang C, Xie D, Cui J, Li Q, Gao Y, Xie K. 2014. FOXM1c promotes pancreatic cancer epithelial-to-mesenchymal transition and metastasis via upregulation of expression of the urokinase plasminogen activator system. Clin Cancer Res 20:1477–1488.

Hyytiäinen M, Penttinen C, Keski-Oja J. 2004. Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. Crit Rev Clin Lab Sci 41:233–264.

Iglesias M, Frontelo P, Gamallo C, Quintanilla M. 2000. Blockade of Smad4 in transformed keratinocytes containing a Ras oncogene leads to hyperactivation of the Ras-dependent Erk signalling pathway associated with progression to undifferentiated carcinomas. Oncogene 19:4134–4145.

Illman SA, Lehti K, Keski-Oja J, Lohi J. 2006. Epilysin (MMP-28) induces TGF-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. J Cell Sci 119:3856–3865.
Ishikawa F, Miyoshi H, Nose K, Shibanuma M. 2010. Transcriptional induction of MMP-10 by TGF-beta, mediated by activation of MEF2A and downregulation of class IIa HDACs. Oncogene 29: 909–919.
Janssens K, ten Dijke P, Janssens S, Van Hul W. 2005. Transforming growth factor-beta1 to the bone. Endocr Rev 26:743–774.
Jenkins G. 2008. The role of proteases in transforming growth factor-beta activation. Int J Biochem Cell Biol 40:1068–1078.
Jo M, Takimoto S, Montel V, Gonias SL. 2009a. The urokinase receptor promotes cancer metastasis independently of urokinase-type plasminogen activator in mice. Am J Pathol 175: 190–200.
Jo M, Lester RD, Montel V, Eastman B, Takimoto S, Gonias SL. 2009b. Reversibility of epithelial-mesenchymal transition (EMT) induced in breast cancer cells by activation of urokinase receptor-dependent cell signaling. J Biol Chem 284:22825–22833.
Jo M, Eastman BM, Webb DL, Stoletov K, Klemke R, Gonias SL. 2010. Cell signaling by urokinase-type plasminogen activator receptor induces stem cell-like properties in breast cancer cells. Cancer Res 70:8948–8958.
Jobling MF, Mott JD, Finnegan MT, Jurukovski V, Erickson AC, Walian PJ, Taylor SE, Ledbetter S, Lawrence CM, Rifkin DB, Barcellos-Hoff MH. 2006. Isoform-specific activation of latent transforming growth factor beta (LTGF-beta) by reactive oxygen species. Radiat Res 166:839–848.
Johansson N, Ala-aho R, Uitto VJ, Grénman R, Fusenig NE, López-Otín C, Kähäri VM. 2000. Expression of collagenase-3 (MMP-13) and collagenase-1 (MMP-1) by transformed keratinocytes is dependent on the activity of p38 mitogen activated protein kinase. J Cell Sci 113:227–235.
Juarez A, Boutet CA, De Frutos PH, Maxwell MJ, Mayol J, Romero J, Nieto MA. 2006. Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. EMBO J 25:5603–5613.
Juárez P, Guise TA. 2011. TGF-β in cancer and bone: implications for treatment of bone metastases. Bone 48:23–29.
Kalluri R, Weinberg RA. 2009. The basics of epithelial-mesenchymal transition. J Clin Invest 119:1420–1428.
Kang JS, Liu C, Derynck R. 2009. New regulatory mechanisms of TGF-β receptor function. Trends Cell Biol 19:385–394.
Kerr LD, Miller DB, Matrisian LM. 1990. TGF-beta 1 inhibition of transin/stromelysin gene expression is mediated through a Fos binding sequence. Cell 61:267–278.
Kessenbrock K, Plaks V, Werb Z. 2010. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141:52–67.
Kim ES, Sohn YW, Moon A. 2007. TGF-beta-induced transcriptional activation of MMP-2 is mediated by activating transcription factor (ATF) 2 in human breast epithelial cells. Cancer Lett 252: 147–156.
Kitamura T, Biyajima K, Aoki M, Oshima M, Taketo MM. 2009. Matrix metalloproteinase 7 is required for tumor formation, but dispensable for invasion and fibrosis in SMAD4-deficient intestinal adenocarcinomas. Lab Invest 89:98–105.
Kocic J, Bugarski D, Santibanez JF. 2013. SMAD3 is essential for transforming growth factor-β1-induced urokinase type plasminogen activator expression and migration in transformed keratinocytes. Eur J Cancer 48:1550–1557.
Krstic J, Santibanez JF. 2014. Transforming growth factor-beta and matrix metalloproteinases functional interplay in cancer, implications in epithelial to mesenchymal transition. Cell Biol Res Ther : 2.
Kubiczkova L, Sedlarikova L, Hajek R, Sevcikova S. 2012. TGF-β an excellent servant but a bad master. J Transl Med 10:183.
Lambert AW, Pattabiraman DR, Weinberg RA. 2017. Emerging biological principles of metastasis. Cell 168:670–691.
Lamouille S, Xu J, Derynck R. 2014. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15:178–196.
Laurenzana A, Biagioni A, Bianchini F, Peppicelli S, Chillà A, Margheri F, Luciani C, Pimpinelli N, Del Rosso M, Calorini L, Fibbi G. 2015. Inhibition of uPAR-TGFβ crosstalk blocks MSC-dependent EMT in melanoma cells. J Mol Med (Berl) 93:783–794.
Lester RD, Jo M, Montel V, Takimoto S, Gonias SL. 2007. uPAR induces epithelial-mesenchymal transition in hypoxic breast cancer cells. J Cell Biol 178:425–436.
Li L, Li H. 2013. Role of microRNA-mediated MMP regulation in the treatment and diagnosis of malignant tumors. Cancer Biol Ther 14:796–805.
Lin CY, Tsai PH, Kandaswami CC, Lee PP, Huang CJ, Hwang JJ, Lee MT. 2011. Matrix metalloproteinase-9 cooperates with transcription factor Snail to induce epithelial-mesenchymal transition. Cancer Sci 102:815–827.
Liu F, Gomez Garcia AM, Meyskens FL Jr. 2012. NADPH oxidase 1 overexpression enhances invasion via matrix metalloproteinase-2 and epithelial-mesenchymal transition in melanoma cells. J Invest Dermatol 132:2033–2041.
Liu Y, Sun X, Feng J, Deng LL, Liu Y, Li B, Zhu M, Lu C, Zhou L. 2016. MT2-MMP induces proteolysis and leads to EMT in carcinomas. Oncotarget 7:48193–48205.
Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ. 1997. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol 139:1861–1872.
Löffek S, Schilling O, Franzke CW. 2011. Series "matrix metalloproteinases in lung health and disease": biological role of matrix metalloproteinases: a critical balance. Eur Respir J 38:191–208.
Lohi J, Lehti K, Valtanen H, Parks WC, Keski-Oja J. 2000. Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. Gene 242:75–86.
López-Otín C, Palavalli LH, Samuels Y. 2009. Protective roles of matrix metalloproteinases: from mouse models to human cancer. Cell Cycle 8:3657–3662.
Luo K. 2017. Signaling cross talk between TGF-β/Smad and other signaling pathways. Cold Spring Harb Perspect Biol 9:pii: a022137.
Lyons RM, Gentry LE, Purchio AF, Moses HL. 1990. Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. J Cell Biol 110:1361–1367.
Lyons JG, Patel V, Roue NC, Fok SY, Soon LL, Halliday GM, Gutkind JS. 2008. Snail up-regulates proinflammatory mediators and inhibits differentiation in oral keratinocytes. Cancer Res 68: 4525–4530.
Macara IG, McCaffrey L. Cell polarity in morphogenesis and metastasis. 2013. Philos Trans R Soc Lond B Biol Sci 368: 20130012.
Massague J. 2012. TGFβ signalling in context. Nat Rev Mol Cell Biol 13:616–630.
Mauviel A, Chung KY, Agarwal A, Tamai K, Uitto J. 1996. Cell-specific induction of distinct oncogenes of the Jun family is responsible for differential regulation of collagenase gene expression by transforming growth factor-beta in fibroblasts and keratinocytes. J Biol Chem 271:10917–10923.
McGuire JK, Li Q, Parks WC. 2003. Matrilysin (matrix metalloproteinase-7) mediates E-Cadherin ectodomain shedding in injured lung epithelium. Am J Pathol 162:1831–1843.
Mekkawy AH, Pourgholami MH, Morris DL. 2014. Involvement of urokinase-type plasminogen activator system in cancer: an overview. Med Res Rev 34:918–956.
Meulmeester E, Ten Dijke P. 2011. The dynamic roles of TGF-β in cancer. J Pathol 223:205–218.
Mittal R, Patel AP, Debs LH, Nguyen D, Patel K, Grati M, Mittal J, Yan D, Chapagain P, Liu XZ. 2016. Intricate functions of matrix metalloproteinases in physiological and pathological conditions. J Cell Physiol 231:2599–2621.
Moirangthem A, Bondhopadhyay B, Mukherjee M, Bandyopadhyay A, Mukherjee N, Konar K, Bhattacharya S, Basu A. 2016. Simultaneous knockdown of uPA and MMP9 can reduce breast cancer progression by increasing cell-cell adhesion and modulating EMT genes. Sci Rep 6:21903.
Moreno-Bueno G, Peinado H, Molina P, Olmeda D, Cubillo E, Santos V, Palacios J, Portillo F, Cano A. 2009. The morphological

and molecular features of the epithelial-to-mesenchymal transition. Nat Protoc 4:1591–1613.
Moustakas A, Heldin CH. 2009. The regulation of TGFβ signal transduction. Development 136:3699–3714.
Moustakas A, Heldin CH. 2016. Mechanisms of TGFβ-induced epithelial-mesenchymal transition. J Clin Med 5:pii: E63.
Munshi HG, Wu YI, Mukhopadhyay S, Ottaviano AJ, Sassano A, Koblinski JE, Platanias LC, Stack MS. 2004. Differential regulation of membrane type 1-matrix metalloproteinase activity by ERK 1/2- and p38 MAPK-modulated tissue inhibitor of metalloproteinases 2 expression controls transforming growth factor-beta1-induced pericellular collagenolysis. J Biol Chem 279:39042–39050.
Murphy G. 2011. Tissue inhibitors of metalloproteinases. Genome Biol 12:233.
Nagamine Y, Medcalf RL, Muñoz-Cánoves P. 2005. Transcriptional and posttranscriptional regulation of the plasminogen activator system. Thromb Haemost 93:661–675.
Narayan S, Thangasamy T, Balusu R. 2005. Transforming growth factor-beta receptor signaling in cancer. Front Biosci 10:1135–1145.
Nguyen HL, Kadam P, Helkin A, Cao K, Wu S, Samara GJ, Zhang Q, Zucker S, Cao J. 2016. MT1-MMP Activation of TGF-β signaling enables intercellular activation of an epithelial-mesenchymal transition program in cancer. Curr Cancer Drug Targets 16:618–630.
Obenauf AC, Massagué J. 2015. Surviving at a distance: organ specific metastasis. Trends Cancer 1:76–91.
Odekon LE, Blasi F, Rifkin DB. 1994. Requirement for receptor-bound urokinase in plasmin-dependent cellular conversion of latent TGF-beta to TGF-beta. J Cell Physiol 158:398–407.
Oft M, Akhurst RJ, Balmain A. 2002. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. Nat Cell Biol 4: 487–494.
Ogawa K, Chen F, Kuang C, Chen Y. 2004. Suppression of matrix metalloproteinase-9 transcription by transforming growth factor-beta is mediated by a nuclear factor-kappaB site. Biochem J 381:413–422.
Orlichenko LS, Radisky DC. 2008. Matrix metalloproteinases stimulate epithelial–mesenchymal transition during tumor development. Clin Exp Metastasis 25:593–600.
Padua D, Massagué J. 2009. Roles of TGFbeta in metastasis. Cell Res 19:89–102.
Park HY, Wakefield LM, Mamura M. 2009. Regulation of tumor immune surveillance and tumor immune subversion by TGF-β. Immune Netw 9:122–126.
Perri F, Longo F, Giuliano M, Sabbatino F, Favia G, Ionna F, Addeo R, Della Vittoria Scarpati G, Di Lorenzo G, Pisconti S. 2017. Epigenetic control of gene expression: potential implications for cancer treatment. Crit Rev Oncol Hematol 111:166–172.
Poliakov A, Tkachuk V, Ovchinnikova T, Potapenko N, Bagryantsev S, Stepanova V. 2001. Plasmin-dependent elimination of the growth-factor-like domain in urokinase causes its rapid cellular uptake and degradation. Biochem J 355:639–645.
Principe DR, Doll JA, Bauer J, Jung B, Munshi HG, Bartholin L, Pasche B, Lee C, Grippo PJ. 2014. TGF-β: duality of function between tumor prevention and carcinogenesis. J Natl Cancer Inst 106:djt369.
Qin G, Luo M, Chen J, Dang Y, Chen G, Li L, Zeng J, Lu Y, Yang J. 2016. Reciprocal activation between MMP-8 and TGF-β1 stimulates EMT and malignant progression of hepatocellular carcinoma. Cancer Lett 374:85–95.
Quintanilla M, del Castillo G, Kocic J, Santibanez JF. 2012. TGF-B and MMPs: a complex regulatory loop involved in tumor progression. In: Oshiro N, Miyagi E, editors. Matrix metalloproteinases: biology, functions and clinical implications. Hauppauge, NY: Nova Science Publishers.
Radisky ES, Radisky DC. 2010. Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. J Mammary Gland Biol Neoplasia 15:201–212.
Radisky ES, Radisky DC. 2015. Matrix metalloproteinases as breast cancer drivers and therapeutic targets. Front Biosci 20: 1144–1163.
Rifkin DB. 2005. Latent transforming growth factor-beta (TGF-beta) binding proteins: orchestrators of TGF-beta availability. J Biol Chem 280:7409–7412.
Rijken DC, Lijnen HR. 2009. New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haemost 7:4–13.
Roberts AB, Anzano MA, Wakefield LM, Roche NS, Stern DF, Sporn MB. 1985. Type beta transforming growth factor: a bifunctional regulator of cellular growth. Proc Natl Acad Sci U S A 82: 119–123.
Roberts AB, Tian F, Byfield SD, Stuelten C, Ooshima A, Saika S, Flanders KC. 2006. Smad3 is key to TGF-beta-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. Cytokine Growth Factor Rev 17:19–27.
Rozanov DV, Deryugina EI, Monosov EZ, Marchenko ND, Strongin AY. 2004. Aberrant, persistent inclusion into lipid rafts limits the tumorigenic function of membrane type-1 matrix metalloproteinase in malignant cells. Exp Cell Res 293:81–95.
Safina A, Vandette E, Bakin AV. 2007. ALK5 promotes tumor angiogenesis by upregulating matrix metalloproteinase-9 in tumor cells. Oncogene 26:2407–2422.
Safina A, Ren MQ, Vandette E, Bakin AV. 2008. TAK1 is required for TGF-beta 1-mediated regulation of matrix metalloproteinase-9 and metastasis. Oncogene 27:1198–1207.
Sánchez-Tilló E, Liu Y, de Barrios O, Siles L, Fanlo L, Cuatrecasas M, Darling DS, Dean DC, Castells A, Postigo A. 2012. EMT-activating transcription factors in cancer: beyond EMT and tumor invasiveness. Cell Mol Life Sci 69:3429–3456.
Sano Y, Harada J, Tashiro S, Gotoh-Mandeville R, Maekawa T, Ishii S. 1999. ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-beta signaling. J Biol Chem 274:8949–8957.
Santibanez JF, Frontelo P, Iglesias M, Martínez J, Quintanilla M. 1999. Urokinase expression and binding activity associated with the transforming growth factor beta1-induced migratory and invasive phenotype of mouse epidermal keratinocytes. J Cell Biochem 74:61–73.
Santibanez JF, Iglesias M, Frontelo P, Martínez J, Quintanilla M. 2002a. Involvement of the Ras/MAPK signaling pathway in the modulation of urokinase production and cellular invasiveness by transforming growth factor-beta (1) in transformed keratinocytes. Biochem Biophys Res Commun 273:521–527.
Santibanez JF, Guerrero J, Quintanilla M, Fabra A, Martínez J. 2002b. Transforming growth factor-beta1 modulates matrix metalloproteinase-9 production through the Ras/MAPK signaling pathway in transformed keratinocytes. Biochem Biophys Res Commun 296:267–273.
Santibanez JF. 2006. JNK mediates TGF-beta1-induced epithelial mesenchymal transdifferentiation of mouse transformed keratinocytes. FEBS Lett 580:5385–5389.
Santibanez JF, Quintanilla M, Bernabeu C. 2011. TGF-β/TGF-β receptor system and its role in physiological and pathological conditions. Clin Sci (Lond) 121:233–251.
Santibanez JF, Kocić J, Fabra A, Cano A, Quintanilla M. 2010. Rac1 modulates TGF-beta1-mediated epithelial cell plasticity and MMP9 production in transformed keratinocytes. FEBS Lett 584:2305–2310.
Santibanez JF. 2013. Transforming growth factor-Beta and urokinase-type plasminogen activator: dangerous partners in tumorigenesis: implications in skin cancer. ISRN Dermatol 2013:597927.
Schwarte-Waldhoff I, Klein S, Blass-Kampmann S, Hintelmann A, Eilert C, Dreschers S, Kalthoff H, Hahn SA, Schmiegel W. 1999. DPC4/SMAD4 mediated tumor suppression of colon carcinoma cells is associated with reduced urokinase expression. Oncogene 18:3152–3158.
Seetoo DQ, Crowe PJ, Russell PJ, Yang JL. 2003. Quantitative expression of protein markers of plasminogen activation system in prognosis of colorectal cancer. J Surg Oncol 82:184–193.
Selvamurugan N, Kwok S, Partridge NC. 2004. Smad3 interacts with JunB and Cbfa1/Runx2 for transforming growth factor-beta1-stimulated collagenase-3 expression in human breast cancer cells. J Biol Chem 279:27764–27773.
Seomun Y, Kim JT, Joo CK. 2008. MMP-14 mediated MMP-9 expression is involved in TGF-beta1-induced keratinocyte migration. J Cell Biochem 104:934–941.
Shi Y, Massagué J. 2003. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685–700.

Shiou SR, Datta PK, Dhawan P, Law BK, Yingling JM, Dixon DA, Beauchamp RD. 2006. Smad4-dependent regulation of urokinase plasminogen activator secretion and RNA stability associated with invasiveness by autocrine and paracrine transforming growth factor-beta. J Biol Chem 28:33971–33981.
Smith HW, Marshall CJ. 2010. Regulation of cell signalling by uPAR. Nat Rev Mol Cell Biol 11:23–36.
Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98:137–146.
STRING: functional protein association networks. Available at: http://string-db.org/.
Symowicz J, Adley BP, Gleason KJ, Johnson JJ, Ghosh S, Fishman DA, Hudson LG, Stack MS. 2007. Engagement of collagen-binding integrins promotes matrix metalloproteinase-9-dependent E-Cadherin ectodomain shedding in ovarian carcinoma cells. Cancer Res 67:2030–2039.
Rijken DC, Lijnen HR. 2009. New insights into the molecular mechanisms of the fibrinolytic system. J. Thromb Haemost 7:4–13.
Tardif G, Reboul P, Dupuis M, Changshan G, Duval N, Pelletier JP, Martel-Pelletier J. 2001. TGF-beta-induced collagenase-3 production in human osteoarthritic chondrocytes is triggered by SMAD proteins: cooperation between AP-1 and PEA-3 binding sites. J Rheumatol 28:1631–1639.
Tatti O, Vehviläinen P, Lehti K, Keski-Oja J. 2008. MT1-MMP releases latent TGF-beta1 from endothelial cell extracellular matrix via proteolytic processing of LTBP-1. Exp Cell Res 314: 2501–2514.
Teicher BA. 2007. From transforming growth factor-B and the immune response to malignant disease. Clin Cancer Res 13: 6247.
Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. 2003. Curr Opin Cell Biol 15:740–746.
Thiery JP, Acloque H, Huang RYJ, Nieto MA. 2009. Epithelial–mesenchymal transitions in development and disease. Cell 139:871–890.
Thunø M, Macho B, Eugen-Olsen J. 2009. suPAR: the molecular crystal ball. Dis Markers 27:157–172.
Tian X, Du H, Fu X, Li K, Li A, Zhang Y. 2009. Smad4 restoration leads to a suppression of Wnt/beta-catenin signaling activity and migration capacity in human colon carcinoma cells. Biochem Biophys Res Commun 380:478–483.
Tobar N, Villar V, Santibanez JF. 2010. ROS-NFkappaB mediates TGF-beta1-induced expression of urokinase-type plasminogen activator, matrix metalloproteinase-9 and cell invasion. Mol Cell Biochem 340:195–202.
Uría JA, Jiménez MG, Balbín M, Freije JM, López-Otín C. 1998. Differential effects of transforming growth factor-beta on the expression of collagenase-1 and collagenase-3 in human fibroblasts. J Biol Chem 273:9769–9777.
Villar V, Kocić J, Santibanez J.F. 2010a. Spred2 inhibits TGF-beta1-induced urokinase type plasminogen activator expression, cell motility and epithelial mesenchymal transition. Int J Cancer 127: 77–85.
Villar V, Kocic J, Bugarski D, Jovcic G, Santibanez JF. 2010b. SKIP is required for TGF-β1-induced epithelial mesenchymal transition and migration in transformed keratinocytes. FEBS Lett 584: 4586–4592.
Vos MC, Hollemans E, Ezendam N, Feijen H, Boll D, Pijlman B, van der Putten H, Klinkhamer P, van Kuppevelt TH, van der Wurff AA, Massuger LF. 2016. MMP-14 and CD44 in Epithelial-to-Mesenchymal Transition (EMT) in ovarian cancer. J Ovarian Res 9:53.
Wakefield LM, Hill CS. 2013. Beyond TGFβ: roles of other TGFβ superfamily members in cancer. Nat Rev Cancer 13:328–341.
Walsh LA, Damjanovski S. 2011. IGF-1 increases invasive potential of MCF 7 breast cancer cells and induces activation of latent TGF-β1 resulting in epithelial to mesenchymal transition. Cell Commun Signal 9:10.
Weiss A, Attisano L. 2013. The TGFbeta superfamily signaling pathway. Wiley Interdiscip Rev Dev Biol 2:47–63.
White LA, Mitchell TI, Brinckerhoff CE. 2000. Transforming growth factor beta inhibitory element in the rabbit matrix metalloproteinase-1 (collagenase-1) gene functions as a repressor of constitutive transcription. Biochim Biophys Acta 1490: 259–268.
Wu YY, Peck K, Chang YL, Pan SH, Cheng YF, Lin JC, Yang RB, Hong TM, Yang PC. 2011. SCUBE3 is an endogenous TGF-β receptor ligand and regulates the epithelial-mesenchymal transition in lung cancer. Oncogene 30:3682–3693.
Xu J, Lamouille S, Derynck R. 2009. TGF-beta-induced epithelial to mesenchymal transition. Cell Res 19:156–172.
Yan C, Boyd DD. 2007. Regulation of matrix metalloproteinase gene expression. J Cell Physiol 211:19–26.
Yang CC, Zhu LF, Xu XH, Ning TY, Ye JH, Liu LK. 2013. Membrane type 1 matrix metalloproteinase induces an epithelial to mesenchymal transition and cancer stem cell-like properties in SCC9 cells. BMC Cancer 13:171.
Yeung KT, Yang J. 2017. Epithelial-mesenchymal transition in tumor metastasis. Mol Oncol 11:28–39.
Yu W, Zhang Y, Ruest LB, Svoboda KK. 2013. Analysis of Snail1 function and regulation by Twist1 in palatal fusion. Front Physiol 4:12.
Yuan W, Varga J. 2001. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. J Biol Chem 276:38502–38510.
Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B, Kern SE. 1998. Human Smad3 and Smad4 are sequence-specific transcription activators. Mol Cell 1:611–617.
Zhao J, Kong Z, Xu F, Shen W. 2015. A role of MMP-14 in the regulation of invasiveness of nasopharyngeal carcinoma. Tumour Biol 36:8609–8615.
Zheng G, Lyons JG, Tan TK, Wang Y, Hsu TT, Min D, Succar L, Rangan GK, Hu M, Henderson BR, Alexander SI, Harris DC. 2009. Disruption of E–Cadherin by matrix metalloproteinase directly mediates epithelial-mesenchymal transition downstream of transforming growth factor-beta1 in renal tubular epithelial cells. Am J Pathol 175:580–591.
Zhong J, Gencay MM, Bubendorf L, Burgess JK, Parson H, Robinson BW, Tamm M, Black JL, Roth M. 2006. ERK1/2 and p38 MAP kinase control MMP-2, MT1-MMP, and TIMP action and affect cell migration: a comparison between mesothelioma and mesothelial cells. J Cell Physiol 207:540–552.
Zhu X, Wang L, Zhang B, Li J, Dou X, Zhao RC. 2011. TGF-beta1-induced PI3K/Akt/NF-kappaB/MMP9 signalling pathway is activated in Philadelphia chromosome-positive chronic myeloid leukaemia hemangioblasts. J Biochem 149:405–414.
Zuo JH, Zhu W, Li MY, Li XH, Yi H, Zeng GQ, Wan XX, He QY, Li JH, Qu JQ, Chen Y, Xiao ZQ. 2011. EGFR activation promotes HNSCC SCC10A cell migration and invasion by inducing EMT-like phenotype change and MMP-9-mediated degradation of E-Cadherin into sE-cad related to activation of ERK-1/2 and PI3K signaling pathways. J Cell Biochem 112:2508–2517.