Role of TGFβ in regulation of the tumor microenvironment and drug delivery (Review)

PANAGIOTIS PAPAGEORGIIS1,2 and TRIANTAFYLLOS STYLIANOPOULOS1

1Cancer Biophysics Laboratory, Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia 1678; 2Department of Health Sciences, Program in Biological Sciences, European University Cyprus, Nicosia 1516, Cyprus

Received September 15, 2014; Accepted October 30, 2014

DOI: 10.3892/ijo.2015.2816

Abstract. Deregulation of cell signaling homeostasis is a predominant feature of cancer initiation and progression. Transforming growth factor β (TGFβ) is a pleiotropic cytokine, which regulates numerous biological processes of various tissues in an autocrine and paracrine manner. Aberrant activity of TGFβ signaling is well known to play dual roles in cancer, depending on tumor stage and cellular context. The crucial roles of TGFβ in modulating the tumor microenvironment, its contribution to the accumulation of mechanical forces within the solid constituents of a tumor and its effects on the effective delivery of drugs are also becoming increasingly clear. In this review, we discuss the latest advances in the efforts to unravel the effects of TGFβ signaling in various components of the tumor microenvironment and how these influence the generation of forces and the efficacy of drugs. We also report the implications of tumor mechanics in cancer therapy and the potential usage of anti-TGFβ agents to enhance drug delivery and augment existing therapeutic approaches. These findings provide new insights towards the significance of targeting TGFβ pathway to enhance personalized tumor treatment.

Contents

1. Introduction
2. TGFβ synthesis and activation
3. TGFβ signaling pathways
4. TGFβ signaling in cancer initiation and tumor progression
5. The effects of TGFβ on the tumor microenvironment
6. TGFβ, tumor desmoplasia and barriers to drug delivery
7. Therapeutic applications of TGFβ targeting
8. Conclusions and future perspectives

1. Introduction

The crucial role of transforming growth factor β (TGFβ) in tumor progression, metastasis and treatment has been well recognized and has become the topic of extensive research. Among the effects, TGFβ can regulate cancer cell proliferation, contribute to epithelial-to-mesenchymal transition (EMT), suppress the function of immune cells compromising immune response, contribute to the conversion of fibroblasts to myofibroblasts and cause overproduction of extracellular matrix (ECM) in the tumor. While it has been known for over two decades that anti-cancer drugs cannot penetrate deep into collagen-rich tumors (e.g., pancreatic cancers) and, more significantly, that depletion of collagen fibers can improve drug delivery, only recently TGFβ has become a target to reduce tumor fibrosis and thus, increase intratumoral drug concentration and treatment efficacy. Preclinical data of this new strategy are promising and it has already reached clinical trials. In this review, we first present a brief description of TGFβ synthesis and activation along with its signaling pathways. Following, we discuss the effects of TGFβ on tumor progression, its pathway alterations in cancer as well as its effects on EMT, immune cells function, fibroblasts behavior and ECM remodeling. Finally, based on the above, we review the barriers to the effective delivery of drugs caused by TGFβ and how regulation of TGFβ signaling can be employed to optimize delivery of therapeutic agents and overall survival (1-3).

2. TGFβ synthesis and activation

The TGFβ superfamily encompasses around 40 secreted cytokines, including TGFβ, bone morphogenetic proteins (BMPs), activins, nodal, lefty, myostatin, anti-Müllerian hormone (AMH) and growth differentiation factors (GDFs). These cytokines regulate a plethora of biological functions such as cell proliferation and apoptosis, embryonic patterning, stem cell maintenance, cell differentiation, migration and immune surveillance. Importantly, the effects of these factors are characterized as cell-type specific as well as context dependent (1-3). The TGFβ isoforms, with most common
being TGFβ1, 2 and 3, are initially synthesized as 75 kDa inactive homodimers, known as pro-TGFβ, which consist of TGFβ associated with latency-associated proteins (LAPs) at the N-terminal part of the pro-peptide. This is part of the TGFβ large latent complex (LLC), comprised of the LAPs and the latency TGFβ-binding proteins (LTBPs) (4-7), and is covalently associated to the ECM via the N-terminal region of LTBPs (8,9) (Fig. 1). While TGFβ is part of the LLC complex, it remains in an inactive form since the high affinity association of LAPs with TGFβ prevents the interaction with its receptors (10). During TGFβ activation, LAPs undergo conformational changes induced by thrombospondin-1 (TSP-1) (11,12) followed by cleavage mediated by furin convertase, plasmin or matrix metalloproteinases MMP-2/9 resulting in the release of the mature 24 kDa TGFβ dimer (13-15). The active ligand is then able to bind and activate TGFβ receptors (TGFβRs) to propagate downstream intracellular signaling events. Therefore, the processing of pro-TGFβ into the active TGFβ ligand is a critical regulatory step which determines its bioavailability.

3. TGFβ signaling pathways

The TGFβ and TGFβ-like cytokines mediate downstream intracellular signaling via the Smad family of proteins, which consists of eight human structurally related members (16-20) (Fig. 1). Smads can be functionally classified into three groups: the receptor activated Smads (R-Smads), which include Smad1, 2, 3, 5, 8; the common mediator Smad (Co-Smad), Smad4; and the inhibitory Smads (I-Smads), Smad6 and 7 (17-21). Three types of TGFβR isoforms are responsible for initiating signaling: TGFβRI, II and III. There are seven TGFβR isoforms known so far. TGFβR isoforms include activin receptor-like kinases 1-7 (ALK1-7), TGFβR isoforms include the TGFβRII, bone morphogenetic protein receptor II (BMPRII), activin receptor II (ACTRII), ACTRIIB, anti-Müllerian hormone receptor II (AMHRII), while betaglycan and endoglin belong to the TGFβRIIIIs (22) and mostly function as co-receptors to enhance activin signaling (23). In most tissues, TGFβ ligands function through heteromeric complex formation between two TGFβRI and two TGFβRII molecules. While both receptors possess Ser/Thr kinase activity, TGFβRII functions as the 'activator' and TGFβRI as the 'signal propagating' component (24). The TGFβRII-ALK5 complex transduces the signal from all three TGFβ isoforms in multiple cell types, whereas association of TGFβRI with ALK1 is involved in endothelial cells and with ALK2 in cardiovascular tissues (25). ALK5 activates Smad2 and 3 via the canonical TGFβ signaling pathway whereas ALK2, 3 and 6 can activate Smad1, 5 and 8, which are transducers of the BMP signaling pathway (26,27). The TGFβ signaling pathways can be classified in two major categories; the canonical or Smad-dependent and the non-canonical or Smad-independent pathways.

Canonical pathway (Smad-dependent). Even though TGFβ isoforms may elicit diverse cellular responses, they all activate signaling via a similar sequence of events. Binding of the active TGFβ ligand to the Ser/Thr kinase TGFβRII followed by recruitment of the ALK5 (TGFβRI) on the cell surface initiates intracellular signaling. Within the heterotetrameric receptor-ligand complex formed, TGFβRII phosphorylates TGFβRI allowing it to interact with the R-Smads (Smad2/3) which, in turn, become phosphorylated at the conserved SXSX C-terminal motif (28,29). Recruitment of R-Smads to the activated TGFβRI is facilitated by Smad anchor for receptor activation (SARA) protein (30). Subsequently, this triggers the formation of a heterotrimeric complex between phosphorylated R-Smads (Smad2/3 and Co-Smad (Smad4), which can translocate into the nucleus to regulate gene expression (3) (Fig. 1). Smads can differentially mediate gene expression by acting as transcription factors in co-operation with co-activators, such as p300/CREB-binding protein (CBP), p300/CBP-associated factor (PCAF), Smad4-interacting factor (SMIF), forkhead transcription factors 1, 3, 4 (FoxO1/3/4), specificity protein 1 (Sp1), c-Jun/c-Fos, Sertad1, or co-repressors, such as E2F4/5-p107, activating transcription factor 3 (ATF3), TGFβ-induced factor (TGIF), Ski, SnO1, forkhead transcription factor G1 (FoxG1), ecotropic viral integration site 1 protein (EVII) and C-terminal binding protein (CTBP) (28,31-47). In addition, Smads are able to epigenetically regulate gene expression either by inducing chromatin remodeling (48,49) or by maintaining DNA methylation and silencing of selected genes (50). Importantly, the I-Smad, Smad7, is a key target gene induced by TGFβ signaling and acts as negative feedback regulator of the pathway (51). In the absence of TGFβ stimulation, Smad7 resides in the cell nucleus and translocates to the plasma membrane upon TGFβ-mediated receptor activation (52). Smad7 is then able to interfere and block interactions between the R-Smads and the activated receptors to inhibit downstream signaling events (53). In addition, Smad7 can target the TGFβRs for proteasomal degradation via the E3-ubiquitin ligase Smurf1 and 2 (54,55). Finally, Smad7 antagonizes the formation of a functional Smad-DNA complex by directly binding to DNA via its MH2 domain and therefore blocks TGFβ-mediated transcriptional responses (56).

Non-canonical pathways (Smad-independent). It is also well established that TGFβ-mediated effects can also be exerted through non-canonical Smad-independent pathways (57). TGFβ has been shown to induce activation of Erk signaling in various tissues including epithelial and endothelial cells, fibroblasts, breast and colorectal cancer cells in order to promote disassembly of adherens junctions and cell migration (58-64). TGFβRI phosphorylation can recruit and activate ShcA, thus promoting the formation of a ShcA/Grb2/Sos complex. In turn, this complex is able to activate Ras on the plasma membrane followed by sequential activation of c-Raf, MEK and Erk (65).

Moreover, TGFβ can mediate the activation of the c-Jun N-terminal kinase (JNK) and p38/mitogen-activated protein kinase (MAPK) pathways, which are responsible for promoting apoptosis or cell migration depending on cellular context (66-68), via the mitogen-activated protein kinase kinase (MKK)4 and 3/6, respectively (69,70). Further upstream, MKKs are phosphorylated by the TGFβ-activated kinase 1 (TAK1) (71,72) which is recruited to the TGFβRs via the scaffold protein TNF receptor-associated factor 6 (TRAF6) (73,74). Besides TAK1, two other
mitogen-activated protein kinase kinase kinases (MAPKKKs), namely MEK1 and mixed lineage kinase 3 (MLK3), were also shown to mediate TGFβ-induced activation of JNK and p38-MAPK by MKK4 and 3/6 (75,76).

The Rho-like small GTPases, predominantly RhoA, Rac and cell division cycle 42 (cdc42), are additional molecules that mediate important TGFβ cellular functions, such as cytoskeletal organization, cell polarity, cell migration and gene expression (77). TGFβ is able to rapidly activate the RhoA and cdc42/Rac1 pathways, in a Smad2/3-independent manner, to promote actin polymerization, formation of stress fibers and EMT (78,79). TGFβ may also downregulate RhoA protein levels by recruitment of Par6 at the TGFβRI-II complex. Phosphorylation of Par6 by TGFβRII triggers binding of the E3 ligase Smurf1 to the complex followed by ubiquitination and degradation of RhoA at sites of cellular protrusions. Subsequently, this leads to the dissolution of tight junctions, rearrangement of actin cytoskeleton and EMT (80).

Some of the effects exerted by TGFβ could also be mediated by activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt (PI3K/Akt) pathway. This is evident from studies showing that TGFβ can rapidly induce PI3K activation followed by phosphorylation of its effector Akt to promote EMT, cell migration and survival (81,82). One of the most important effector molecules downstream of PI3K/Akt pathway appears to be the mammalian target of rapamycin (mTOR), a key regulator of protein synthesis, which can subsequently phosphorylate S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) (83). Activation of the mTOR pathway by TGFβ is thought to be important for regulating cell size, EMT and invasion (84) (Fig. 1).

4. TGFβ signaling in cancer initiation and tumor progression

It is well established that the multipotent actions of TGFβ are highly context dependent. The complexity of these functions is increased due to the fact that TGFβ exerts distinct effects depending on the tissue type as well as the genetic and epigenetic background of cells (85). It is clearly evident that
TGFB plays dual roles during carcinogenesis. In early stages TGFB promotes growth inhibition and apoptosis of normal epithelial and lymphoid cells as well as pre-malignant tumors, whereas during late stages TGFB acquires pro-oncogenic and pro-metastatic roles, which are associated with a progressive increase in the locally secreted TGFB levels (86-88). Therefore, one of the hallmarks of cancer is that the vast majority of cases exhibits insensitivity to TGFB-mediated growth inhibition.

**Regulation of cell proliferation.** It has long been noted that TGFB has a cytostatic effect on normal epithelial (89), endothelial (90,91) and neuronal cells (92) as well as certain cells of the immune system, such as T cells (93). These functions of TGFB are extremely important for physiological tissue homeostasis in order to restrain cell proliferation and prevent the generation of hyperproliferative disorders, like cancer. These anti-proliferative effects primarily control the G1/S phase transition events (94) and are mediated via induction of the cyclin-dependent kinase inhibitors CDKN2B (encoding p15/INK4B) (95), CDKN1A (encoding p21/Cip/Waf1) (96) and p27/Kip1 (97) by TGFB. Cell cycle arrest can also be achieved by repression of the proliferation-inducing transcription factors c-Myc (98) and the family of inhibitor of DNA-binding proteins ID1, 2 and 3 (36,99). On the other hand, the effects of TGFB in proliferation can be opposing, depending on the tissue type. It is also well recognized that TGFB enhances proliferation of fibroblasts (89) and it is often mediated indirectly by TGFB-induced connective tissue growth factor (CTGF) secretion, which is responsible for stimulating fibroblast proliferation and ECM synthesis (100). It is now unambiguously accepted that cancer-associated fibroblasts (CAFs) play critically important roles in the tumor microenvironment and cancer progression and their functions are further discussed below.

**Pathway alterations in human cancers.** Numerous human studies have identified that components of the TGFB pathway become genetically or epigenetically altered in various tumor types thus explaining, at least in part, the escape from TGFB-mediated growth control. Loss of function or truncating mutations in TGFβRI and TGFβRII as well as in Smad2 and Smad4 have been detected in colorectal, pancreatic, gastric and prostate cancers (18,101-105). In addition, loss of the 18q21 chromosome region, harboring the Smad4 gene, is commonly observed in ~60% of pancreatic and 30% of colorectal cancers (106-109) has been shown to promote angiogenesis and tumor growth by inducing vascular endothelial growth factor (VEGF) expression (60,110). However, in other tumor types like breast, the frequency of Smad gene mutations is rare (18,104,105) suggesting that alternative gene, is commonly observed in ~60% of pancreatic and 30% of breast cancer patients was found to suppress TGFB-mediated growth inhibition (114). Finally, another mechanism which TGFB may exploit in order to switch from a tumor suppressor to a metastasis-promoting factor is through differential regulation of the ID1 gene. While ID1 expression is suppressed by TGFB in normal tissues, it was found to be induced in patient-derived metastatic breast cancer cells (115).

**EMT and cancer metastasis.** EMT is an integral process during embryonic development which can be abnormally reactivated in adult tissues under pathological conditions, such as cancer and fibrosis (116). It involves the activation of a coordinated reversible transcriptional program whereby epithelial cells undergo dissolution of cell junctions, lose their polarity and epithelial characteristics concomitantly with acquisition of mesenchymal features and dramatic remodeling of their cytoskeleton. During this process, the expression of epithelial genes, such as E-cadherin, γ- and β-catenin, zonula occludens (ZO), and claudins is suppressed with concurrent expression of mesenchymal components, such as N-cadherin, vimentin, fibronectin and α-smooth muscle actin (α-SMA) (50,117,118). This program can be initiated by several pleiotropically acting transcription factors regulated by signaling pathways such as TGFB, Wnt and receptor tyrosine kinases (RTKs). Some of the better characterized examples include Snail (119), Slug (120), zinc-finger E-box binding homeobox 1 (ZEB1/SEF1) (121), zinc-finger E-box binding homeobox 2/Smad interacting protein 1 (ZEB2/SIP1) (122), Twist (117), high mobility group AT-hook 2 (HMGA2) (123) and forkhead box protein C2 (FOXC2) (124). In addition, recent studies indicate that overactive TGFB-TGFβR-Smad2 signaling axis could further contribute to the establishment of an EMT phenotype by maintaining the epigenetic silencing of epithelial genes during this process (50). Besides Smads, other signaling pathways have also been implicated in TGFB-induced EMT, including Erk, PI3K/Akt, RhoA, p38-MAPK and cofilin (125-127). Induction of EMT is one of the major mechanisms by which TGFB has been shown to promote cell motility, invasiveness and metastasis of cancer cells (128). EMT significantly enhances intravasation of carcinoma in situ cells through the basement membrane, survival in the circulation, extravasation at the distal tissues and formation of micrometastases in secondary organs (116,117,129).

**5. The effects of TGFB on the tumor microenvironment**

Under physiological conditions, the sustained local release of basal TGFB levels is sufficient to maintain normal tissue homeostasis. However, under conditions of tissue injury, the local TGFB secretion from stromal cells and blood platelets is rapidly increased to facilitate wound repair as well as to prevent uncontrolled regenerative cell proliferation and inflammation (130,131). A similar situation is commonly observed in pre-malignant tumors where TGFB is secreted in the microenvironment initially to control proliferation and cancer progression, but it is ultimately utilized by cancer cells to promote their malignant properties. Local TGFB release produces a tumor microenvironment which is conducive to tumor growth, invasion and metastasis (132). Secretion of TGFB can be derived from epithelial cancer cells thus regulating their own properties within the tumor mass in an autocrine or paracrine fashion (125). Moreover, infiltrating stromal cells, including fibroblasts, leukocytes, macrophages, bone-marrow derived endothelial, mesenchymal and myeloid...
precursor cells, is another major source of this cytokine (133). Finally, TGFβ can be stored in the ECM of the bone and can be activated during development of osteolytic metastatic lesions (134). In the following paragraphs, we summarize the effects of TGFβ on the main and better characterized components of the tumor microenvironment and particularly on fibroblasts, immune cells and the ECM.

Effect of TGFβ on immune cells. TGFβ exhibits immunosuppressive effects on all arms of the immune system because it functions as antagonist of several functions of the immune cells (132,135). As a result, the anti-tumor immune response is compromised, reducing cancer cell recognition and clearance. Specifically, TGFβ affects the function of natural killer cells, CD4+ and 8+ T cells, macrophages, neutrophils, dendritic, mast and B cells (136-138). Specifically, a TGFβ-rich tumor microenvironment is a suppressor of T-cell proliferation, reduces their effector function and inhibits the maturation of Th helper cells (137,139,140). It also induces macrophage M2 polarization from a type I to a type II phenotype, which hinders the suppression of monocyte-mediated cell death, reduces effector function and increases chemotaxis (141,142). Additionally, TGFβ induces an N2 neutrophil phenotype which, as with the macrophages, reduces effector function and increases secretion of inflammatory cytokines (143). Finally, high levels of TGFβ can cause apoptosis of B cells, inhibit the maturation of dendritic and natural killer cells and induce chemotaxis of mast cells (144-146). The combined immunosuppressive effects of TGFβ compromise the ability of the host to resist tumor progression and thus consist a barrier to immunotherapy.

Effect of TGFβ on fibroblasts. A primary role of TGFβ in modulating the tumor microenvironment is its contribution to the conversion of fibroblasts to myofibroblasts, also known as CAFs (147,148). Specifically, the compressive forces developed inside a tumor, due to its growth in the confined space of the host tissue, can facilitate the conversion of fibroblasts to proto-myofibroblasts. Subsequently, TGFβ increases the levels of collagens I and III and fibronectin, which promote cellular adhesion to extracellular fibers, and thus, enhances the communication of mechanical signals between the ECM of the tumor and the fibroblasts (149,150). As a result, the mechanical forces are more actively transmitted in the interior of the cell and contribute to the conversion of proto-myofibroblasts to differentiated myofibroblasts. Myofibroblasts are characterized by more extensively developed stress fibers in the cytoskeleton compared to proto-myofibroblasts, presumably to balance the extracellular forces, and by the de novo expression of α-SMA. The contraction of myofibroblasts is sustained by α-SMA stress fibers and it is regulated by Rho/ROCK signaling activation. The produced contractile forces remodel the ECM due to the ability of fibroblasts to stretch collagen fibers and produce ECM molecules (151,152). Additionally, these forces can be transmitted to the LLC via integrins. LLC is also bound to extracellular fibers (Fig. 1), which resists the pulling of the LLC by myofibroblasts and gives rise to a mechanically-induced liberation of TGFβ (147). The stiffer the ECM, the stronger the interactions among myofibroblasts, LLC and extracellular fibers and thus, the release of TGFβ becomes more pronounced. Therefore, myofibroblast contraction within a collagen-rich, and thus, stiff microenvironment further stimulates the release of active TGFβ from its latent form.

Effect of TGFβ on ECM. TGFβ upregulates the expression and synthesis of many matrix proteins, primarily through the recruitment of myofibroblast. Proteins upregulated by TGFβ include collagens I-V, basement membrane proteins (laminin, entactin, perlecain) and ECM proteins (fibronectin, osteopontin, thrombospondin, tenascin, osteonectin/SPARC, elastin, biglycan, decorin, and hyaluronan) (153). Additionally, in the early stages of carcinogenesis, TGFβ stimulates myofibroblasts and other stromal cells to enhance the synthesis of collagen crosslinking enzymes, particularly lysyl oxidase, which increases the rigidity of the collagen network (154). On the contrary, TGFβ downregulates the synthesis of matrix-depleting proteins, such as matrix metalloproteinases (MMP-1, -8, -13). As a result, the increase in matrix protein synthesis and decrease in matrix proteinase activity, owing to the TGFβ activity, contributes to the remodeling of the tumor ECM and can result in a fibrotic response, known as desmoplasia, which is commonly observed in many types of tumors and particularly in pancreatic, colon and breast cancers as well as in various sarcomas (155,156).

Tumor fibrotic response stiffens the tumor tissue, and as a result, it increases the compressive physical forces in the interior of the tumor (157). Compression of cancer cells alters their gene expression profile to enhance their invasive and metastatic phenotype (158,159). Furthermore, as mentioned previously, matrix stiffening along with the high contractile forces of myofibroblasts, cause further liberation of TGFβ from the LLC. These events suggest a positive feedback loop between TGFβ activation, myofibroblast contraction and ECM remodeling and production (Fig. 2A) (148). Finally, compression of intratumoral blood vessels reduces tumor perfusion, and thus, the delivery of oxygen (160). Hypo-perfusion and hypoxia, in turn contribute to immune-evasion, promote malignant progression and metastasis, and reduce the efficacy of a number of therapies including radiation treatment and systemic administration of chemo- and nanotherapy (161-163).

6. TGFβ, tumor desmoplasia and barriers to drug delivery

The desmoplastic reaction of solid tumors hinders all three transport steps of the systemic delivery of drugs, namely vascular, transvascular and interstitial transport (156,163). As mentioned above, increased levels of collagen in the ECM, result in intratumoral blood vessel compression and hypo-perfusion. Hypo-perfusion, in turn, reduces the concentration of the drug that can reach the tumor site. Apart from compromised drug delivery, hypo-perfusion also decreases the supply of oxygen rendering the tumor hypoxic, which in turn reduces the efficacy of radiation therapy. Additionally, desmoplasia reduces the hydraulic conductivity of the tumor interstitial space, i.e., the ease with which the interstitial fluid percolates through the interstitial space of a tissue. High hydraulic conductivity allows fluid to rapidly flow in the interstitial space and be drained by peripheral lymphatic vessels. The accumulation of collagen and other ECM proteins in tumors decrease the available spaces for interstitial fluid flow and because the fluid cannot freely
move, the interstitial fluid pressure (IFP) increases. Interstitial hypertension is a hallmark of tumor pathophysiology. IFP reaches and even exceeds micro-vascular fluid pressure, which eliminates pressure gradients across the tumor vessel wall and thus, the transvascular transport of drugs (164). Therefore, the only mechanism of transport is through diffusion (i.e., due to a concentration difference), which is inversely proportional to the size of the therapeutic agent. Chemotherapeutic agents, with a size <1 nm, are able to diffuse fast and exit the tumor vasculature. Nanoparticles, however, with sizes >60 nm cannot effectively extravasate into the tumor interstitial space (165).

Furthermore, the dense interstitial matrix of desmoplastic tumors hinders the homogeneous distribution of large nanoparticles. As with transvascular transport, nanoparticles with a size >60 nm often cannot penetrate deep into the tumor because their size is comparable to the size of the pores of the interstitial collagen network and they often get trapped (166). Therefore, even if large nanoparticles extravasate from the leaky vessels of the tumor, they will not be able to effectively diffuse into the tissue but they will concentrate in the perivascular regions, causing only local effects. Apart from these steric interactions between the interstitial matrix and nanoparticles, the increased levels of collagen and hyaluronan give rise to electrostatic interactions. Indeed, hyaluronan has a highly negative charge, while collagen fibers carry a slight positive charge. Nanoparticles of a non-neutral surface charge density can be attracted electrostatically and bind to these proteins, which further inhibits their uniform delivery inside the tumor (167).

7. Therapeutic applications of TGFβ targeting

Pharmacological inhibition of TGFβ has been used in preclinical and clinical studies as a therapeutic strategy to either hinder tumor progression directly or modify the tumor microenvironment in order to improve perfusion and drug delivery and thus, increase indirectly the efficacy of the treatment. There is a large number of TGFβ inhibitory drugs employed in these studies (137). Particularly, targeting with TGFβ agents (e.g., 1D11, AP12009, SD-208) as well as non-specific
targeting with other TGFβ inhibitory drugs (e.g., tranilast) have shown to reduce tumor progression and metastasis in vivo, mainly owing to augmentation of the immune response and inhibition of EMT (132,168-171). However, there are also studies that relate inhibition of TGFβ with promotion of tumor progression owing to an increase in inflammatory cell infiltration (172). Particularly, it has been shown that inflammatory infiltrates mediate the pro-tumorigenic functions of fibroblasts that lack TGFβ signalling. Clinical trials for the use of TGFβ inhibitory drugs have been in progress (ClinicalTrials.gov identifiers: NCT00368082, NCT01582269 and NCT00844064), but their results are not conclusive yet, presumably owing to differences in the degree of desmoplasia among tumor types or even among tumors of the same type, but also owing to the various effects of TGFβ on tumor biology.

Targeting of TGFβ to reduce desmoplasia has the ability to alleviate physical forces in tumors, decompress tumor blood vessels and improve perfusion (Fig. 2B) (160). Restoration of tumor perfusion, however, can increase nutrients supply to the tumor, and thus, increase its growth rate. Also, the decompressed vessels could allow more metastatic cells to leave the primary tumor. Indeed, in some cases, inhibition of TGFβ has been shown to facilitate tumor progression and metastases in mouse tumor models (173,174), whereas other studies, not related to TGFβ, have shown a correlation between improved perfusion and increased metastases (175,176). Therefore, based on this rationale, judicious doses of TGFβ inhibitory drugs should be used to alleviate physical forces, decompress blood vessels and improve perfusion when these agents are combined with cytotoxic treatments, such as chemo-, nano-, immuno- and radiotherapy. In these combined treatments the role of the anti-TGFβ drug is to enhance the delivery of the cytotoxic agent and thus, optimize its efficacy. This therapeutic strategy is known as stress alleviation treatment (156,163,165).

Detailed in vivo studies have shown that re-purposing the anti-hypertensive, angiotensin receptor blocker (ARB) drug losartan reduced expression of TGFβ1 and decreased stromal collagen and hyaluronan production, in doses that did not affect blood pressure. Reduction of collagen and hyaluronan, in turn, reduced stress levels in the tumor decompressing intratumoral blood vessels and improving perfusion. Furthermore, reduction of the ECM components improved the interstitial fluid flow and thus, reduced levels of IFP. Improved perfusion and reduced IFP enhanced the delivery and efficacy of chemotherapy in orthotopic breast and pancreatic murine tumor models (160). Also, in another study combined treatment of mice bearing tumors with losartan and nanomedicine (Doxil) increased the distribution of the drug and the overall survival of the mice (177). Furthermore, retrospective analyses of clinical data have shown increased survival in patients with lung or renal cancers treated with ARBs (178,179). Similar retrospective analysis has shown that patients with pancreatic ductal adenocarcinomas (PDACs) receiving ARBs survived ~6 months longer than those who did not (180). These preclinical and clinical data have led to a phase II clinical trial with losartan and FOLFIRINOX in PDAC patients (ClinicalTrials.gov identifier NCT01821729). Apart from the use of ARBs, the TGFβ neutralizing antibody ID11 improved the distribution and efficacy of therapeutics in breast carcinomas by reducing the tumor stroma (181). Additionally, re-purposing the drug pifrenidone, a TGFβ inhibitor clinically approved for the treatment of idiopathic pulmonary fibrosis, was shown to suppress desmoplasia in mice bearing pancreatic tumors and improve the efficacy of chemotherapy (182). Apart from chemotherapy, radiation therapy has been also improved after treatment with TGFβ inhibitors. Efficacy of radiotherapy depends on the oxygenation of the tissue, which is regulated by tumor perfusion (183,184).

8. Conclusions and future perspectives

Owing to the pleiotropic effects of TGFβ on tumor microenvironment and progression, targeting TGFβ signaling to directly treat tumor growth remains controversial. Recent studies have suggested an alternative therapeutic strategy, which involves the use of anti-TGFβ agents in a stress alleviation treatment. The scope of this strategy is to hinder but not completely inhibit the activation of TGFβ ultimately aiming to reduce tumor desmoplasia and particularly the levels of collagen. As described in this review, reduced collagen levels can lead to improved delivery of both chemo- and nano-therapeutics by alleviating mechanical forces and decompressing intratumoral blood vessels. Thus, blocking of TGFβ can improve indirectly the efficacy of conventional treatments. It is promising that many anti-TGFβ agents exist that are already clinically approved for other diseases (e.g., ARBs for hypertension). Re-purposing of these drugs can lead to more effective anti-cancer therapies. Therefore, we need to identify safe and well-tolerated pharmaceuticals that may complement the treatment regimen of cancer patients. Anti-TGFβ agents are not the only drugs that have the ability to modify the tumor microenvironment. In principle, any clinically approved agent that has the ability to reduce collagen levels could be employed as an alternative strategy. Also, collagen is not the only target for the stress alleviation treatment. Reduction of stromal cells or hyaluronan has also the potential to enhance drug delivery through the same mechanism (157).

Acknowledgements

We thank Dr Christiana Polydorou for useful discussions. This study received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement no. 336839-ReEngineeringCancer.

References

1. Derynck R and Akhurst RJ: Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol 9: 1000-1004, 2007.
2. Wakefield LM and Hill CS: Beyond TGFβ: roles of other TGFβ superfamily members in cancer. Nat Rev Cancer 13: 328-341, 2013.
3. Massagué J, Seoane J and Wotton D: Smad transcription factors. Genes Dev 19: 2783-2810, 2005.
4. Annes JP, Munger JS and Rifkin DB: Making sense of latent TGFβ activation. J Cell Sci 116: 217-224, 2003.
5. Gleizes PE, Beavis RC, Mazzieri R, Shen B and Rifkin DB: Identification and characterization of an eight-cysteine repeat of the latent transforming growth factor-beta binding protein-1 that mediates bonding to the latent transforming growth factor-beta1. J Biol Chem 271: 29891-29896, 1996.
6. Miyazkondo K, Olofsson A, Colosetti P, and Heldin CH: Role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. EMBO J 10: 1091-1101, 1991.

7. Arikawa Y, Takai N, and Keshi-Oja J: Association of the small latent transforming growth factor-beta with an eight cysteine repeat of its binding protein LTBP-1. EMBO J 15: 245-253, 1996.

8. Unsöld C, Hyytiäinen M, Bruckner-Tuderman L, and Keshi-Oja J: Latent TGF-beta binding protein LTBP-1 contains three potential extracellular matrix interacting domains. J Cell Sci 114: 187-197, 2001.

9. Nunes I, Gleizes PE, Metzen C, and Rifkin DB: Latent transforming growth factor-beta binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-beta. J Cell Biol 136: 1151-1163, 1997.

10. Derynck R, Pozas M, and Kiyonari J: Normal embryo fibroblasts release transforming growth factors β-like molecules by plasmin during coculture. J Cell Biol 109: 303-315, 1989.

11. Yu Q and Stamenkovic I: Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 14: 163-176, 2000.

12. Derynck R, Zhang Y, and Feng X: Smads: transcriptional activators of TGF-beta responses. Cell 95: 737-740, 1998.

13. Massagué J: TGF-beta signal transduction. Annu Rev Biochem 67: 10618-10624, 1999.

14. Sato Y and Rifkin DB: Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor beta-1-like molecule by plasmin during coculture. J Cell Biol 109: 303-315, 1989.

15. Dubois CM, Laprise MH, Blanchette F, Gentry LE, and Leduc R: Processing of transforming growth factor beta 1 precursor by human furin convertase. J Biol Chem 270: 10618-10624, 1995.

16. Shimizu K, Hyytiäinen M, Bruckner-Tuderman L, and Kiyonari J: Normal embryo fibroblasts release transforming growth factors β-like molecules by plasmin during coculture. J Cell Biol 109: 303-315, 1989.

17. Massagué J: TGF-beta signal transduction. Annu Rev Biochem 67: 753-791, 1998.

18. Riggins GJ, Thiagalingam S, Rozenblum E, Riggins GJ, et al: Identification of Smad7, a Smad family member induced by TGF-beta signals, as a negative regulator of TGF-beta signaling. Nature 389: 631-635, 1997.

19. Sun Y, Liu X, Eaton EN, Lane WS, Lodish HF, and Weinberg RA: Interaction of the Ski oncoprotein with Smad3 regulates TGF-beta signaling. Nat Cell Biol 4: 181-190, 2002.

20. Liu K, Luo X, and Massagué J: The Smad transcriptional corepressor TGIF recruits Smad3. Cell Growth Diff 12: 457-463, 2001.

21. Derynck R, Zhang Y, and Feng X: Smads: transcriptional activators of TGF-beta responses. Cell 95: 737-740, 1998.

22. Akiko T, Nishimura M, Bruckner-Tuderman L, and Kiyonari J: Normal embryo fibroblasts release transforming growth factors β-like molecules by plasmin during coculture. J Cell Biol 109: 303-315, 1989.

23. Heldin CH, Miyazkondo K, and Ten Dijke P: TGF-beta signalling from cell membrane to nucleus through Smad proteins. Nature 383: 832-836, 1996.

24. Nakao A, Immamura T, Tsouchelnitsky S, and Yamauchi T: TGF-beta-receptor mediated signal transduction through Smad2, Smad3 and Smad4. EMBO J 16: 5353-5362, 1997.

25. Heldin CH, Miyazkondo K, and Ten Dijke P: TGF-beta signalling from cell membrane to nucleus through Smad proteins. Nature 390: 465-471, 1997.

26. Biere B and Moses HL: Tumour microenvironment: TGFbeta: the molecule. J Clin Pathol 50: 506-520, 2006.

27. Lewis KA, Gray PC, Blount AL, et al: Smad nuclear translocation is required for tissue-specific expression of a poised chromatin platform for TGF-β access to master regulators. Cell 147: 1511-1524, 2011.

28. Ross S, Cheung E, Petrakis TG, Howell M, Kraus WL, and Hill CS: Smads orchestrate specific histone modifications and chromatin remodelling to activate transcription. EMBO J 25: 4490-4502, 2006.

29. Papageorgis P, Lambert AW, Ozturk S, et al: Smad signaling is required to maintain epigenetic silencing during breast cancer progression. Cancer Res 70: 968-978, 2010.

30. Nakao A, Afraikhte M, Moren A, et al: Identification of Smad7, a latent TGF-beta-inducible and Smad7 antagonist transforming growth factor beta signal transducer. J Biol Chem 278: 27678-27685, 2003.

31. Derynck R and Zhang Y: Smad-dependent and Smad-independent pathways in TGF-beta family signaling. Nat Rev 425: 577-584, 2002.

32. Sabolala S, Macias-Silva M, Tsukazaki T, Hayashi H, Attisano L, and Wrana JL: TGF-beta1 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

33. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

34. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

35. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

36. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

37. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

38. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

39. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

40. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

41. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

42. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.

43. Zhang Y, Feng X, and Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature 383: 168-172, 1996.

44. Tsukazaki T, Chiang TA, Davison AF, Attisano L, and Wrana JL: Smad2 phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signalling. J Biol Chem 272: 27678-27685, 1997.
57. Zhang YE: Non-Smad pathways in TGF-beta signaling. Cell Res 19: 128-139, 2009.

58. Hartsoog MT and Mulder KM: Transforming growth factor beta activates rhoa in proliferating cultures of epithelial cells. J Biol Chem 270: 7117-7124, 1995.

59. Frey RS and Mulder KM: TGFbeta regulation of mitogen-activated protein kinases in human breast cancer cells. Cancer Lett 117: 41-50, 1997.

60. Papageorgis P, Cheng K, Ozturk S, et al: Smad4 inactivation promotes malignancy and drug resistance of colon cancer. Cancer Res 71: 98-1008, 2011.

61. Finlay GA, Thannickal VJ, Fambarg BL and Paulson KE: Transforming growth factor-beta 1-induced activation of the ERK pathway/activator protein -1 in human lung fibroblasts requires the increased induction of basic fibroblast growth factor. J Biol Chem 275: 27650-27656, 2000.

62. Vinals F and Pouyssegur J: Transforming growth factor beta (TGF-beta) promotes endothelial cell survival during in vitro angiogenesis via an autocrine mechanism implicating TGF-alpha signaling. Mol Cell Biol 21: 7218-7230, 2001.

63.Ellenrieder V, Hendler SF, Boeck W, et al: Transforming growth factor beta1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extra-cellular signal-regulated kinase 2 activation. Cancer Res 61: 4222-4227, 2001.

64. Xie L, Law BK, Chytil AM, Brown KA, Aakre ME and Moses HL: Activation of the Erk pathway is required for TGF-beta-induced EMT in vitro. Neoplasia 6: 603-610, 2004.

65. Lee MK, Pardoux C, Hall MC, et al: TGF-beta activates Erk MAP kinase signaling through direct phosphorylation of ShcA. EMBO J 16: 3957-3967, 2007.

66. Liao JH, Chen JS, Chai MQ, Zhao S and Song JG: The involvement of p38 MAPK in transforming growth factor beta-induced apoptosis in murine hepatocytes. Cell Res 11: 89-94, 2001.

67. Kimura N, Matsuo R, Shibuya H, Nakashima K and Taga T: BMP2-induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6. J Biol Chem 275: 17647-17652, 2000.

68. Bakin AV, Rinehart C, Tomlinson AK and Arteaga CL: p38 mitogen-activated protein kinase is required for TGF-beta-mediated fibroblast transdifferentiation and cell migration. J Cell Sci 115: 3193-3206, 2002.

69. Hocevar BA, Brown TL and Howe PH: TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. EMBO J 18: 1345-1356, 1999.

70. Yu L, Hébert M, Zhang YE: TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. EMBO J 20: 3749-3753, 2001.

71. Yamaguchi K, Shirakabe K, Shibuya H, et al: Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science 270: 2008-2011, 1995.

72. Liao JH, Flegel T, Paschal AE, et al: p38 MAPK, but not TAK1 or TAB2, plays an essential role in multiple signaling pathways in vivo. Genes Dev 19: 2668-2681, 2005.

73. Sorrentino A, Thakur N, Grimsby S, et al: The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. Nat Cell Biol 10: 1199-1207, 2008.

74. Yamashita M, Fatyol K, Jin C, Wang X, Liu Z and Zhang YE: TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. Mol Cell 31: 918-924, 2008.

75. Zhang L, Wang W, Hayashi Y, et al: A role for MEK kinase 1 in TGF-beta/activin-induced epithelial morphogenesis and embryonic cell differentiation through a rhoA-dependent mechanism. Mol Biol Cell 12: 4433-4434, 2001.

76. Kim KY, Kim BC, Xu Z and Kim SJ: Mixed lineage kinase 3 (MLK3)-activated p38 MAP kinase mediates transforming growth factor-beta-induced apoptosis in hematopoietic cells. J Biol Chem 279: 29478-29484, 2004.

77. Jaffe AB and Hall A: Rho GTases: biochemistry and biology. Annu Rev Cell Dev Biol 21: 247-269, 2005.

78. Bhowmick NA, Giassi M, Bakin A, et al: Transforming growth factor beta mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. Mol Biol Cell 12: 27-36, 2001.

79. Edlund S, Landström S, Heldin CH and Aspénstrom P: Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA. Mol Biol Cell 13: 902-914, 2002.

80. Omarizad-Razavi IA, Wang HR, Zhang Y and Wranas J: Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. Science 307: 1603-1609, 2005.

81. Bakin AV, Tomlinson AK, Bhowmick NA, Moses HL and Arteaga CL: Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. J Biol Chem 275: 36083-36080, 2000.

82. Shin I, Bakin AV, Rodeck U, Brunet A and Arteaga CL: Transforming growth factor beta enhances epithelial cell survival via Akt-dependent regulation of FKHR1. Mol Biol Cell 12: 3328-3339, 2001.

83. Hidalgo M and Rowinsky EK: The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene 19: 6680-6686, 2000.

84. Lamouille S and Derynck R: Cell size and invasion in TGF-beta-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. J Cell Biol 178: 437-451, 2007.

85. Roberts AB and Wakefield LM: The two faces of transforming growth factor beta in carcinogenesis. Proc Natl Acad Sci USA 100: 8621-8623, 2003.

86. Tang B, Yu M, Booker T, et al: TGF-beta switches from tumor suppressor to prometastatic factor in a model of breast cancer progression. J Clin Invest 112: 1116-1124, 2003.

87. Wakefield LM and Roberts AB: TGF-beta signaling: positive and negative effects on tumorigenesis. Curr Opin Genet Dev 12: 429-220, 2002.

88. Siegel PM, Shu W, Cardiff RD, Muller WJ and Massague J: Transforming growth factor beta signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis. Proc Natl Acad Sci USA 100: 8430-8435, 2003.

89. Siegel PM and Massague J: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 3: 807-821, 2003.

90. Choi ME and Ballermann BJ: Inhibition of capillary morphogenesis and associated apoptosis by dominant negative mutant transforming growth factor-beta receptors. J Biol Chem 270: 21144-21150, 1995.

91. Hyman KM, Seghezzi G, D'Antonio GA, et al: Transforming growth factor-beta induces apoptosis in vascular endothelial cells by activation of mitogen-activated protein kinase. Surgery 132: 173-179, 2002.

92. Rich NJ, Zhang M, Datto MB, Bigner DD and Wang XF: Transforming growth factor-beta-mediated p15INK4B induction and growth inhibition in astrocytes are SMAD3-dependent and a pathway prominently altered in human glioma cell lines. J Biol Chem 278: 35053-35058, 1999.

93. Yang X, Letterio JJ, Lechleider RJ, et al: Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J 18: 1280-1291, 1999.

94. Laiho M, Kallio T, et al: TGF-beta 1 inhibition of CD4+ T cell responses to TGF-beta. EMBR J 18: 1280-1291, 1999.

95. Laiho M, DeCaprio JA, Ludovico GW, Livingstone DM and Massague J: TGF-beta1 inhibition of T cell responsiveness. Cell 83: 2114-21150, 1995.

96. Hyman KM, Seghezzi G, D'Antonio GA, et al: Transforming growth factor-beta induces apoptosis in vascular endothelial cells by activation of mitogen-activated protein kinase. Surgery 132: 173-179, 2002.

97. Rich NJ, Zhang M, Datto MB, Bigner DD and Wang XF: Transforming growth factor-beta-mediated p15INK4B induction and growth inhibition in astrocytes are SMAD3-dependent and a pathway prominently altered in human glioma cell lines. J Biol Chem 278: 35053-35058, 1999.

98. Yang X, Letterio JJ, Lechleider RJ, et al: Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J 18: 1280-1291, 1999.

99. Laiho M, Kallio T, et al: TGF-beta 1 inhibition of CD4+ T cell responses to TGF-beta. EMBR J 18: 1280-1291, 1999.

100. Laiho M, Kallio T, et al: TGF-beta 1 inhibition of CD4+ T cell responses to TGF-beta. EMBR J 18: 1280-1291, 1999.
104. Markowitz S, Wang J, Myeroff L, et al.: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 268: 1336-1338, 1995.

105. Riggin GE, Kinzler KW, Vogelstein B and Thiagalingam S: Frequency of Smad gene mutations in human cancers. Cancer Res 57: 2578-2580, 1997.

106. Eppert K, Scherer SW, Ozcelik H, et al.: MIDR2 maps to 18q21 and encodes a TGF-beta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 86: 543-552, 1996.

107. Hahn SA, Hoque AT, Moskaluk CA, et al.: Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res 56: 490-493, 1996.

108. Hahn SA, Schutte M, Hoque AT, et al.: DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271: 350-353, 1996.

109. Thiagalingam S, Lengauer C, Leach FS, et al.: Evaluation of candidate tumor suppressor genes on chromosomes 18 in colorectal carcinomas. Nat Genet 13: 343-346, 1996.

110. Schwartz-Waldhoff I, Volpert OV, Bouck NP, et al.: Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. Proc Natl Acad Sci USA 97: 9624-9629, 2000.

111. Kretzschmar M, Doodj, Timokhina I and Massagué J: A mechanism of repression of TGF-beta signaling by oncogenic Ras. Genes Dev 13: 804-816, 1999.

112. Kretzschmar M, Doodj and Massagué J: Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad. Nature 389: 618-622, 1997.

113. Massagué J, Doolabh, and MAPK pathways: a link and a linker revisited. Genes Dev 17: 2993-2997, 2003.

114. Gomis RR, Alarcón C, Nadal C, Van Poznak C and Massagué J: C/EBPbeta at the core of the TGFbeta cytosstatic response and its evasion in metastatic breast cancer cells. Cancer Cell 10: 203-214, 2006.

115. Padua D, Zhang XH, Wang Q, et al.: TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. Cell 133: 66-77, 2008.

116. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442-445, 2002.

117. Yang J, Mani SA, Donaher JL, et al.: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117: 927-939, 2004.

118. Xu J, Lamouille S and Derynck R: TGF-beta-induced epithelial to mesenchymal transition. Cell 19: 156-172, 2009.

119. Comijn J, Berx G, Vermassen P, et al.: The two-handed E box binding zinc finger protein SPI1 downregulates E-cadherin and induces invasion. Mol Cell 7: 1267-1278, 2001.

120. Thuault S, Valcourt U, Petersen M, Manfioletti G, Heldin CH and Moustakas A: Transforming growth factor-beta employs EGF signalling pathways to induce alternative M2 mononuclear phagocytes. Trends Immunol 23: 549-555, 2002.

121. Yamanaka Y, Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C and Brown RA: TGFbeta. Nat Rev Drug Discov 11: 790-811, 2012.

122. Derynck R, Scherz MD and Weaver VM: Dynamic interplay between the collagen scaffold and tumor evolution. Cell 174: 1311-1323, 2017.

123. Wrzesinski SH, Svejda JA, Koo K, et al.: TGF-beta signaling pathway in disease. Nat Rev Drug Discov 11: 790-811, 2012.

124. Derynck R, Scherz MD and Weaver VM: Dynamic interplay between the collagen scaffold and tumor evolution. Cell 174: 1311-1323, 2017.
156. Stylianopoulos T and Jain RK: Combining two strategies to improve perfusion and drug delivery in solid tumors. Proc Natl Acad Sci USA 110: 18632-18637, 2013.

157. Stylianopoulos T, Martin JD, Chauhan VP, et al: Causes, consequences, and remedies for growth-induced solid stress in murine and human tumors. Proc Natl Acad Sci USA 109: 15101-15108, 2012.

158. Demou ZN: Gene expression profiles in 3D tumor analogs indicate compressive strain differentially enhances metastatic potential. Ann Biomed Eng 38: 3509-3520, 2010.

159. Tsu JM, Cheng G, Tyrrell JA, et al: Mechanical compression drives cancer cells toward invasive phenotype. Proc Natl Acad Sci USA 109: 911-916, 2012.

160. Chauhan VP, Martin JD, Liu H, et al: Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumor blood vessels. Nat Commun 4: 2516, 2013.

161. Facciabene A, Peng X, Hagemann IS, et al: Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. Nature 475: 226-230, 2011.

162. Wilson WR and Hay MP: Targeting hypoxia in cancer therapy. Nat Rev Cancer 11: 393-410, 2011.

163. Jain RK, Martin JD and Stylianopoulos T: The role of mechanical forces in tumor growth and therapy. Annu Rev Biomed Eng 16: 321-346, 2013.

164. Jain RK and Stylianopoulos T: Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 7: 653-664, 2010.

165. Chauhan VP and Jain RK: Strategies for advancing cancer nanomedicine. Nat Mater 12: 958-962, 2013.

166. Popović Z, Liu W, Chauhan VP, et al: A nanoparticle size series for in vivo fluorescence imaging. Angew Chem Int Ed Engl 49: 8649-8652, 2010.

167. Stylianopoulos T, Poh MZ, Insin N, et al: Diffusion of particles in the extracellular matrix: the effect of repulsive electrostatic interactions. Biophys J 99: 1342-1349, 2010.

168. Zhong Z, Carroll KD, Policarpo D, et al: Anti-transforming growth factor beta receptor II antibody has therapeutic efficacy against primary tumor growth and metastasis through multi-effects on cancer, stroma, and immune cells. Clin Cancer Res 16: 1191-1205, 2010.

169. Uhl M, Aulwurm S, Wischhusen J, et al: SD-208, a novel transforming growth factor beta receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo. Cancer Res 64: 7954-7961, 2004.

170. Kim S, Buchlis G, Fridlender ZG, et al: Systemic blockade of transforming growth factor-beta signaling augments the efficacy of immunogene therapy. Cancer Res 68: 10247-10256, 2008.

171. Chakrabarti R, Subramaniam V, Abdalla S, Jothy S and Prud’homme GF: Tranilast inhibits the growth and metastasis of mammary carcinoma. Anticancer Drugs 20: 334-345, 2009.

172. Achyut BR, Bader DA, Robles AI, et al: Inflammation-mediated genetic and epigenetic alterations drive cancer development in the neighboring epithelium upon stromal abrogation of TGF-β signaling. PLoS Genet 9: e1003251, 2013.

173. Bragado P, Estrada Y, Parikh F, et al: TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signalling. Nat Cell Biol 15: 1351-1361, 2013.

174. Biswas T, Gu X, Yang J, Ellies LG and Sun LZ: Attenuation of TGF-β signaling supports tumor progression of a mesenchymal-like mammary tumor cell line in a syngeneic murine model. Cancer Lett 346: 129-138, 2014.

175. Stockmann C, Doedens A, Weidemann A, et al: Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. Nature 456: 814-818, 2008.

176. Rhim AD, Mirek ET, Aiello NM, et al: EMT and dissemination precede pancreatic tumor formation. Cell 148: 349-361, 2012.

177. Diop-Frimpong B, Chauhan VP, Krane S, Boucher Y and Jain RK: Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanotherapeutics in tumors. Proc Natl Acad Sci USA 108: 2909-2914, 2011.

178. Wilpos S, von Hobe S, Crysandt M, Esser A, Osieka R and Jost E: Impact of angiotensin I converting enzyme inhibitors and angiotensin II type 1 receptor blockers on survival in patients with advanced non-small-cell lung cancer undergoing first-line platinum-based chemotherapy. J Cancer Res Clin Oncol 135: 1429-1435, 2009.

179. Keizman D, Huang P, Eisenberger MA, et al: Angiotensin system inhibitors and outcome of sunitinib treatment in patients with metastatic renal cell carcinoma: a retrospective examination. Eur J Cancer 47: 1955-1961, 2011.

180. Nakai Y, Isayama H, Iijichi H, et al: Phase I trial of gemcitabine and candesartan combination therapy in normotensive patients with advanced pancreatic cancer: GECA1. Cancer Sci 103: 1489-1492, 2012.

181. Liu J, Liao S, Diop-Frimpong B, et al: TGF-β blockade improves the distribution and efficacy of therapeutics in breast carcinoma by normalizing the tumor stroma. Proc Natl Acad Sci USA 109: 16618-16623, 2012.

182. Kozono S, Ohuchida K, Eguchi D, et al: Pirfenidone inhibits pancreatic cancer desmoplasia by regulating stellate cells. Cancer Res 73: 2345-2356, 2013.

183. Bouquet F, Pal A, Pilones KA, et al: TGFβ1 inhibition increases the radiosensitivity of breast cancer cells in vitro and promotes tumor control by radiation in vivo. Clin Cancer Res 17: 6754-6765, 2011.

184. Zhang M, Kleber S, Röhrich M, et al: Blockade of TGF-β signaling by the TGFβR-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. Cancer Res 71: 7155-7167, 2011.