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REVIEW

Transforming growth factor-β signaling in tumor initiation, progression and therapy in breast cancer: an update

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Abstract Transforming growth factor-β (TGF-β) is a ubiquitous cytokine playing an essential role in cell proliferation, differentiation, apoptosis, adhesion and invasion, as well as in cellular microenvironment. In malignant diseases, TGF-β signaling features a growth inhibitory effect at an early stage but aggressive oncogenic activity at the advanced malignant state. Here, we update the current understanding of TGF-β signaling in cancer development and progression with a focus on breast cancer. We also review the current approaches of TGF-β signaling-targeted therapeutics for human malignancies.

Keywords TGF-β signaling · Epithelial-mesenchymal transition · Tumorigenesis · Breast cancer · Cancer therapy

Abbreviations

CAF	Carcinoma-associated fibroblasts
EMT	Epithelial-mesenchymal transition
CdGAP	Cdc42 GTPase-activating protein
MEC	Mammary epithelial cell
DMR	DNA mismatch repair
MSC	Mesenchymal stem cell
PRD	Proline-rich domain
PTK	Protein tyrosine kinase
RANKL	Receptor activator of NF-κB ligand
TGF-β	Transforming growth factor-β
TβRI	Type I TGF-β receptor
TβRII	Type II TGF-β receptor
VEGF	Vascular endothelial growth factor

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Introduction

Transforming growth factor-β (TGF-β) is a key player in embryogenesis and cell proliferation, differentiation, apoptosis, adhesion and invasion, as well as in the cellular microenvironment (Heldin et al. 2009; Ikushima and Miyazono 2010; Massague 2008; Yang et al. 2010). Three TGF-β isoforms have been identified thus far: TGF-β1, TGF-β2 and TGF-β3. Together with inhibins, bone morphogenetic proteins (BMPs), activins, *Drosophila* decapentaplegic and *Xenopus* Vg-1, these TGF-β isoforms consist in a family of ligand proteins (Herpin et al. 2004; Massague 2008). TGF-β functions as a secreted polypeptide, signaling by binding to cell-surface TGF-β receptors (Pennison and Pasche 2007). Type I TGF-β receptor (TβRI) and type II TGF-β receptor (TβRII) are two major types of TGF-β receptors. TGF-β binds to TβRII and recruits TβRI to form a heterotetrameric complex, leading to phosphorylation and activation of TβRII and TβRI. The activated TGF-β/receptor complex in turn phosphorylates Smad2 and Smad3. Phosphorylated Smad2 and Smad3 (active forms) associate with Smad4, translocate into nuclei and regulate target gene expression (Attisano and Wrana 2002; Pennison and Pasche 2007).

The role of TGF-β in cancer is complicated. At the early stage of tumorigenesis, TGF-β plays an inhibitory effect

whereas tumor cells at advanced stages can evade antiproliferative control and undergo tumorigenic progression in response to TGF-β. Therefore, TGF-β plays a tumor-suppressive or promoting role depending on the cell and tissue contexts (Inman 2011; Joshi and Cao 2010; Meulmeester and Ten Dijke 2011). In this review article, we update the studies on TGF-β signaling and review its potential avenues toward cancer therapies, with a focus on breast cancer.

TGF-β signaling

TGF-β is a multifunctional polypeptide with 390 amino acids encoded by the *TGF-β1* gene. The product pre-polypeptide is cleaved into a latency-associated peptide (LAP) and TGF-β1 that form an inactive, non-covalently associated small latent TGF-β complex (SLC) (Gentry et al. 1988). The latent TGF-β binding proteins (LTBPs) bind to SLC by disulphide bonds and form the large latent TGF-β complex (LLC). LTBPs play a role in localization of LLC in the extracellular matrix through interaction with matrix proteins (Massague 1998; Rifkin 2005). Upon appropriate signals, the latent complex is cleaved to release a mature, bioactive TGF-β that binds to cell-surface receptors to elicit signal transduction. TGF-β receptors are transmembrane serine/threonine kinase receptors. Two major groups of TGF-β receptors are involved in its signaling. They are type I TGF-β receptors (TβRI) and type II TGF-β receptors (TβRII) (Attisano and Wrana 2002; Massague 1998; Rahimi and Leof 2007). In vertebrates, the type I receptor family of the TGF-β family consists in three groups that share similar kinase domains and signaling activities. Group 1 includes TβRI, ActR-IB and ALK7; Group 2 contains BMPR-IA and IB; and Group 3 is composed of ALK1 and ALK2 (Glasgow and Mishra 2008; Rubenstein et al. 2009; Tang et al. 2003; Yun et al. 2008). Vertebrate type II receptor family consists in TβRII, ActR-II, ActR-IIB, BMPR-II and AMHR (Anti-Mullerian hormone receptor). ActR-II and IIB bind to activins when expressed alone or jointly with activin type I receptors, or bind to BMPs 2, 4 and 7 and GDF5 in concert with BMPR-I (Buijs et al. 2007). In addition to these two major types of receptors, type III TGF-β receptor (TβRIII) or betaglycan is reported as an accessory receptor (Ajiboye et al. 2010). This TβRIII is ubiquitously expressed but does not have an intrinsic signaling function. TβRIII participates in the regulation of TGF-β signaling and has recently been identified as a tumor suppressor (Ajiboye et al. 2010; Lee et al. 2010; You et al. 2009).

TGF-β transduces its signals through a group of small, evolutionarily conserved intracellular effector proteins, termed Smads (Xu 2006). Three types of Smads have been identified: receptor-activated Smads (R-Smads, e.g., Smad1, Smad2, Smad3, Smad5 and Smad8), a common mediator Smad (Co-Smad, e.g., Smad4) and inhibitory Smads (I-Smads, e.g., Smad6 and Smad7) (Moustakas et al. 2001; Xu 2006). Smads are modular proteins containing a conserved N-terminal Mad-homology 1 (MH1) domain, an intermediate linker and a C-terminal MH2 domain. The MH1 domain participates in nuclear localization, DNA-binding and protein–protein interaction. The linker accepts regulatory phosphorylation by other signaling kinases, such as mitogen-activated protein kinases (MAPKs) or cyclin-dependent kinases (CDKs) and recruits ubiquitin ligases that regulate Smad and TGF-β receptor half-lives. The MH2 is a major protein–protein interaction domain, possessing phospho-serine-binding activity (Massague and Wotton 2000; Moustakas et al. 2001). The activated, catalytically active TβRI phosphorylates C-terminal serine residues of Smad2 and Smad3, two distinct proteins that play non-redundant functions in TGF-β signaling. Receptor-phosphorylated R-Smads exhibit high affinity to Co-Smad (Smad4). The Co-Smad is not phosphorylated by receptors but rapidly oligomerizes with phosphorylated Smad2 or Smad3 to form functional protein complexes (Broderick et al. 2007; Derynck and Zhang 2003; Giehl et al. 2007; Moustakas et al. 2001; Xu 2006). Monomeric Smad proteins constantly shuttle in and out of the nucleus but the activated R-Smad/Co-Smad complexes favor nuclear accumulation, where they associate with a plethora of transcription factors (co-activators or co-repressors) and bind to the Smad-binding element, activating or repressing a diverse array of gene expression (Xu 2006) (Fig.1).

TGF-β signaling and cancer development and progression

The role of TGF-β in cancer development and progression has been extensively investigated. In breast cancer, TGF-β, TβRII and the phospho-Smad2 expression are associated with the earlier age of onset and tumor characteristics (Figueroa et al. 2010). TGF-β signaling status may stratify the prognosis of estrogen receptor (ER)-positive patients and a high TGF-β level may be associated with worse survival (Bierie et al. 2009; Grau et al. 2008). However, it is universally accepted that TGF-β plays a differential role in carcinogenesis and progression upon the stage of tumors (Joshi and Cao 2010).

TGF-β signaling as a suppressor of cancer development

A body of evidence has shown that TGF-β inhibits proliferation of normal epithelial cells and early tumors through down-regulating proto-oncogene c-myc or cyclin-dependent kinases (CDKs) and up-regulating CDK inhibitors, including p15 and p21 (Feng et al.

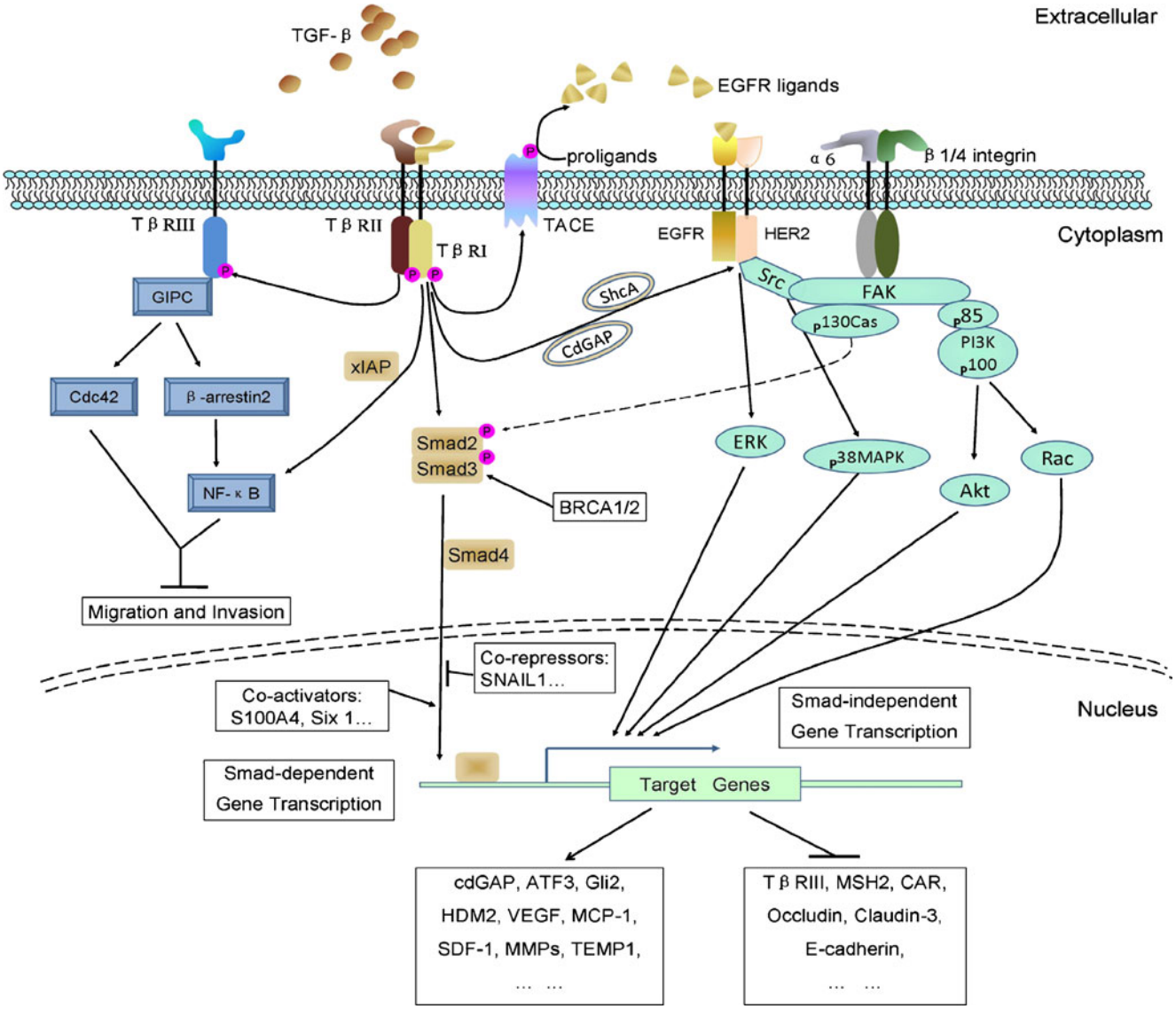

Fig. 1 TGF-β signaling pathway in breast cancer cells. Autocrine or paracrine TGF-β binds to Ser/Thr protein kinase receptors and initiates the canonical TGF-β signaling pathway and complicated cross-talks with other signal molecules or pathways. Activated TβRI induces membrane-proximal clustering of HER2 and integrins via activating Src-FAK and this process is probably mediated by distinct effectors, including TACE, ShcA and CdGAP. Src-FAK activation leads to MAP kinase (e.g., ERK, p38 MAPK) and PI3K-mediated signaling that are called noncanonical or non-Smad pathways. In addition, activated TβRII can phosphorylate TβRIII and activate Cdc42 and NF-κB through GIPC, inhibiting cancer migration and invasion. As a result, TGF-β signaling regulates a large number of gene expressions, including up-regulation of cdGAP, ATF3, Gli2, SDF-1 and MMPs and down-regulation of TβRIII, MSH2 and CAR to influence the progression of cancers. On the other hand, a variety of molecules (e.g., S100A4, Six1, SNAIL1, $p130^{Cas}$ and BRCA1/2) mediate the activity of TGF-β signaling effectors

2002; Glasgow and Mishra 2008; Juarez and Guise 2010; Malliri et al. 1996; Massague 2008). In TGF-β transgenic mice, primary tumor numbers induced by chemical carcinogens are significantly less than that in wild-type control (Guarino 2007; Pierce et al. 1995). In contrast, spontaneous epithelial tumors are significantly increased in mice with mammary epithelium-specific expression of dominant negative TβRII (Gorska et al. 2003). In human neoplastic breast lesions, TβRII down-regulation is correlated with progression and aggression of both in situ and invasive breast carcinomas (Gobbi et al. 1999, 2000). Reduced nuclear levels of phosphorylated Smad2/3 are associated with high tumor grades and larger tumor size (Yin et al. 2010). These data suggest the suppressive role of TβRII in breast tumorigenesis. TβRI and TβRIII also play a suppressive role in mammary gland tumorigenesis. In mice carrying the *Neu* oncogene, active TβRI expressed specifically in the mammary epithelium significantly reduces epithelial tumor occurrence (Siegel et al. 2003). In canine mammary tumors, TβRIII and latent TGF-β-binding

protein-4 (LTBP-4) are significantly decreased; ectopic expression of TβRIII inhibits breast cancer cell migration and invasion (Klopfleisch et al. 2010).

TGF-β signaling as a promoter of cancer progression

The oncogenic function of TGF-β signaling in mammary tumor progression has also been well documented. TGF-β paradoxically stimulates cancer cell proliferation, epithelial-mesenchymal transition (EMT) and invasion and metastasis. As a master regulator of autocrine and paracrine signaling pathways between the tumor and microenvironment, TGF-β is implicated in microenvironment modifications, angiogenesis and immunosuppression (Jakowlew 2006; Stover et al. 2007; Tang et al. 2003). In breast cancer patients with lymph node and distant metastasis, TGF-β levels in plasma are increased and may be predictive of local and distant metastases (Ivanovic et al. 2009; Yu et al. 2011). Knockdown of TβRIII impairs mobility and invasion of metastatic cancer cells (Criswell et al. 2008). TGF-β signaling also contributes to the resistance of breast cancer cells to DNA-damaging chemotherapeutic agents through modulating the MutS homolog 2 (MSH2) expression (Yu et al. 2011). In anti-estrogen therapy, TGF-β may induce immunosuppression and lead to resistance and relapses of breast cancer (Joffroy et al. 2010).

Molecular mechanisms of the tumor-promoting function of TGF-β are complicated. TGF-β induces expression and translocation of the nuclear factor of activated T cells (NFAT) into the nucleus, stimulating c-Myc expression (Singh et al. 2010). TGF-β stimulates the expression of activating transcription factor-3 (ATF3), an enhancer of breast cancer-initiating cell features (Yin et al. 2010) but down-regulates FXYD-domain-containing ion transport regulator-3 (FXYD3), a suppressor of breast tumorigenesis (Yamamoto et al. 2011). In bone metastasis of breast cancer, TGF-β increases secretion of important osteolytic factors, such as the parathyroid hormone-related protein (PTHrP) (Johnson et al. 2011). In addition, TGF-β-activated Smad3/4 can specifically bind to the promoter of *HDM2* and enhance HDM2 expression, destabilizing p53 in human breast cancer cells. In fact, approximately 65% of late-stage carcinomas are positive for activated Smad3 and HDM2 (Araki et al. 2010). TGF-β may also affect survival of certain types of cancer cells through the TGF-β-Foxc1-Bim pathway (Hoshino et al. 2011).

TGF-β signaling, epithelial-mesenchymal transition and breast cancer stem cells

Epithelial–mesenchymal transition (EMT) is an essential process during which epithelial cells lose polarity and cell–cell contacts and undergo a dramatic cytoskeleton remodeling, resulting in a highly motile mesenchymal morphology (Kalluri and Weinberg 2009). EMT is an indispensable process that is associated with normal tissue development, tissue remodeling and wound healing (Micalizzi and Ford 2009). In tumor progression, inappropriate reactivation of EMT readily contributes to cancer cell invasion and metastasis (Guarino 2007; Lenferink et al. 2010). TGF-β is a critical player of EMT, mediating cancer metastasis (Vincent et al. 2009; Voulgari and Pintzas 2009; Wendt et al. 2009a).

Regulatory mechanisms of TGF-β signaling in EMT are complicated. Transcriptional repressor SNAIL1 in Wnt signaling is a promoter of EMT. Smad3 and Smad4 interact with SNAIL1 and repress the expression of CAR, occludin, claudin-3 and E-cadherin in breast epithelial cells, inducing EMT (Vincent et al. 2009). TGF-β/TβRI induces phosphorylation and translocation of the TNF-alpha-converting enzyme (TACE) to the cell surface, where TACE cleaves ErbB proligands to ErbB. The ErbB then initiates autocrine and paracrine signaling and activates phosphatidylinositol-3 kinase (PI3K)/Akt and p38 MAPK, enhancing cytoskeleton rearrangement, survival and migration of breast cancer cells. FAK, a protein tyrosine kinase ubiquitously expressed, plays a synergistic role in the cross-talk between the Neu/ErbB-2 and TGF-β signaling pathways (Wang et al. 2008, 2009; Wendt and Schiemann 2009; Wendt et al. 2010). In addition, TGF-β also stimulates EMT by up-regulating ATF3, a transcription factor mediating morphology, EMT marker expression and cancer-initiating features of breast cells (Yin et al. 2010).

Breast cancer stem cells are a subpopulation of cells with $CD44^{+}/CD24^{-/low}$ surface antigens. This population of cells is characterized by their capability of self-renewal, tumor-initiation, mammosphere formation and differentiation into myoepithelial or luminal epithelial cells (Blick et al. 2010; Mani et al. 2008; May et al. 2011). TGF-β cross-talks with Wnt, Her2 and FAK, the regulators of EMT and breast cancer stem cells and promotes the formation of cancer stem cells (Jain and Alahari 2011; Taube et al. 2010). It has been reported that, in $CD44^{+}/CD24^{-/low}$ stem cells, TGF-β signaling is activated and up-regulates vimentin, CTGF, SERPINE1, SPARC and TGFBR2 expression (Shipitsin et al. 2007). Of note,contrary results have been reported that TGF-β may suppress the mammosphere-forming ability of breast cancer stem cells (Tang et al. 2007). Further investigations are needed to clarify the role of TGF-β in the formation of breast cancer stem cells.

TGF-β signaling and microenvironment

Up to 50% or more of a tumor bulk consists in non-parenchymal cells that are often referred to as the tumor microenvironment. The non-parenchymal cells include

immune cells, microvascular cells and fibroblasts. TGF-β exerts complicated roles in the mammary tumor microenvironment to influence tumor growth, migration and invasion. For instance, TGF-β enhances tumor vascularity via regulating the expression of Cathepsin G, vascular endothelial growth factor (VEGF) and monocyte chemotactic protein (MCP)-1 and promotes immune evasion and extracellular matrix degradation (Wilson et al. 2010) (Fig. 2).

Fibroblasts

Using a co-culture system of human metastatic breast cancer cells (MCF10CA1a) and normal murine dermal fibroblasts, Stuelten et al. (2010) found that the medium of this co-culture increases migration and scattering of MCF10CA1a cells. This function is dependent on small amounts of active TGF-β secreted by fibroblasts under the influence of the tumor cells. Stromal fibroblasts and α-SMA-positive myofibroblasts are collectively termed carcinoma-associated fibroblasts (CAFs), which function as a tumor promoter. During tumor progression, mammary fibroblasts progressively convert into CAF myofibroblasts and TGF-β stimulates this conversion, promoting tumor progression (Casey et al. 2008; Kojima et al. 2010).

Immune cells

Myeloid-derived suppressor cells (MDSC) are a population of $CD11b^{+}Gr\text{-}1^{+}$ myeloid cells expanded dramatically during tumor progression. MDSC can inhibit T cells and dendritic cells, contributing to tumor immune escape. The MDSC in the peripheral blood, spleen and tumors are directly associated with mammary tumor sizes and inversely correlated with the T cell number (Abe et al. 2010; Donkor et al. 2009). TGF-β binds to MDSC, leading to MDSC-mediated suppression of natural killer (NK) cells. Inhibition of TGF-β decreases the MDSC number and increases the activity of dendritic cells, NK cells and tumor antigen-specific T cells (Li et al. 2009a; Llopiz et al. 2009). TGF-β also affects regulatory T cell activity (Yoshimura et al. 2010) through a neuropilin-1 (Nrp1)-mediated mechanism and supports breast cancer growth (Glinka and Prud'homme 2008; Glinka et al. 2010). Moreover, TGF-β can actively subvert the $CD8^{+}$ arm of the immune system to promote mammary cancer growth through an IL-17-dependent mechanism (Nam et al. 2008a).

Extracellular matrix

The bone tendency and osteolytic capability of breast cancer cells are derived from their interaction with stromal cells. Microarray analysis (Nannuru et al. 2010) indicated that MMP13, receptor activator of the NF-κB ligand (RANKL) and integrin binding sialoprotein are up-regulated at the tumor–bone interface. Knockdown of MMP13 expression leads to a decrease of active MMP9, RANKL and TGF-β signaling and a significant reduction in bone destruction and in the number of activated osteoclasts at the tumor–bone interface. Cathepsin G, a protease up-regulated at the tumor–bone interface of breast cancer, activates pro-MMP9 and sequentially latent TGF-β, promoting tumor growth, osteoclast activation and subsequent bone resorption (Wilson et al. 2009). Consistent with this, TGF-β induces MMP2 and MMP9 expression in malignant breast epithelial cells through a Smad3- and Smad4-dependent manner, forming a vicious cycle (Wiercinska et al. 2010).

Regulatory factors of the TGF-β signaling pathway

TGF-β signaling cross-talks with the EGF and integrin and is regulated by multiple co-operators/activator and suppressors (Fig. 1).

Co-operators and enhancers of TGF-β signaling

Many proteins act as a positive regulator of TGF-β signaling through interaction with the key effectors in this pathway. S100A4, also called metastatin-1, is a metastasis-associated protein that interacts with Smad3 at the N-terminus and increases TGF-β-induced MMP-9 expression in breast cancer cells, co-operatively promoting cancer metastasis (Matsuura et al. 2010). Similarly, BRCA1, a regulator of cell cycle checkpoints and DNA damage repair networks, binds to Smad3 at 207-426aa and affects its activity (Li et al. 2009b). In addition, TGF-β associates with HER2 and elevates BRCA2 expression. These three factors in turn function synergistically to promote breast cancer progression (Li et al. 2011).

Neuropilin-1 (Nrp1) acts as a co-receptor for TGF-β and augments latent and active TGF-β signaling. Nrp1 was identified as a co-receptor for VEGF and class 3 semaphorins but recent studies have shown that Nrp1 has a high affinity to TβRI and TβRII and forms a complex together with LAP-TGF-β, augmenting canonical Smad2/3 signaling (Glinka et al. 2010).

$p130^{Cas}$ (also known as breast cancer resistance-1) functions as a molecular scaffold within focal adhesion complexes. $p130^{Cas}$ regulates the balance between Smad2/3 and p38MAPK signaling, which is critical to maintain the tumor suppressor function of TGF-β during breast cancer progression. Over-expression of $p130^{Cas}$ in mammary epithelial cells (MECs) can diminish the ability of TGF-β to activate Smad2/3 but increases its coupling with p38

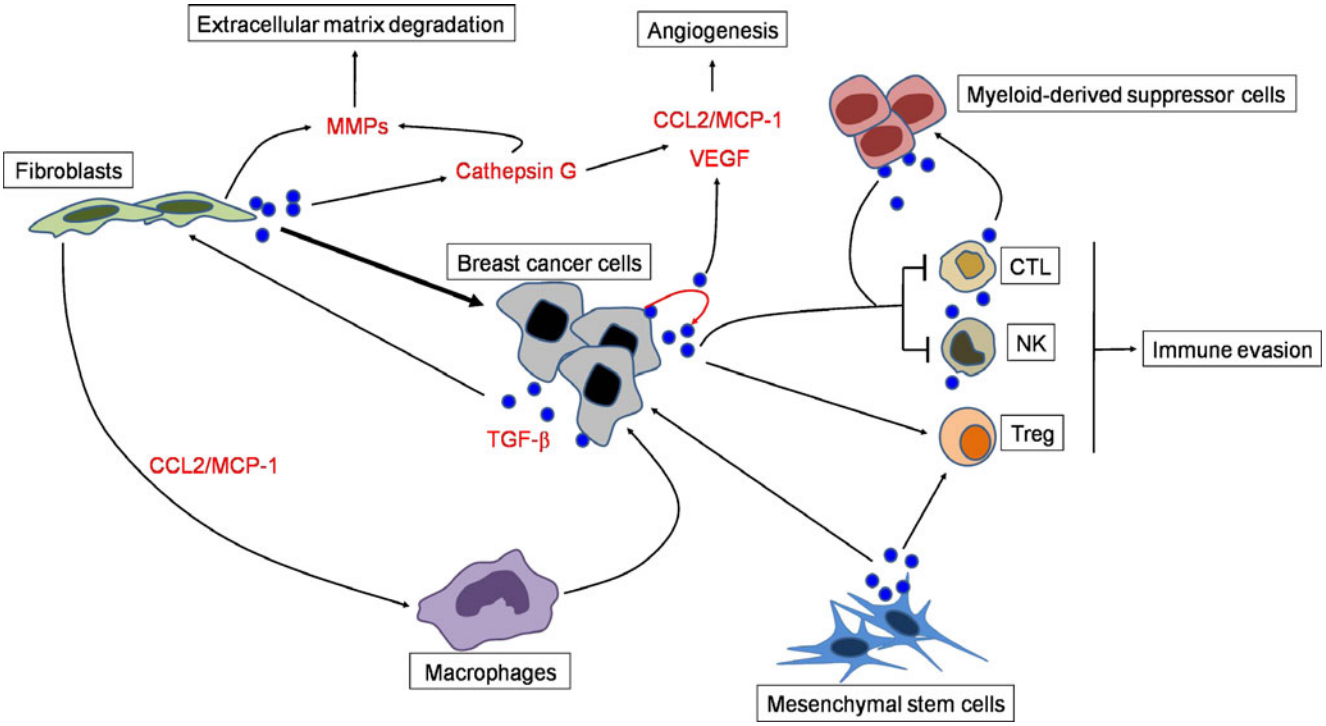

Fig. 2 TGF-β and tumor microenvironment. TGF-β is produced by cancer and stromal cells (e.g., fibroblasts and tumor-associated macrophages) and exerts immunosuppression against immune cells involved in the antitumor response, extracellular matrix degradation and angiogenesis. CCL2/MCP-1, cathepsin G, MMPs and VEGF are implicated in this process and contribute to TGF-β-induced cancer cell growth, migration and invasion. There exist positive loops of TGF-β signaling between cancer and stromal cells in the tumor microenvironment. *CTL* cytotoxic T lymphocytes, *Treg* regulatory T cells, *NK* natural killer cells

MAPK. In contrast, deficiency of $p130^{Cas}$ in MECs enhances TGF-β-stimulated Smad2/3 activity, restoring TGF-β-induced growth inhibition both in vitro and in mammary tumors produced in mice (Wendt et al. 2009b). Cdc42 GTPase-activating protein (CdGAP) is a serine- and proline-rich Rho GAP protein showing GAP activity against both Cdc42 and Rac1. CdGAP is phosphorylated by the MEK-ERK pathway in response to serum and is required for normal cell spreading and polarized lamellipodia formation. Recent studies have demonstrated that CdGAP protein and mRNA levels are highly upregulated by TGF-β, acting as a synergistic mediator of TGF-β and Neu/ErbB-2 signaling pathways in breast cancer cells (He et al. 2011). The proline-rich, not GAP, domain of CdGAP is essential to mediate TGF-β-induced cell motility and invasion. In addition, CD151 may regulate compartmentalization of TβRI/ALK-5 and specifically controls TGF-β-induced activation of p38 MAPK (Sadej et al. 2011).

Six1 homeodomain protein is a developmental transcription factor implicated in tumor onset and progression. This protein seems a critical switch of tumor suppression of TGF-β to tumor promotion. Six1 induces TβRI expression, activates TGF-β signaling and induces EMT in breast cancer development (Micalizzi et al. 2009, 2010). TMEPAI is a TGF-β target transmembrane protein overexpressed in several cancers. This protein may also function in breast cancer as a molecular switch of TGF-β as a tumor suppressor to a promoter (Singha et al. 2010).

Suppressors of TGF-β signaling

Endoglin (CD105) is a co-receptor of TGF-β and modulator of TGF-β signaling in endothelial cells. The loss of endoglin in MCF10A cells enhances migration and invasion into a 3D matrix induced by activated oncogenes. In contrast, ectopic expression of endoglin in endoglin-negative MDA-MB-231 cells blocks TGF-β-induced cell motility and invasion and reduces colonization of the cells in lung parenchyma in an in vivo metastasis study (Henry et al. 2010). c-Abl is a multifunctional non-receptor protein tyrosine kinase (PTK) that localizes at the plasma membrane, cytoplasm and nucleus. c-Abl is essential for maintaining normal MEC morphology and c-Abl inactivation results in morphological, transcriptional and migrational changes stimulated by TGF-β. Therefore, the measures enforcing c-Abl activation may represent a novel means to abrogate the oncogenic activity of TGF-β in breast cancer (Allington et al. 2009; Allington and Schiemann 2011).

Estrogen can disrupt the TGF-β signaling network in breast cancer cells via a G protein-coupled receptor 30 (GPR30)-mediated mitogen-activated protein kinase (MAPKs) pathway (Kleuser et al. 2008). A ligand-independent cross-talk between ER-alpha and TGF-β is identified in breast cancer cells and may influence the development and/or progression of breast cancer (Band and Laiho 2011; Ren et al. 2009; Stope et al. 2010). Finally, cystatin C (CystC) is found to be a novel antagonist of TGF-β signaling in normal and malignant cells, reducing murine and human cell responsiveness to TGF-β (Tian and Schiemann 2009).

MicroRNAs in TGF signaling

MicroRNAs are a group of small-molecule regulatory RNAs. Recent studies have revealed that microRNAs are implicated in TGF-β signaling. For instance, TGF-β regulates the sphere-initiating stem cell-like feature in breast cancer cells through miRNA-181 (miR-181) (Wang et al. 2010) and miR-155 may play an important role in TGF-β-induced EMT and cell migration and invasion by targeting RhoA (Kong et al. 2008). TGF-β up-regulates miR-21 that targets the 3' untranslated region of MSH2 mRNA and down-regulates its expression. Together with p53 (a transcriptional regulator of miR-21 and MSH2), TGF-β may affect the net output of MSH2 through the posttranscriptional mechanism (Yu et al. 2011).

TGF-β signaling and breast cancer therapy

The therapeutic potential of the TGF-β signaling pathway is ascribed to its supportive function in late stage tumors, i.e., the promotion of tumor invasion and metastasis, neoangiogenesis and escape of immunosurveillance (Pinkas and Teicher 2006). A number of approaches to inhibit TGF-β or its signaling effectors have shown promise in anticancer treatment. They are generally classified into four categories: (1) TGF-β expression inhibition at the translational level with antisense oligonucleotides (ASO); (2) blockade of TGF-β interaction with its receptors using monoclonal antibodies; (3) small molecule inhibitors; and (4) peptide aptamers to Smad proteins (Kelly and Morris 2011).

TGF-β neutralizing antibodies

GC1008, 1D11 and 2G7 are three high-affinity monoclonal antibodies capable of neutralizing all three TGF-β isoforms. In vitro, antibody 1D11 shows efficacy in reducing migration and invasion of MDA-MB-231 cells (Ganapathy et al. 2010) and in vivo, 1D11 demonstrates significant suppression activity towards the metastasis of 4T1 murine breast cancer cells to the lung (Nam et al. 2006; Tan et al. 2009). Antibody 1D11 also reduces mammary tumor-induced osteolysis at the tumor–bone interface (Futakuchi et al. 2009). It seems that 1D11 suppression on metastasis is attributed to the effect on both tumor parenchyma and microenvironment, increasing NK and T cell infiltrations and $CD8^+$ T cell functions (Nam et al. 2008b). Antibody 2G7 also reduces tumor growth and lung metastases of MDA-MB-231 cells and 4T1 carcinoma cells (Arteaga et al. 1993; Carano et al. 2004), as well as angiogenesis (Tan et al. 2009).

TGF-β receptor kinase inhibitors

Several different chemical classes of TβR kinase inhibitors have been developed and been shown to block TGF-β signaling in mammary epithelial cells at sub-micromolar concentrations (Kelly and Morris 2010). Ki26894, a TβRI kinase inhibitor, is a representative of small-molecule inhibitors. This chemical can block Smad2 phosphorylation and inhibit TGF-β responsive reporter activity. In vitro, Ki26894 reduces the motility and invasion of MDA-231-D cells induced by TGF-β and the systemic administration of Ki26894 1 day before the inoculation of MDA-231-D cells significantly decreased the bone metastasis in BALB/cnu/nu female mice. Moreover, Ki26894 can prolong the survival of mice inoculated with MDA-231-D cells (Ehata et al. 2007)

Other TβRI antagonists under investigation include LY364947, LY2157299, SB-431542, SD-093, SD-208, SM16 and IN-1130, all of which exhibit antitumor activity in vitro and in vivo. For instance, the antagonist LY364947 can significantly block TGF-β-induced cellular migration (Gauger et al. 2011) and systemic administration of LY364947 via intraperitoneal injection effectively reduces the number and size of lung metastasis in both orthotopic xenografts and experimental metastatic models of human breast carcinomas (Bandyopadhyay et al. 2006). LY2157299 inhibits breast tumor growth in an animal study (Bueno et al. 2008). Similarly, chemical SB-431542 inhibits the invasion of Ras-transformed mammary epithelial cells (Wiercinska et al. 2010) and SD-093 and SD-208 blocks TGF-β-induced phosphorylation of Smad2 and Smad3 in a dose-dependent manner. SM16 is a novel TβRI kinase inhibitor that inhibits Smad2 phosphorylation in cultured 4T1 cells and primary and metastatic 4T1 tumors (Rausch et al. 2009). In addition, the novel TβRI inhibitor IN-1130 can suppress renal and hepatic fibrosis and shows an anti-metastasis effect in breast cancer-bearing MMTV-*cNeu* mice (Kim et al. 2008).

LY2109761 is a TβRI and II inhibitor, blocking TGF-β-induced phosphorylation of Smads (Ganapathy et al. 2010). LY2109761 reduces the growth and invasion of HER2

mutant mammary epithelial cells in vitro (Wang et al. 2009) and synergistically enhances the anticancer activity of paclitaxel (Bauer et al. 2010).

Soluble TGF-β receptors

Recombinant DNA technology is used to generate the extracellular TGF-β-binding domain of TβRII fused to an IgG1 Fc domain (TβRIIFc). This TβRIIFc has stable high affinity to TGF-β and inhibits tumor cell motility, intravasation and metastasis (Muraoka et al. 2002). TβRIIFc also shows activity of inhibiting bone metastasis and osteolysis and thus may be an effective agent for the treatment of bone metastasis (Hu et al. 2010).

Other agents targeting TGF-β signaling

As a hormone secreted by the pineal gland in the brain, melatonin exerts an oncostatic action in estrogen-responsive breast cancer. Recent studies have indicated that melatonin induces a biphasic apoptotic response in MCF-7 breast cancer cells. Melatonin triggers early apoptosis of MCF-7 cells in a TGF-β-independent manner but the late apoptosis elicited by melatonin is a TGF-β-dependent process (Cucina et al. 2009).

Secreted clusterin acts as an important extracellular promoter of EMT and antibodies targeting secreted clusterin inhibit TGF-β-induced EMT of BRI-JM01 cells, as well as the invasive phenotype of several other breast and prostate tumor cell lines, such as 4T1, MDA-MB231LM2 and PC3 (Lenferink et al. 2010). In addition, TGF-β signaling often promotes metastasis by activating survival signals, such as epidermal growth factor receptors (Caja et al. 2007; Jechlinger et al. 2006). Therefore, Gleevec, a specific tyrosine kinase receptor inhibitor, can effectively block TGF-β-induced proliferation of human osteosarcoma cells (Matsuyama et al. 2003).

Side effects and safety concerns of TGF-β signaling targeted therapy

TGF-β is a multifunctional cytokine implicated in many pathophysiological processes. In particular, TGF-β plays a butterfly effect in the cancer development and progression. Therefore, side effects of TGF-β signaling targeted therapy are a critical concern and promising TGF-β signaling targeted agents successfully undergoing clinical trials are still limited after decades of efforts. Reported side effects of TGF-β signaling targeted therapy include generalized inflammation, autoimmune reactions and even increased tumorigenic risk and teratogenic fetal rates (Cheng et al. 2008; Kim et al. 2001). However, some promising experimental data have been reported in animal studies. Inflammatory phenotypes are observed in a dominant-negative TGF-β receptor type II expressing transgenic mouse model but not considerably in TGF-β signaling antagonist treatment. This may be ascribed to their inhibitory effect on excessive rather than basal homeostatic TGF-β activity (Gorelik and Flavell 2000). Side effects in mice are also acceptable in the treatment against metastasis with soluble type II TGF-β receptor: Fc fusion protein (Yang et al. 2002). Monoclonal antibody 1D11 causes only minimal immune parameter changes in mice (Ruzek et al. 2003). In humans, TβRI antagonist LY2157299 may induce premalignant skin lesions (Calvo-Aller et al. 2008). Nevertheless, in view of the limited information of the TGF-β signaling targeted therapy, it is too early to make any convincing conclusions about its safety. More extensive efforts are needed to define the safety issue.

Conclusion

In this review, we update the latest understanding of TGF-β signaling, with a focus on breast cancer. We also summarize the applications of the TGF-β signaling pathway for cancer treatment but the current understanding is limited in preclinical stages. In general, TGF-β signaling exerts oncogenic action at advanced mammary tumors via enhancing tumor cell invasion and metastasis, neoangiogenesis and escape of immunosurveillance. At this stage, TGF-β signaling is a potential target for cancer treatment. At the early stage of mammary tumorigenesis, however, TGF-β signaling often acts as a tumor suppressor and should not be targeted for tumor management. Specific antagonists have been developed to target various steps of TGF-β signaling and have shown promise in preclinical studies. Compared to small-molecule receptor kinase inhibitors, large-molecules (such as peptides and monoclonal antibodies) are characterized with specificity but limited tissue penetration is a potential concern for clinical applications. Small-molecule inhibitors penetrate cells better but tumor-selectivity and side effects are a more considerable concern. In addition, tumor stages are critical for TGF-β signaling targeted cancer intervention. Therefore, this is a major challenge and should be directed in future studies to circumvent shortcomings of these antagonists and develop criteria for patient selection for TGF-β signaling targeted treatment.

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