The roles of TGFβ in the tumour microenvironment

Michael Pickup, Sergey Novitskiy and Harold L. Moses

Abstract | The influence of the microenvironment on tumour progression is becoming clearer. In this Review we address the role of an essential signalling pathway, that of transforming growth factor-β, in the regulation of components of the tumour microenvironment and how this contributes to tumour progression.

Epithelial-to-mesenchymal transition
The process by which epithelial cells lose their epithelial characteristics, such as cellular polarity and cell–cell junctions, in favour of mesenchymal characteristics, which include high cellular motility.

Vanderbilt University Medical Center, Vanderbilt-Ingram Comprehensive Cancer Center, Medicine and Pathology, Cancer Biology, 2220 Pierce Avenue, 691 Preston Research Building, Nashville, Tennessee 37232, USA. Correspondence to H.L.M. e-mail: hal.moses@vanderbilt.edu
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The transforming growth factor-β (TGFβ) pathway has been established as essential for cancer progression, because of its prominent role in the regulation of cell growth, differentiation and migration. Through the canonical and non-canonical arms of the signalling pathway, TGFβ instigates cellular phenotypical changes that mediate its role as both a tumour suppressor and a tumour promoter. Indeed, the first described phenotypical effect of TGFβ signalling was the induction of a cellular cytostatic programme[1] and this provided the first evidence of the TGFβ pathway being tumour suppressive[2]. However, there was also evidence to the contrary, such as increased tumour progression in carcinomas that overexpressed the TGFβ1 ligand[3,4]. The initial *in vitro* evidence for the pro-tumorigenic effects of TGFβ consisted of the induction of a mesenchymal phenotype in epithelial tumour cells (commonly known as an epithelial-to-mesenchymal transition (EMT)) after prolonged exposure to TGFβ[5,6].

These early studies into the functional outcome of active TGFβ signalling underlie the difficulties in implementing clinically efficacious treatment regimens that target the TGFβ pathway. The contextual cues that drive the tumour suppressor and tumour promoter roles of TGFβ, as well as the switch between these two phenotypes, are not fully understood. As the understanding of tumour progression has increased, the importance of the tumour microenvironment has been clearly shown. It is interesting to note that TGFβ signalling, both endogenously in human disease and in genetically engineered mouse models of cancer, is associated with the characteristic epithelial changes outlined above, as well as with substantial changes in the tumour stroma[7–9]; for example, TGFβ1 expression in invasive breast cancer correlates with markers of tumour progression, such as metastasis, extracellular matrix (ECM) deposition and the infiltration of immune cells[7]. Such findings have laid the groundwork for recent studies that have established TGFβ signalling as an important mediator not only of changes to the epithelial phenotype but also of changes in the stromal environment that are essential for tumour progression. In this Review, we address the functional role of TGFβ signalling in modulating the tumour microenvironment and how these changes affect tumour progression.

The basics of TGFβ signalling

TGFβ1, TGFβ2 and TGFβ3 ligands function as the primary mediators of TGFβ signalling[1,10,11] and are secreted as inactive homodimeric polypeptides that can bind to latent TGFβ-binding proteins, which promote extracellular sequestration[12]. On activation, the ligands bind to the type 2 TGFβ receptor (TGFBR2), which causes recruitment and phosphorylation of TGFBR1 (also known as ALK5), resulting in downstream signalling activation[13] (FIG. 1). The strength of this signal depends on which ligand is bound, as the ligands vary in their binding affinity for TGFBR1. This variation in ligand binding promotes differential ligand presentation to TGFBR2 (REF. 14). TGFBR3 can augment the initialization of the signalling cascade by promoting differential ligand binding[15]. The final heterotetrameric form of the active receptors initiates downstream signalling through either SMAD-mediated canonical signalling or SMAD-independent non-canonical signalling[13]. The canonical signalling pathway involves phosphorylation of the carboxy-terminal serine residue of the internal modulator SMAD proteins, SMAD2 or SMAD3, by the activated receptors[16,17]. This phosphorylation induces oligomerization of SMAD2 or SMAD3 with SMAD4, which is necessary for nuclear translocation[18]. Through interactions with a variety of transcription cofactors, the nuclear-localized SMAD complex initiates transcriptional activation or transcriptional repression of several genes. The non-canonical branch of the signalling

Key points

- During the early stages of tumorigenesis, transforming growth factor-β (TGFβ) functions as a tumour suppressor. However, as tumours progress, tumour cells may lose their growth-inhibitory response to TGFβ and may instead respond by initiating epithelial-to-mesenchymal transition and by increasing cell migration.
- Experimental data support the idea that both loss of and gain of TGFβ signalling is pro-tumorigenic, as mouse models show that the overexpression of TGFβ and the abrogation of signalling results in increased tumour cell metastasis. It is beginning to be appreciated that the effects of TGFβ signalling in the tumour epithelium extend beyond tumour cell-autonomous mechanisms into the tumour microenvironment.
- Nearly every cell in the tumour microenvironment responds to TGFβ in a unique way, and these diverse biological responses have a variety of effects on tumour progression.
- Given the pleotropic nature of TGFβ signalling, the numerous downstream signalling events of TGFβ offer new and intriguing targets for therapeutic intervention.
- Successful therapeutic targeting of TGFβ itself remains a highly desirable goal and the considerable advances in understanding TGFβ signalling, not only in the tumour epithelium but also in the tumour microenvironment, bring the realization of this goal closer.

pathway[19] involves activation of the PI3K–AKT, RHOA and MAPK pathways, among others, by the activated heterotetrameric receptors[20].

The outcome of these signalling pathways in epithelial cells is either suppression of cell proliferation or induction of cellular migration and invasion. Studies investigating the cytostatic phenotype induced by TGFβ have established numerous pathways through which this is achieved, including repression of *MYC* and cyclin-dependent kinase 4 (*CDK4*), as well as the induced expression of CDK inhibitors p21 (also known as CIP1; encoded by *CDKN1A*) and INK4B (also known as p15)[21–23]. Furthermore, the SMAD-dependent activation of Krueppel-like factor 10 (also known as TIEG1), death-associated protein kinase 1 (DAPK1) and the pro-apoptotic protein BCL-2-interacting mediator of cell death (BIM; also known as BCL2L11), among others, triggers programmed cell death[24]. Studies elucidating the tumour suppressor role of TGFβ corroborated evidence that the loss of TGFβ-signalling components was associated with carcinoma progression[25,26]. The signalling pathway downstream of EMT induction by TGFβ has also been partly mapped. Inhibitor of DNA binding 1 (ID1; a transcriptional regulator) is inhibited by TGFβ. This results in decreased expression of E-cadherin (also known as cadherin 1) and zona occludens 1 (ZO1; also known as TJP1), which are two factors known to help maintain an epithelial phenotype. TGFβ signalling also induces the expression of EMT-associated transcription factors, such as snail family zinc finger 1 (*SNAI1*), *SNAI2*, zinc finger E-box binding homeobox 1 (*ZEB1*), *ZEB2*, and lymphoid enhancer-binding factor 1 (*LEF1*), which help to promote the loss of cellular adhesions and cytoskeletal rearrangement[27]. Non-canonical signalling pathways activated by TGFβ, particularly the RHO–ROCK1 (RHO-associated protein kinase 1) and the AKT pathways, were also shown to be essential in the promotion of the migratory and invasive cellular phenotypes observed after treatment of epithelial cells with TGFβ[28]. RHO–ROCK1 signalling that is induced by TGFβ is particularly important in single-cell migration. Interestingly, abrogation of TGFβ signalling did not completely stop epithelial cell migration, but rather it switched the cells towards a cohesive migratory phenotype[29]. Thus, the dichotomous effects of TGFβ signalling are associated with tumour suppression early in tumour development, through initiation of growth arrest, and with tumour promotion in late-stage tumours, through the induction of EMT and of cellular migration and invasion.

However, the effects of epithelial TGFβ signalling on cells that constitute the tumour microenvironment have more recently aided our understanding of the diverse effects that TGFβ has on tumour development and progression. These effects have also started to put in perspective why many studies cite increased metastasis and poor patient prognosis in cancers that lack intact TGFβ signalling.

Disruption of TGFβ signalling

Even without the introduction of oncogenic changes, altered TGFβ-signalling in normal epithelial cells results in phenotypical changes in the stroma; for example, expression of constitutively active TGFBR1 in the mammary epithelium results in mammary glands with greater collagen deposition around the ductal epithelium[30]. Notably, mammary and pancreatic carcinoma cells that harbour activated TGFBR1 induce a significant increase in angiogenesis following implantation into mice[31,32] (FIG. 2). Active TGFβ signalling in mammary tumour cells also promotes tumour progression through the SMAD-independent induction of the expression of matrix metalloproteinases (MMPs), which results in increased angiogenesis and in tumour cell invasion[31]. One of the more interesting effects of disrupted TGFβ signalling in the malignant epithelium on the tumour microenvironment is related to the alteration of microRNA (miRNA) regulation. miRNAs, which are potent modulators of mRNA, are aberrantly expressed in numerous cancer types and are linked with several pro-tumorigenic and antitumorigenic functions[33]. TGFβ signalling can inhibit the function of specific miRNAs; for example, TGFβ expression in hepatocellular carcinoma (HCC) cells induces the expression of CC-chemokine ligand 22 (CCL22) through the inhibition of miR-34a expression[34]. This promotes the recruitment of regulatory T (T_{Reg}) cells. TGFβ can also have direct effects on cells that form part of the tumour stroma; for example, in human vascular smooth muscle cells (VSMCs), TGFβ can promote the cleavage and the maturation of pri-mir-21 sequences by inducing the formation of a complex that involves SMAD3 and ATP-dependent RNA helicase DDX5, which is a component of the DROSHA microprocessor complex[35]. This increases the expression of miR-21, which alters the contractile properties of the VSMCs. TGFβ signalling also increases the expression of miR-29a in human and mouse endothelial cells, which thereby increases the induction of angiogenesis in a non-tumour model[36]. miR-29a targets phosphatase and tensin homolog (*PTEN*) mRNA, and it thereby increases the activity of the AKT signalling pathway. TGFβ can also increase the expression of miR-494 in

Cohesive migratory phenotype
Contrary to single-cell invasion, this is a form of invasion by which cells maintain their cell–cell contacts and by which cells migrate as a unit.

MicroRNA
(miRNA). 20–30 nucleotide long non-coding RNA that modulates gene expression by binding to complementary sequences in the 3′ untranslated region of target genes, which induces repression of gene translation or mRNA degradation.

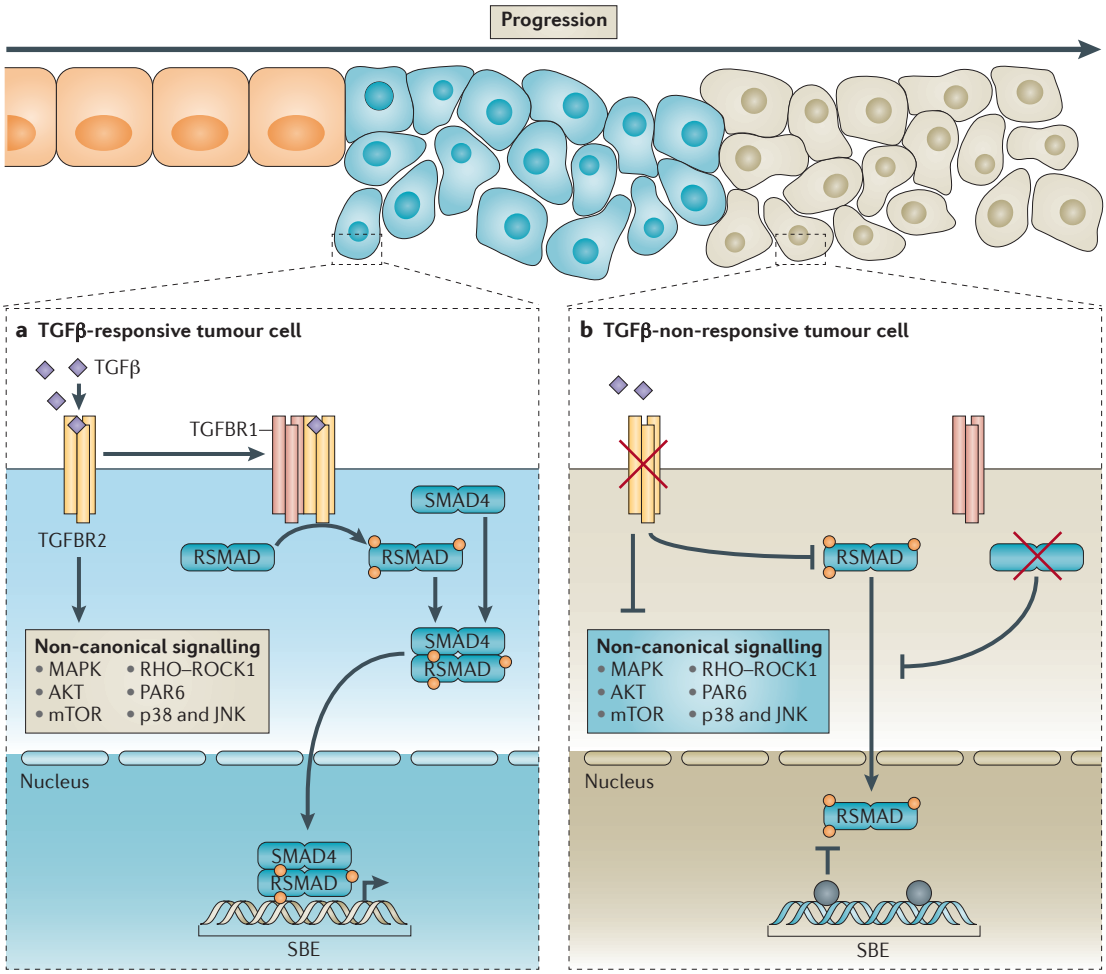

Figure 1 | **Epithelial TGFβ signalling during tumour progression.** **a** | Normal transforming growth factor-β (TGFβ) signalling in TGFβ-responsive cells (blue cells) feeds through the type 2 TGFβ receptor (TGFBR2) to activate downstream signalling targets. Canonical signalling is activated through phosphorylation of TGFBR1 to induce nuclear localization and transcriptional activity of SMADs. Non-canonical signalling can occur independently of SMAD proteins and includes activation of RHOA, AKT and MAPK pathways. Early in tumorigenesis, TGFβ functions as a tumour suppressor, partly through the SMAD-dependent induction of cell cycle arrest. **b** | One hypothesis is that selective pressure leads to the expansion of the population of tumour cells that harbour inactivating mutations in the TGFβ pathway, thus allowing them to overcome the growth-inhibitory effects of active TGFβ signalling. As outlined by Levy *et al.*[48], loss of TGFβ responsiveness (green cells) can occur through loss-of-function mutations, loss of expression or promoter methylation of genes (shown by the grey circles) that encode TGFβ receptors or SMADs. JNK, JUN N-terminal kinase; mTOR, mammalian target of rapamycin; PAR6, partitioning defective 6 homologue; ROCK1, RHO-associated protein kinase 1; RSMAD, receptor SMAD; SBE, SMAD-binding element.

myeloid-derived suppressor cells (MDSCs). This increases the CXC-chemokine receptor (CXCR4)-mediated chemotaxis of MDSCs to the tumour and it reduces the expression of PTEN. The reduced expression of PTEN results in increased AKT activity and in increased expression of pro-tumorigenic liver arginase 1 (ARG1), MMP2, MMP13 and MMP14, which contribute to increased tumour cell invasion and metastasis[37].

Induction of TGFβ signalling through genetic alterations or through the treatment of carcinoma cells with TGFβ has identified numerous gene targets, many of which are conserved in various cancer types[38–41]. These experiments have begun to address the functional importance of gene expression induced by TGFβ by correlating these changes with patient data that predict poor patient outcome[38,42]. Importantly, many of these gene expression targets have well-characterized functions in the modification of tumour microenvironments and they link with phenotypical changes seen on alteration of TGFβ signalling in mouse models of cancer (FIG. 2). TGFβ signalling in epithelial cells induces the expression of numerous ECM genes, including collagen type 1 α1 (*COL1A1*) and *COL4A1*, as well as matrix modifying enzymes *MMP2*, *MMP9* and lysyl oxidase homologue 4 (*LOXL4*). These same studies also provide supporting evidence for the observed pro-angiogenic phenotypes that are induced by TGFβ activation in epithelial cells. Gene expression analysis showed a significant increase in the expression levels of vascular endothelial growth factor A (VEGFA) and thrombospondin 1 (REFS 38–41). Given the

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous population of cells that are defined by their myeloid origin, immature state and ability to potently suppress T cell responses.

Chemotaxis
The function of a cell responding to a chemokine gradient to promote directional migration towards sites of tissue injury or into tumour microenvironments.

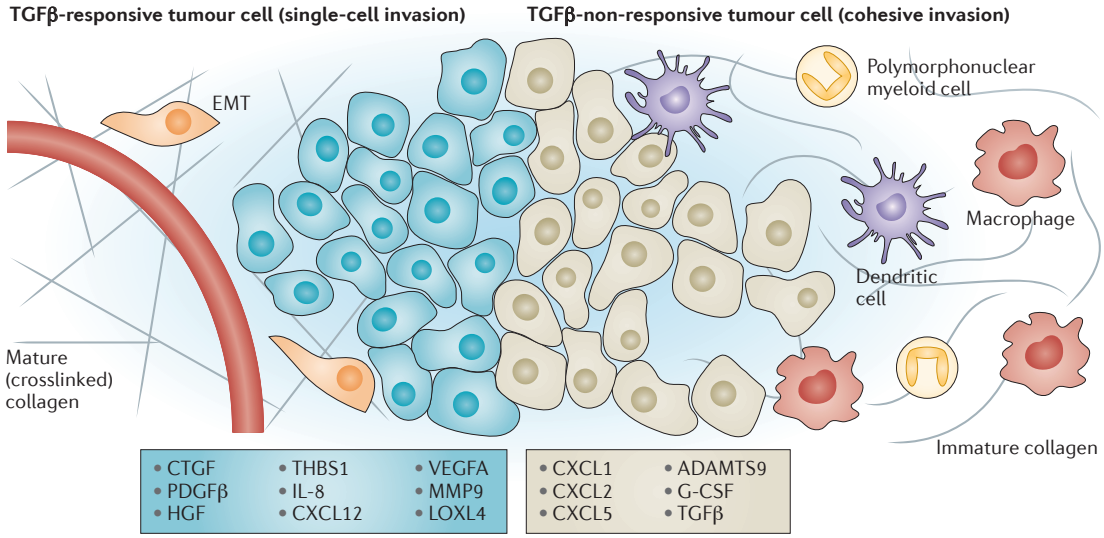

Figure 2 | **TGFβ signalling in tumour cells determines microenvironmental modification.** Transforming growth factor-β (TGFβ) signalling in tumour cells induces the expression of numerous mediators of extracellular change. Tumours in which cells show increased TGFβ activity are characterized by increased extracellular matrix (ECM) deposition; they show increased secretion of matrix proteins and maturation of these proteins through ECM-modifying enzymes such as lysyl oxidase homologue 4 (LOXL4). In addition, TGFβ signalling in tumour cells drives the induction of endothelial cell recruitment and proliferation, which promote increased angiogenesis. Conversely, TGFβ suppresses the expression of numerous cytokines and chemokines such as the CXC-chemokine ligand 1 (CXCL1) and CXCL5. Loss of TGFβ responsiveness relieves this suppression and results in enhanced immune cell infiltration. These microenvironmental changes promote epithelial cell and stromal cell phenotypical responses, which substantially affect tumour progression. The figure shows the phenotypical changes that specifically result from the epithelial-derived factors listed. ADAMTS9, a disintegrin and metalloproteinase with thrombospondin motifs 9; CTGF, connective tissue growth factor; EMT, epithelial-to-mesenchymal transition; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; IL-8, interleukin-8; MMP, matrix metalloproteinase; PDGFβ, platelet-derived growth factor-β; THBS1, thrombospondin 1; VEGFA, vascular endothelial growth factor A.

EMT and migratory phenotypical changes induced following activation of TGFβ signalling, as well as the epithelial cell TGFβ gene signature associated with tumour recurrence, the identification of stromal changes confirms epithelial cell TGFβ signalling as a pro-tumorigenic signalling pathway. However, such investigations do not distinguish the epithelial and stromal effects of epithelial cell TGFβ signalling on the promotion of tumour cell metastasis, and thus they do not address the specific contribution of each effect to the correlation of TGFβ signalling with poor patient prognosis.

As tumours progress, the growth-inhibitory effects of TGFβ are overcome by the loss of TGFβ pathway elements or of downstream signalling targets[10,43] (FIG. 1). Loss of TGFβ responsiveness in tumour cells has substantial effects on tumour progression, not only through altered epithelial cell characteristics but also through gene expression changes that affect the tumour microenvironment. Activated TGFβ signalling has primarily been associated with an increase in metastasis and with poor patient prognosis, because EMT is induced[29,44]. However, abrogation of TGFβ signalling in carcinoma cells can also result in increased metastasis[45]. Loss of TGFβ signalling components in both mouse models of cancer and in human cancer has been associated with poor prognosis as a result of increased progression and metastasis[25,46–49]. Similarly to receptor activation in the normal mammary epithelium, the pancreatic epithelium expressing a dominant-negative TGFBR2 results in increased desmoplasia and angiogenesis in adult mice[25]. The abrogation or attenuation of TGFβ signalling, either at the receptor or at the SMAD level in mouse models of cancer, has various expected epithelial effects, such as loss of growth inhibition, but it also induces several stromal changes, including activation of stromal fibroblasts, deposition of collagenous ECM, infiltration of a variety of immune cells and increased angiogenesis[50]. Recent work has shown that the infiltration of MDSCs is increased in tumours in which epithelial TGFβ signalling is abrogated[51] (FIG. 2). The increased recruitment of MDSCs is primarily associated with the increased expression of the CXC-chemokine ligand 1 (CXCL1) and CXCL5, the expression of which is normally inhibited by TGFβ. Although this altered chemokine expression pattern is associated with increased myeloid cell infiltration into the tumour microenvironment, these chemokines can also drive the activation of stromal fibroblasts through the induction of connective tissue growth factor (CTGF) expression[52]. Perhaps the most interesting induced change in gene expression to result from abrogation of TGFβ signalling in epithelial cells is the change in the expression of the TGFβ ligand itself[53,54]. Given that the epithelium expressing TGFβ can no longer respond to it, any effects derived from its expression would either be on a separate population of epithelial cells that retain their ability to respond to TGFβ or on cells found in the stromal microenvironment.

Dominant-negative
A defective protein product that effectively inhibits the function of its wild-type counterpart by retaining essential interaction capabilities without having a desired effector function.

Desmoplasia
The accumulation of extracellular matrix proteins, usually through the enhanced activation of stromal fibroblasts.

The epithelial loss of TGFBR2 results in increased expression of CXCL1, CXCL5 and bone marrow stromal antigen 2 (BST2), and in a reduction in the expression of genes such as *CXCL12*, platelet-derived growth factor-β (*PDGFB*) and *CTGF*[55] (FIG. 2). These gene expression changes significantly correlate with a worse outcome in patients with lymph node-positive, oestrogen receptor (ER)-positive luminal A-type breast cancer. These findings introduce an interesting concept that explains many of the counter-intuitive findings regarding impairment of epithelial cell TGFβ signalling that ultimately promotes metastasis. If, as many studies have shown, the stromal microenvironment is a prognostic factor for metastasis, it could override the previously established cell-autonomous signalling events that are associated with tumour cell migration, invasion and metastasis[56]. Thus, even though tumour cells that lack TGFβ responsiveness no longer gain any epithelial-centric metastatic advantages from TGFβ production, stromal alterations as a result of altered TGFβ expression might continue to promote metastasis. Tumours are heterogeneous and therefore probably contain cells with and without the ability to respond to TGFβ — a concept that is yet to be fully appreciated in terms of its effects on tumour progression.

The effects of TGFβ on stromal cells

Although the effects of increased TGFβ1 expression on epithelial tumour progression have been well established, consideration of the tumour stroma has recently garnered a considerable amount of momentum. Although they give no indication of whether they are causal or merely an effect of progression, specific gene expression changes in the stroma are noted at defined stages of breast cancer progression[57]. Thus, much like the Vogelgram identified the specific genetic changes that are associated with the stages of colon cancer progression[58], we are beginning to appreciate that gene expression changes that correspond with tumour progression also occur in the stroma of tumours. A recent review examined the various effects of stromal components on each hallmark of cancer and highlighted the importance of stromal cells in influencing tumour progression[59]. However, the mechanisms behind these changes and the pathways that drive specific gene expression changes have yet to be fully elucidated. Given the pleiotropic nature of TGFβ and its established role in regulating the development and the function of numerous stromal cells that have an effect on tumour progression, in this section we discuss components of the tumour microenvironment and examine the role of TGFβ signalling in each.

MSCs. TGFβ has numerous roles in the maintenance and the function of mesenchymal stem cells (MSCs). MSCs have origins in both the bone marrow and a variety of other tissues and have been ascribed numerous functions in normal physiology, including maintaining a homeostatic bone marrow environment and differentiating into various cells that are required for the physiological function of the bone marrow[60]. In terms of normal physiological function, TGFβ promotes MSC proliferation through induction of the nuclear accumulation of β-catenin, which is mediated by phosphorylation of SMAD3 (REF. 61). In addition, an essential function of MSCs in the bone marrow is the preservation of haematopoietic stem cells in an undifferentiated and inactive state[60]. This is achieved through the secretion of numerous factors, including TGFβ. Another known function of MSCs is that in various conditions they have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes and fibroblasts[62]. This differentiation maintains the bone marrow microenvironment, mediating bone turnover and cartilage stability, and facilitating adaptability of tissue homeostasis through the modulation of cell population dynamics. In both normal and malignant conditions, TGFβ mediates the differentiation process, primarily the induction of chondrocyte and fibroblast differentiation and the inhibition of adipocyte differentiation[63–65]. Although many functions in MSCs are driven by TGFβ, the contextual cues that mediate these differential responses remain unknown. As seen in MSC proliferation, it is probable that the induction of different responses is due to cooperation of the TGFβ pathways with other signalling pathways. TGFβ also modulates MSC function by promoting chemotaxis to sites of tissue damage and to neoplastic lesions[63,66].

In tumours, MSCs have been shown to promote tumour progression both on their own and through their ability to differentiate into other cell types[67,68]. MSCs secrete numerous cytokines that increase tumour cell proliferation, that drive angiogenesis and that affect tumour cell resistance to chemotherapeutic drugs. MSCs in particular can increase tumour metastasis through interactions with carcinoma cells to induce CCL5 expression[62,69]. Interestingly, this does not seem to be dependent on the ability of CCL5 to function as a chemoattractant for immune cells but rather on its ability to promote cell colonization in the lungs. In addition, as we discuss below, MSCs can inhibit T cell proliferation and activation, which substantially increases their tumour-promoting effects. Another normal physiological function of MSCs is their ability to differentiate into other cells of mesenchymal origin[70]. Reports indicate that approximately 20% of cancer-associated fibroblasts (CAFs) have origins in the bone marrow, arising from the differentiation of MSCs[70]. Once differentiated, these fibroblasts promote tumour progression in a TGFβ-dependent manner; indeed, inhibition of TGFβ-mediated MSC differentiation into CAFs through the expression of BAMBI (BMP and activin membrane-bound inhibitor homologue) has been shown to abolish the ability of MSCs to promote tumour progression[71].

Fibroblasts. One of the most common features of carcinomas that overexpress TGFβ is a desmoplastic stromal environment[7,72–75]. Increased ECM deposition corresponds well with the known functions of TGFβ in fibroblast activation and cancer[11,50]. TGFβ has previously been shown to induce myofibroblast differentiation in fibroblasts, which leads to increased collagen deposition and ECM remodelling[76,77]. Expression of a constitutively active TGFBR1 in dermal fibroblasts in mice resulted in increased fibrosis and increased expression of known fibroblast-specific TGFβ target genes in chemically

Vogelgram
A model for colorectal cancer development in which specific genetic changes are acquired, which mark progression of the disease from benign to malignant.

Myofibroblast
A generalized term for an activated fibroblast. In tumour biology, these cells function to deposit and to remodel the extracellular matrix as well as to alter tumour progression through paracrine protein signalling.

induced tumours[78]. Thus, an abundance of TGFβ in a tumour would probably elicit a similar response. This has been shown to be true as tumours that are characterized as having increased levels of TGFβ are associated with increased fibroblast activation and collagen deposition. In addition, fibroblasts derived from small-cell lung carcinomas show enrichment in TGFβ signalling compared with normal lung fibroblasts, and the TGFβ-enriched gene signature of the carcinoma-derived fibroblasts predicts poor patient outcome[79]. In fact, it has been shown that the increased TGFβ activation is caused by interactions between stromal fibroblasts and colon carcinoma cells inducing the expression of numerous MMPs and known TGFβ target genes[80]. As discussed above, numerous studies show that TGFβ-mediated signalling mechanisms are involved in the reciprocal interactions of tumour cells and the stroma. However, only recently have the effects of these interactions on the metastatic potential of tumours been appreciated. Overexpression of TGFβ in a TGFβ-non-responsive colon carcinoma cell line drives increased metastasis of these tumour cells[81]. In this case, TGFβ expression altered the expression of TGFβ target genes in fibroblasts, including interleukin-11 (*IL11*), angiopoietin-like 4 (*ANGPTL4*) and *CTGF* (TABLE 1). Importantly, the TGFβ-driven gene signature from these fibroblasts was used to predict recurrence in human patients with colon cancer[81]. As Calon *et al.*[81] comment, such TGFβ-induced gene expression changes have also been ascribed to epithelial carcinoma cells; thus, regardless of the ability of the tumour cells to respond to TGFβ, the pro-tumorigenic effects of the TGFβ-induced gene expression profile are seen in tumours that express high levels of TGFβ.

Table 1 | **TGFβ signalling affects central components of fibroblast function**

Factors affected by TGFβ	Expression levels increased	Expression levels decreased
Chemokines	• IL-6 • IL-11 • LIF	• CXCL1 • IFNγ • TNF • COX2 • iNOS
Growth factors	• CTGF • VEGF • CXCL12 • TGFβ	• HGF • EGF
Matrix deposition	• Tenascin C • Fibronectin • Elastin • Collagen type 1	• Collagen type 4 • Decorin • Vitronectin • Fibrullin
ECM remodelling	• MMP2 • MMP3 • MMP9 • TIMP3 • TIMP4 • LOX • THBS1	• ADAMTS1 • ADAMTS5 • MMP13

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; COX2, cyclooxygenase 2; CTGF, connective tissue growth factor; CXCL, CXC-chemokine ligand; EGF, pro-epidermal growth factor; HGF, hepatocyte growth factor; IL, interleukin; IFNγ, interferon-γ; LIF, leukaemia inhibitory factor; LOX, lysly oxidase; MMP, matrix metalloproteinase; iNOS, inducible nitric oxide synthase; TGFβ, transforming growth factor-β; THBS1, thrombospondin 1; TIMP, tissue inhibitor or metelloproteinase; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Much like epithelial cell TGFβ signalling, work investigating the role of fibroblast TGFβ on tumour progression has showed a contradictory role for the pathway with respect to tumour progression. Although there is a preponderance of evidence supporting a tumour-promoting role for TGFβ in stromal fibroblasts, there is also a considerable amount of work showing a tumour-suppressive role. The foundation of this work lies in the paper from Bhowmick *et al.*[82],which shows that specific deletion of *Tgfbr2* in mouse fibroblasts results in spontaneous carcinoma initiation. Further work has shown that fibroblasts that lack TGFβ signalling increase the progression of breast, prostate and squamous cell cancers, as well as melanoma[83–85]. The major gene expression changes that are associated with the loss of this signalling axis in fibroblasts are in genes that encode cytokines and chemokines. In particular, increased expression of *CXCL1*, *CXCL5*, *CXCL12* and *TGFB1* is observed in fibroblasts that have abrogated TGFβ signalling[86] (TABLE 1). This indicates that modulation of the tumour microenvironment through increased infiltration of immune cells is a primary driver of the enhanced tumour progression seen when fibroblasts lose TGFβ responsiveness[9,87]. Consistent with this idea, recent work shows that a significant increase in inflammation is observed in spontaneous forestomach carcinomas that develop as a result of abrogation of TGFβ signalling in fibroblasts[88]. Interestingly, this inflammatory response seems to drive tumour formation through the silencing of *CDKN1A* via epigenetic promoter methylation (BOX 1). Administration of anti-inflammatory drugs significantly delayed tumour onset and increased overall survival — a result that supports immune cell infiltration as a facilitator of tumour development. This work is in agreement with *TGFBR2* expression having an important role during the progression of breast cancer from normal to ductal carcinoma *in situ* (DCIS) and then to invasive ductal carcinoma (IDC). Stromal *TGFBR2* expression decreases as these tumours progress towards invasiveness[57]. Interestingly, immunohistochemical studies in patients with colon cancer have shown a similar trend, but have gone one step further to show that low expression of *TGFBR2* in the stroma is an independent predictor of poor patient prognosis[87].

The innate immune system. The innate immune system is the first line of defence during an immune response. It comprises various phagocytic and antigen-presenting cellular components, including macrophages, neutrophils, natural killer (NK) cells and dendritic cells. TGFβ has a substantial role in regulating the recruitment, activation and function of these cells[89]. TGFβ functions as an antagonist of major immune functions, decreasing tumour cell recognition and clearing[90]. One of the most notable functions of TGFβ in this regard is the induction of M2 macrophage polarization[91]. The expression of inducible nitric oxide synthase (iNOS), which is a driver of the nitric oxide production that suppresses monocyte-mediated cell death, is associated with this polarization[92]. TGFβ has a similar effect on

Box 1 | **TGFβ induces epigenetic changes to modulate cellular functions**

Epigenetic modulation of gene expression primarily occurs through modification of histones to promote access to specific stretches of DNA and through DNA methylation of CpG islands in gene promoter regions to modulate transcription factor binding and promoter activity[130]. The occurrence of altered chromatin structure and of promoter hypermethylation is important in numerous cancer types. In this regard, tumour progression is associated with an increase in histone deacetylase (HDAC) and DNA methyltransferase (DNMT) activity, which drives these changes[131,132]. Most transforming growth factor-β (TGFβ) silencing in tumours is a direct result of promoter methylation of TGFβ signalling components[133–135].

TGFβ and the TGFβ signalling pathway have been linked with histone and promoter modifications[136]. The hypermethylation of promoter regions of a gene mainly results in the silencing of that gene. This seems to be essential to overcome the tumour-suppressor actions of TGFβ signalling in epithelial cells. Numerous cancer types show hypermethylation of runt-related transcription factor 3 (*RUNX3*), which results in attenuation of its growth-suppressive functions[137]. Active TGFβ signalling is required for the maintenance of this methylation, as tumours overexpressing SMAD7 (which inhibits TGFβ signalling), lose methylation of promoters and reverse the effects of gene silencing[138]. Conversely, it has recently been shown that TGFβ downregulates DNMT expression in fibroblasts[139]. Fibroblasts have an inverse requirement for promoter methylation with regard to induction of TGFβ target gene expression. DNMT inhibitors enhance the expression of α-smooth muscle actin, as well as collagen type 1, following TGFβ treatment[139,140]. Thus, in the context of TGFβ signalling, the use of DNMT inhibitors might provide efficacious results with respect to epithelial cells but might ultimately result in increased microenvironmental desmoplasia in these tumours.

The transcriptional activation of SMAD2 target genes requires histone acetylation by p300 before assembly of transcriptional machinery to drive gene expression[141]. Conversely, induction of a myofibroblast phenotype by TGFβ requires histone deacetylation by HDAC4 (REF. 142). Interestingly, although histone deacetylation is required for TGFβ-mediated fibroblast activation, histone methylation is needed to drive the expression of the TGFβ target genes connective tissue growth factor (*CTGF*), collagen type 1 α1 (*COL1A1*) and plasminogen activator inhibitor type 1 (*PAI1*)[143]. Such data support the hypothesis that tumour-promoting or -suppressing functions of TGFβ are dependent not only on the microenvironmental context of the tumour but also on the epigenetic histone modifications found in these cells. Although there is a big push for the use of HDAC and DNMT inhibitors in tumour therapy, it is interesting to note that treatment with an HDAC inhibitor might inhibit TGFβ activation in epithelial cells; however, this might increase the response of stromal cells to TGFβ and promote stromal activation.

neutrophils as it has on macrophages[93]. A recent publication shows that exposure of neutrophils to TGFβ results in a pro-tumorigenic N2 neutrophil expression profile and that inhibition of TGFβ signalling switches this cellular phenotype to an antitumorigenic N1 phenotype[94]. N2 neutrophils are associated with the expression of pro-tumorigenic factors, such as MMP9, CXCL1 and ARG1.

Rather than inducing a different activation state in NK cells, TGFβ has been identified as a factor that inhibits the functional maturation of this cell population[95]. Immature NK cells do not respond to foreign antigens, thus exposure of NK cells to TGFβ will impair the recognition and the clearance of tumour cells. The inhibition of maturation also prevents the systemic effects of NK cells, for example, activation of antigen-presenting dendritic cells and inhibition of interferon-γ (IFNγ) secretion, which drives T helper 1 cell (T_H1 cell) maturation[96,97]. However, TGFβ also has antagonistic effects on dendritic cells that inhibit their antitumorigenic properties[98]. Recent work has shown that impaired TGFβ signalling in dendritic cells significantly increases the ability of these cells to present foreign antigens and to activate the adaptive immune system[99]. Such actions would promote the killing of tumour cells but, in TGFβ-rich environments where dendritic cells respond to TGFβ, their maturation would be impaired, thus promoting tumour growth and progression. Infiltrating dendritic cells in human lung cancer samples are characterized as immature compared with peritumoural dendritic cells, and this immature state has been shown to promote the differentiation of T_{Reg} cells, which increases tumour progression[100,101].

T helper 1 cell
(T_H1 cell). A primary effector cell of the adaptive immune system, which is classically defined as antitumorigenic.

The adaptive immune system. Early work investigating the function of TGFβ established that it is essential in the regulation of the adaptive immune system; indeed, systemic loss of TGFβ1 expression results in the development of autoimmune-mediated inflammation in numerous organs[102,103]. In addition, abrogation of TGFβ signalling specifically in T cells results in a similar autoimmune response[104,105]. The mechanisms proposed for regulation of adaptive immunity by TGFβ are the SMAD-mediated inhibition of T cell proliferation and the suppression of a T_H1 cell phenotype[106]. Recent work has also shown that TGFβ mediates the immunosuppressive differentiation of T cells[89]. Treatment of naive T cells with TGFβ induces the expression of the transcription factor forkhead box protein P3 (FOXP3), which drives the phenotypical conversion of a naive T cell to a T_{Reg} cell[107,108]. Interestingly, T_{Reg} cell-induced suppression of the adaptive immune response is also mediated through the expression of TGFβ[109–111]. Addition of IL-6 to the TGFβ treatment of naive T cells induces a completely different cellular phenotype to that of T_{Reg} cells[112]. IL-6 and TGFβ induce T_H17 cell differentiation through the suppression of *FOXP3* and the activation of the transcription factor retinoic acid receptor-related orphan receptor γt (*RORgt*)[113]. Various pro-tumorigenic and antitumorigenic functions of T_H17 cells are primarily mediated through the expression of IL-17 in these cells. Interestingly, the function of IL-17 is also context dependent: IL-17 functions as a tumour promoter by inducing angiogenesis and tumour cell proliferation in immune-deficient hosts, but functions as a tumour suppressor in hosts with intact immune systems by enhancing anti-tumour immune functions. In addition to the secretion of IL-17, the presence of T_H17 cells has been associated with

1D11
An antibody which is specifically directed towards the three transforming growth factor-β ligands and used to sequester these ligands, thus inhibiting TGFβ signalling.

Losartan
An angiotensin II receptor antagonist that has been shown to have the off-target effects of downregulating expression of type I transforming growth factor-β receptor (*Tgfbr1*) and *Tgfbr2*.

increased tumorigenesis that is mediated by neutrophil recruitment and differentiation to an N2 phenotype, and by the induction of IL-6 secretion[114].

Despite the numerous pro-tumorigenic functions that are induced by TGFβ stimulation of adaptive immune cells, work using TGFβ signalling-deficient T cells supports an antitumorigenic function for TGFβ signalling in T cells. Thus, as in epithelial cells and fibroblasts, TGFβ signalling in T cells seems to have a contextual dependence that drives its dichotomous functions in tumour progression. Attenuation of TGFβ signalling in $CD8^+$ T cells, through the expression of a dominant-negative TGFBR2, results in uncontrolled cellular proliferation[152]. Seminal work from the Letterio laboratory[115] has shown that genetic deletion of *Smad4* in T cells results in the spontaneous development of gastrointestinal tumours. The abundant expression of T_H2 cell cytokines has been shown to mediate this phenotype. Just as TGFBR2-deficient fibroblasts are capable of inducing epithelial hyperplasia, so are T cells that are deficient in TGFβ signalling. Counter-intuitively, analysis of SMAD4-deficient T cells shows a significant enrichment of T_H17 cells[116]. Tumours that arise in mice with *Smad4*-null T cells are characterized by an increased expression of IL-6 and IL-11. This increased expression seems to be sufficient to induce T_H17 cell conversion in T cells despite the previously established requirement for TGFβ in this process. However, these studies only examined the canonical TGFβ pathway. The requirement for the non-canonical pathway in the induction of T_H17 cells has yet to be addressed.

Although the individual components of both the innate and the adaptive immune system undoubtedly have an important role in tumour progression, it is important to think systemically when considering therapeutic intervention; all cells involved in an immune response interact with and respond to the other components; granulocytes secrete chemokines to promote recruitment of phagocytes and antigen-presenting cells; dendritic cells clear abnormal cells and activate T cells; and so on. Taking a macroscopic view in the context of TGFβ signalling, it can be concluded that TGFβ signalling in the immune system primarily functions as an antagonist that suppresses the recognition and the clearance of tumour cells. It is this feature that has driven current efforts to use anti-TGFβ therapeutic regimens to activate T cell activity and thus to improve current chemotherapeutics.

Conclusions and therapeutic perspectives

Epithelia that lack the ability to respond to TGFβ have various developmental defects, but the loss of TGFβ signalling in selected organs is not, in itself, tumorigenic. However, introduction of oncogenic manipulations causes these cells to be more aggressive and metastatic than their TGFβ-responsive counterparts[45,46,49,53]. Although many of the pro-tumorigenic effects of TGFβ have been ascribed to the ability of the protein to promote an EMT in epithelial cells, the recently identified effects of this cytokine on the stroma have proved to be an equally important pro-metastatic mechanism. Given the important effects seen in tumour progression following manipulation of TGFβ responsiveness, efficacious intervention in the TGFβ pathway remains a highly sought-after goal for many researchers. As a result of evidence that supports the idea that loss of TGFBR in epithelial cells has a profound effect on the stroma, as well as showing that increased ligand expression by the tumour is associated with poor patient prognosis, the tumour microenvironmental effects of this pathway are increasingly relevant. Rakesh Jain has long been a proponent of the stromal normalization hypothesis, which states that inhibition and reversal of the stromal changes that occur during tumour formation would slow tumour progression and would aid therapeutic intervention[117]. The use of VEGF inhibitors and relaxin, which is a pregnancy-related hormone shown to induce collagen remodelling, has given credence to this hypothesis; TGFβ inhibitors have recently been added to this list[118,119]. The use of conventional chemotherapeutics with the specific TGFβ-targeted antibody 1D11 as well as nonspecific inhibitors such as losartan (which is an angiotensin II receptor inhibitor that has been shown to reduce active TGFβ levels in tumours), significantly increases the delivery and the efficacy of chemotherapeutic regimens. The use of these inhibitors in mouse models promoted vessel stability, decreased collagen deposition and significantly improved the efficacy of doxorubicin and doxil treatments[120].

Numerous studies have shown that systemically intervening in the TGFβ pathway during cancer progression elicits antitumorigenic effects on epithelial cell phenotypes and through microenvironmental changes (TABLE 2). Several Phase I and Phase II clinical trials of TGFβ-neutralizing antibodies and of small-molecule inhibitors to both ligand and receptor have so far been shown to be safe and efficacious; many trials are still accruing patients[121–124]. For a complete and comprehensive examination of therapeutic treatments targeting the TGFβ pathway in malignant and non-malignant disease progression, see REF. 125.

Interestingly, studies show that the effects of TGFβ inhibition are efficacious even in the treatment of tumours that lack the central mediator of canonical TGFβ signalling, SMAD4 (REF. 126). Such data highlight the importance not only of the microenvironment but also of microenvironmental TGFβ signalling on tumour progression. TGFβ-inhibitory treatment regimens tested in numerous mouse models of different cancer types, as well as in early-stage clinical trials, have shown benefits; increased activity of antitumoural adaptive immune cells is thought to be responsible[127,128]. However, inhibition of the TGFβ signalling pathway in specific components of the microenvironment has once again introduced controversial findings into the field of TGFβ research: suppression of TGFβ signalling in fibroblasts, in an effort to reduce the desmoplastic response in these cells, promoted tumour progression through the augmentation of inflammatory cell infiltration[81,88]. Despite this, clinical trials involving TGFβ inhibition, either through genetic abrogation of TGFβ sensitivity (such as by

Table 2 | **Identified microenvironmental effects of TGFβ inhibitors in preclinical tumour models**

Drug name	Cancers tested	Microenvironmental effects	Refs
TGFβ ligand trap			
P144	Melanoma	Increased dendritic cell, natural killer cell and T cell activity	144
1D11	Lung, breast and glioblastoma	• Increased natural killer cell infiltration and $CD8^+$ T cell-mediated tumour cell killing • Decreased blood vessel area	119,145, 146
TGFβ antisense			
AP12009	Pancreatic	Increased immune cell-mediated cytotoxicity	123
Receptor inhibitors			
GW788388	Oesophageal squamous cell carcinoma	• Decreased fibroblast activation • Decreased vascular development	147
LY2109761	Hepatocellular carcinoma and glioblastoma	• Decreased angiogenesis • Increased pericyte coverage	148,149
LY364947	Glioblastoma	Increased vascular permeability	146
LY3022859	Breast, pancreas and colon	• Increased natural killer cell and cytotoxic T cell-mediated tumour cell killing • Decreased T_{Reg} cell presence • Decreased MDSC infiltration	126
SB431542	Breast	Increased dendritic cell maturation	98
SD-208	Breast cancer bone metastasis and glioma	• Decreased osteoclast differentiation • Increased immune cell-mediated cytotoxicity	127
SX-007	Glioma	Increased presence of $CD3^+$ T cells	150
SM-16	Lung	• Increased number of activated $CD8^+$ T cells • Increased presence of $CD4^+$ T cells	151

MDSC, myeloid-derived suppressor cells; TGFβ, transforming growth factor-β; T_{Reg}, regulatory T.

the adoptive transfer of T cells expressing dominant-negative forms of the TGFBR) or through the use of inhibitory antibodies, have progressed (ClinicalTrials.gov identifiers: NCT00368082, NCT01582269 and NCT00844064). Although modest and mixed results are consistently obtained from these trails, this is probably due to varying stromal compositions and to the pleiotropic effects of TGFβ.

Studies that elucidate the function of TGFβ signalling in specific cellular components of the stroma and that establish an efficacious means of specifically targeting the pathways in these cells are imperative in the resolution of the contextual dependence of TGFβ on the tumour microenvironment. Indeed, it is probable that, as with epithelial cell TGFβ signalling, there is a contextual dependence that controls the tumour-promoting or tumour-suppressing actions of TGFβ in the tumour microenvironment. As cellular components and their interactions drive this microenvironmental context, future studies will need to address not only signalling but also the stromal milieu in the observed response. Without a thorough understanding of the contextual dependence of TGFβ signalling in the stroma it is probable that the implementation of systemic therapeutics that target the TGFβ pathway will yield inconclusive results because of tumour heterogeneity. Thus, novel methods of delivery that facilitate specific targeting to a cellular type or evasion of pathway activation are being investigated, including the adoptive transfer of T cells that have an abrogated TGFβ response, thus circumventing the antitumorigenic response to TGFβ[129]. This highly specific manipulation should provide beneficial effects on tumour progression, without introducing controversial results by affecting TGFβ signalling in other stromal and epithelial cells.

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