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REVIEWS

Spotlight

Targeting TGFβ Signalling in Cancer: Toward Context-Specific Strategies

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The transforming growth factor beta (TGFβ) signalling pathway regulates a range of important cellular processes in a context-dependent manner. Recent discoveries have provided important insights into the regulation of the TGFβ pathway and its change from an antitumorigenic to a protumorigenic pathway. These findings may have important implications for cancer stem cell (CSC) functions and therapeutic strategies.

The TGFβ signalling pathway has an important role in regulating cell growth and differentiation, inflammatory responses, immune evasion, and tumorigenesis [1]. The pathway displays tissue and cell context-dependent effects and can also switch from an antitumorigenic function at early stages of tumorigenesis to a protumorigenic function at later stages. Consequently, the TGFβ signalling pathway is a potentially attractive target for pharmacological intervention in cancer; however, current therapeutics have been relatively unsuccessful due to low specificity and toxicity [2]. Three recent studies [2–4] have sought to better understand how contextual signals from other pathways can impinge on the regulation of TGFβ signalling at multiple levels, from signalling activation at the cell surface via ligand–receptor interactions [3] to signalling crosstalk and gene expression regulation [4,5]. These findings are revealing new strategies to target the pathway with more specificity.

The TGFβ signalling pathway is tightly regulated. Early during TGFβ ligand synthesis, its prodomain [latency-associated peptide (LAP)] is cleaved and forms a latent complex that hinders the binding of mature TGFβ ligand to its transmembrane receptor [6]. Hence, the general notion has been that TGFβ needs to dissociate from LAP to enable ligand–receptor interaction and pathway activation [7]. This mechanism of global inhibition of TGFβ has also been the basis of current therapeutic efforts [2]. However, an approach to increase specificity and limit toxicity by targeting TGFβ activation could improve therapies directed against this pathway.

Integrin αvβ8 is important for TGFβ activation and plays roles in T cell, myeloid, and endothelial cell differentiation during development as well as antitumour immunity through suppression of regulatory T (Treg) cells, which is a major mechanism of tumour immune evasion [2]. Therefore, understanding how integrin αvβ8 binds to and activates TGFβ may help to inform better therapeutic strategies. In a recent study, Campbell *et al.* [3] examined the αvβ8 integrin complex with latent TGFβ (L-TGFβ) that is still bound to LAP and showed, by a combination of cryoelectron microscopy (cryo-EM), structure-guided mutagenesis, and cell-based assays, that TGFβ signalling activation via αvβ8/L-TGFβ does not require the release and diffusion of mature TGFβ as shown by previous activation models. Unexpectedly, αvβ8-mediated TGFβ activation directs TGFβ signalling to the opposing L-TGFβ/GARP-expressing cells by forming a large, multicomponent cell–cell protein complex. In colon carcinoma cells, αvβ8-expressing cancer cells can lead to immunosuppressive differentiation of L-TGFβ/GARP-expressing Treg and myeloid cells [8]. αvβ8 expression is particularly elevated in a range of human carcinomas, such as pancreatic ductal adenocarcinoma (PDAC). Therefore, targeting αvβ8 rather than mature free TGFβ or TGFβ receptors with therapeutic compounds would increase specificity and efficacy while reducing the risks of global TGFβ inhibition due to low specificity.

In addition to mediating immunosuppression in tumour microenvironments, TGFβ signalling plays an important role in regulating epithelial-to-mesenchymal transition (EMT) during development and cancer. Oncogenic mutational activation of KRAS in PDAC is known to stimulate EMT via TGFβ signalling [4]. Oncogenic KRAS induces the transcription factor SNAIL via the TGFβ pathway, which in turn represses *KLF5* expression while inducing EMT. The absence of KLF5 in such cells allows SOX4 to initiate a phenotype checkpoint by inducing programmed cell death [9]. Therefore, during pancreatic cancer formation the progenitor-PDAC cells with oncogenic *KRAS* activation need to genetically alter the TGFβ pathway to inactivate signalling or escape from its apoptosis-inducing function, while retaining its protumorigenic functions that lead to immune evasion, invasion, and metastasis. Switching from an antitumorigenic to a protumorigenic function may be a general function of cancer development, because many tumours from different tissues retain a functional TGFβ pathway.

The mechanisms that abolish the antitumorigenic effect of TGFβ while retaining its proinvasive and immunosuppressive effects have been largely unclear. Huang *et al.* show that elevated transcription of *ID1*, known as an inhibitor of progenitor cell differentiation, is selected for during pancreatic tumorigenesis because it protects pancreatic cells from TGFβ-induced apoptosis. The authors find that PI3K–

AKT activation selectively prevents TGFβ-mediated *ID1* repression, which in turn decouples TGFβ-induced EMT from apoptosis in PDAC cells. This function seems to be achieved by PI3K–AKT signalling-mediated exclusion of FOXO1 and FOXO3a from the nucleus, preventing their binding to regulatory regions close to the *ID1* locus. Hence, *ID1* expression in PDAC cells provides resistance to apoptotic responses during the concerted activation of KRAS and TGFβ signalling. TGFβ also depends on RAS and mitogen-activated protein kinase (MAPK) pathway inputs for the induction of EMT in PDAC [4], providing another therapeutic strategy. Su *et al.* identified RAS-responsive element binding protein 1 (RREB1) as a novel cofactor of SMAD2/3, responsible for the recruitment of SMAD2/3 to the *SNAIL* locus [5]. RREB1 cooperates with SMADs to induce developmental EMT during gastrulation and fibrogenic EMT during tumorigenesis via regulating different transcription programs. Identifying the key partners (e.g., transcriptional cofactors, chromatin-associated RNA) that bind with the RREB1–SMAD2/3 complex in cancer cell populations would help understanding the regulation of EMT and metastatic processes. Both ID1 and RREB1 are therefore novel and key mediators of a TGFβ-induced EMT in cancer that crosstalks extensively with the KRAS signalling pathway.

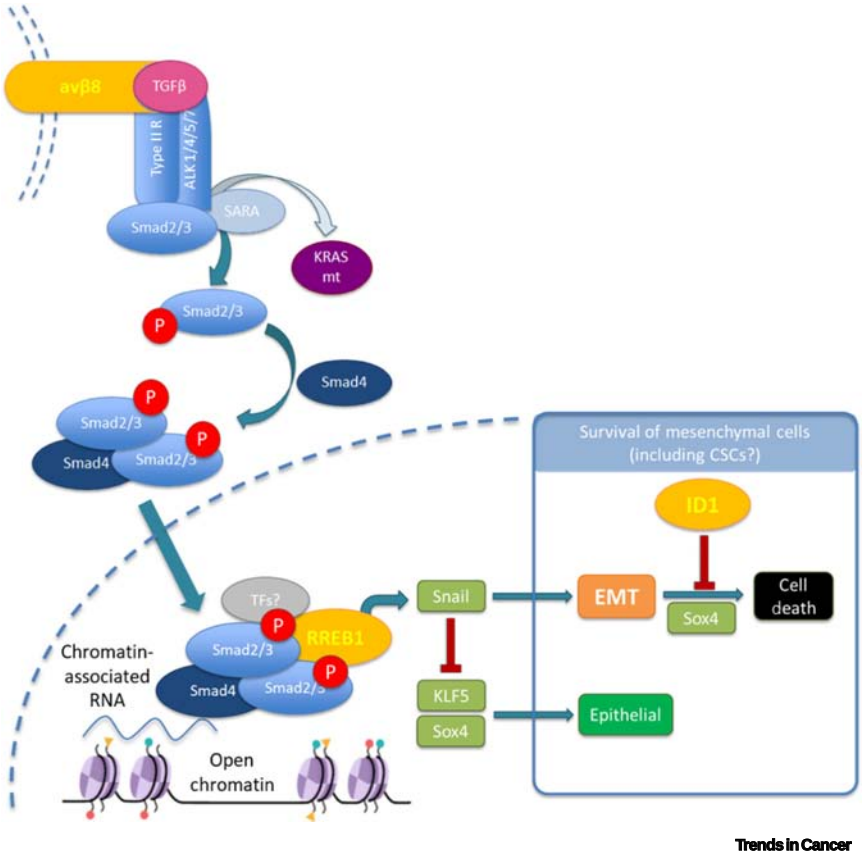

Figure 1. Regulation of Transforming Growth Factor Beta (TGFβ) Signalling in Tumorigenesis. Integrin vβ8-mediated TGFβ activation directs TGFβ signalling to the opposing latent TGFβ (L-TGFβ)/GARP-expressing cells by forming a multicomponent cell–cell protein complex that leads to the phosphorylation of the Smad2/3 transcription factors in the tumour cell. In the nucleus, Smad2/3 form a transcriptional complex with Smad4 and RAS-responsive element binding protein 1 (RREB1) that induces the expression of *Snail*, which induces a fibrogenic epithelial-to-mesenchymal transition (EMT) during tumorigenesis. This EMT can lead to programmed cell death via Sox4-mediated mechanisms that can exert antitumorigenic effects in early stages of tumorigenesis and hence maintain an epithelial cell phenotype (green box). To escape the TGFβ-induced programmed cell death (black box) caused by EMT, elevated expression of ID1 protein is selected for during cancer progression. This decouples TGFβ-induced EMT from apoptosis in pancreatic ductal adenocarcinoma (PDAC) cells and enables metastasis. The coordination of EMT and stem cell-like features could be particularly relevant to certain subpopulations such as cancer stem cells (CSCs) that are efficient in giving rise to metastasis. The described mechanisms are attractive targets for context-specific cancer therapies.

Together these finding [3–5] not only build a clearer picture of the TGFβ pathway (Figure 1) but also suggest a link with tumour cell heterogeneity and CSCs. CSCs have been strongly associated with EMT and a TGFβ-induced EMT is a known promoter of the CSC phenotype [10]. Furthermore, cells that undergo a partial EMT with E-cadherin and Zeb1 expression acquire stem-like properties, enter into circulation, and initiate PDAC dissemination [11]. Cellular heterogeneity could arise from the differential expression of L-TGFβ/GARP and αvβ8 on cancer cells. Given the function of αvβ8-expressing cancer cells in mediating the immunosuppressive differentiation of L-TGFβ/GARP-expressing Treg and myeloid cells [8], there will be selective pressure for the expression of αvβ8. The CSC niche would benefit from αvβ8 expression either on cancer cells or surrounding stromal cells to promote immune evasion and immunosuppression while promoting CSC invasion and metastasis by inducing TGFβ signalling-mediated EMT. Also, cellular heterogeneity in ID1 activity could arise on the decoupling of the proapoptotic function of TGFβ from its EMT-inducing activity during pancreatic tumorigenesis. Therefore, the protumorigenic switch to the survival of mesenchymal cells, mediated by RREB1 and ID1, could serve as a mechanism of CSC generation and maintenance that may contribute significantly to PDAC tumour progression. Since

EMT is a key process accompanying the metastatic dissemination of pancreatic cancer cells, this switch could be particularly relevant for the development of CSCs responsible for metastasis. In addition, ID1 is expressed in embryonic stem cells and functions to inhibit differentiation, suggesting a role for this protein in CSCs.

These studies are significant not only for the identification of novel therapeutic targets specific to the protumorigenic side of the TGFβ pathway, but also because these proteins have a strong potential to be candidate factors for CSC targeting. The evolution of cellular heterogeneity in the aforementioned processes could also serve as an important consideration in the development of combined therapeutic strategies. Conventional chemotherapeutics could be used in parallel with inhibitors of αvβ8, ID1, or RREB1 to target stem-like cells as well as non-stem-like cancer cells and bring about long-sought-after targeting of protumorigenic TGFβ signalling that has so far remained elusive due to the nonspecific effects and toxicity of general TGFβ inhibitors *in vivo*.

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Forum

Epigenetic Biomarkers in Gallbladder Cancer

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Gallbladder cancer (GBC) is associated with various nongenetic and genetic factors. Lack of specific and sensitive diagnostic markers has significantly impacted the mortality of this disease. Here we discuss the recent discovery of epigenetic changes that show great promise as diagnostic biomarkers as well as potential therapeutic targets for GBC.

Gallbladder Cancer

Gallbladder cancer (GBC) is a relatively uncommon but aggressive cancer with distinct geographical and ethnic occurrence and strong female bias. Several environmental or nongenetic and genetic causes are suggested, which likely have a role in inducing carcinogenesis of the gallbladder, with gallstones (cholelithiasis) being reported as the most predominant risk factor for GBC (reviewed in [1]). Due to a lack of early detection markers and an effective treatment strategy, mortality has remained high. Recent reports on the epigenetic mechanisms in cancer, specifically GBC, present a novel approach to biomarker discovery in GBC, with likely implications in targeted therapy (epitherapy).

Epigenetics refers to heritable changes in the gene expression (phenotype) without involving a mutational event (genotype). Epigenetic changes occur naturally and regularly, but can also be caused by environmental or extrinsic factors. The role of epigenetics in cancer, including GBC, is widely implicated, where loss of function or inappropriate expression of genes is the hallmark. In cancer, different epigenetic modifications, including promoter DNA methylation (e.g., 5-methylcytosine) and histone modifications (e.g., acetylation, methylation, phosphorylation, SUMOylation), aberrantly alter gene expression, chromatin structure, and ensue chromatin remodeling, leading to local or global transcriptional deregulation (reviewed in [2]). Analysis of histone modifications and/or detection of methylation of target genes individually or using genome-wide methylome analysis of tumor tissue samples, body fluids, or circulating blood can help identify early markers and, thus, early disease diagnosis/prognosis (reviewed in [3,4]; Figure 1). The focus of this forum article is to discuss our current understanding of the epigenetic regulation of GBC and identification of potential biomarkers of diagnostic/prognostic and therapeutic significance.

Epigenetic Biomarkers of GBC

A biomarker of clinical significance must be stable, specific, and sensitive. Selection of clinically useful marker(s) suitable for diagnosis, prognosis, and therapy is challenging and requires aggressive validation *in vitro* and *in vivo*. Such markers may be derived from tumor tissue, body