



Growth Factor Signalling

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TGF- β is a pleiotropic cytokine that plays many important physiological roles. It is the most potent negative growth factor in mammals and abrogation of the TGF- β mediated cytostatic response is considered one of the hallmarks of cancer. As well as avoiding the tumour suppressive activities of TGF- β , cancer cells can also switch their response to this cytokine and utilise it as a promoter of motility, survival, invasion, vascularisation, metastasis and immunosuppression. We are studying the regulation of TGF- β signal transduction pathways to try to understand how, where and when it acts as a tumour suppressor or promoter. Our ultimate goals are to develop therapeutics that selectively target the pro-oncogenic actions of TGF- β and identify patient selection criteria for their deployment.

TGF- β family signalling

We wish to develop targeted therapeutics that block the tumour promoting aspects of TGF- β signalling, while maintaining its normal physiological roles. This requires a detailed understanding of TGF- β signalling and biological responses in normal and tumour tissues. TGF- β family

members signal via transmembrane serine/threonine kinase receptors. These receptors activate several signalling pathways including the canonical Smad pathways (Fig. 1). Smad2 and Smad3 act as direct substrates of the TGF- β type I receptor ALK5, the Activin type I receptor ALK4 and the Nodal type I receptor ALK7. Smad1, Smad5 and Smad8 are substrates of the BMP type I receptors ALK1, ALK2, ALK3 and ALK6. Following phosphorylation, these receptor-regulated Smads form complexes with Smad4, accumulate in the nucleus and, in conjunction with cofactors and other sequence specific transcription factors, regulate target gene expression. We have been studying the molecular mechanisms of how Smad complexes activate gene expression and have revealed that this involves recruitment of histone modifying transcriptional co-activators.

TGF- β can also activate several non-Smad signalling pathways in a cell type and context-dependent fashion. In collaboration with Robert Grosse (University of Heidelberg), we have been studying the regulation of RhoA by TGF- β . We have found that autocrine TGF- β regulates basal RhoA activity and actin-cytoskeletal dynamics in rodent and v^{12} HaRas transformed human fibroblasts (Fig. 2). Excitingly, we have discovered that this activity of TGF- β is required for efficient v^{12} HaRas and v^{600E} BRAF oncogene-mediated transformation (Fleming *et al.*, *Oncogene*, in press). We are now investigating the roles autocrine TGF- β may play in the proliferation and spread of tumours. Using the ALK5 inhibitor SB-431542 and shRNA knockdown approaches, we have found that many human tumour cell lines are 'addicted' to autocrine TGF- β and require it for efficient proliferation *in vitro* and *in vivo*. We are defining the signalling pathways regulated by autocrine TGF- β that underlie this addiction. We are also correlating the mutational status of these cell lines with TGF- β dependence to define criteria that can predict tumours that exhibit TGF- β addiction, thus enabling us to identify patients that would benefit from TGF- β signalling-targeted therapeutics.

Mechanisms of TGF- β mediated tumour suppression

The tumour suppressive activities of TGF- β are attributed to its ability to act as a potent cytostatic factor and inducer of apoptosis. Studies in mice have indicated that these effects may be particularly important in the stringent control of B and T cell activation and development, but little is known about how TGF- β regulates B and T cell proliferation. In collaboration with Louise Clark and colleagues (Southern General, Stobhill and Golden Jubilee Hospitals), we are studying TGF- β signalling in normal primary human B cells and model Burkitt's Lymphoma (BL) cell lines. Studies of others have defined the minimal TGF- β driven cytostatic in epithelial cells. Central to this programme is the downregulation of c-Myc and the induction of cyclin dependent kinase inhibitors (CDKIs). We have observed that TGF- β can act as an inducer of growth arrest in human BL cell lines. Mechanistically, we have determined that this is entirely independent of repression of c-Myc and activation of CDKIs but is mediated by transcriptional repression of E2F-1 (Spender and Inman, *J. Biol. Chem.* 2009; 284: 1435).

In the majority of BL cell lines, TGF- β acts as a potent inducer of apoptosis. Our detailed molecular analyses have revealed that TGF- β employs a novel apoptotic paradigm involving the coordinated transcriptional regulation of Bcl2 family members to induce cell death. TGF- β acts to induce transcription of the pro-apoptotic gene BIK and repress transcription of the anti-apoptotic gene BCL-X_L, and the coordinated regulation of these genes is required for TGF- β mediated apoptosis. BL tumours are derived from germinal centre B cells and importantly we have found that TGF- β regulates BIK and BCL-X_L expression and acts as a physiological regulator of normal human centroblastic B cell death (Spender *et al.*, *Cell Death Differ.*, in press).

Switching TGF- β from tumour suppressor to promoter

The switch of the TGF- β response from tumour suppressive to tumour promoting involves epistatic and genetic changes in the cancer cell genome. We are screening for epigenetic changes that modulate TGF- β signalling during tumorigenesis and have identified one gene that is silenced by promoter methylation in many primary squamous cell carcinomas. Loss of expression of this gene correlates with the development of metastatic disease and a poor prognosis in head and neck cancer patients. We have also found that this gene is downregulated in breast cancer and may predict site-specific metastasis. Remarkably, using RNAi and stable re-expression technologies, we have discovered that loss of expression of this gene switches the TGF- β response from tumour suppressive to tumour promoting. Our efforts are now concentrating on determining the molecular mechanisms of how the product of this gene regulates both the TGF- β response, and tumour development and dissemination.

BMP signalling in cancer

Recent mutation and expression studies have implicated

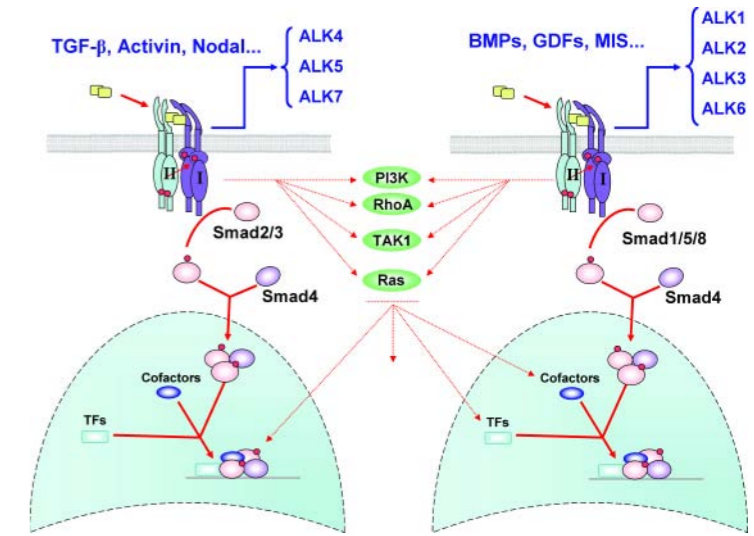


Fig. 1: Schematic representation of TGF- β family signalling. TGF- β family members signal through a heteromeric complex of type II and type I receptors that directly phosphorylate Smads 1, 2, 3, 5 and 8. These Smads form hetero-oligomers with Smad4, accumulate in the nucleus and, in conjunction with transcription factors (TFs) and coregulators, modulate target gene expression. These growth factors also activate other signal transduction pathways in a context-dependent fashion.

aberrant BMP signalling as potentially playing roles in both tumour suppression and promotion in human malignancy. Based on receptor expression profiles, we are investigating the potential oncogenic role of BMP signalling in ovarian cancer. We have developed a sensitive bioassay for measuring multiple BMPs present in biological fluids to assess autocrine production of these cytokines by tumour cell lines, and potentially their presence in patient serum samples. We have found that some BMPs act as potent mitogens for ovarian cancer cell lines. Using blocking antibodies, soluble receptors, kinase inhibitors and siRNA approaches, we have been 'pathway walking' to determine how these BMPs regulate ovarian cancer cell proliferation. Our results indicate that BMP receptor inhibitors may have clinical use in the treatment of ovarian cancer.

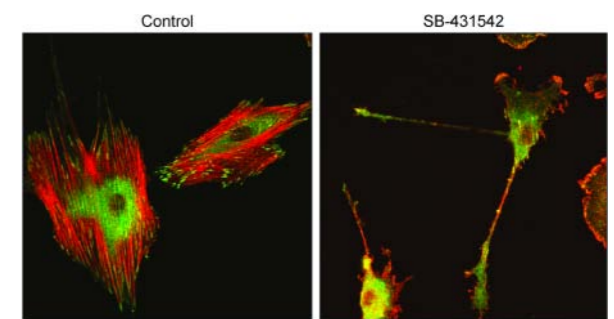


Fig. 2: Autocrine TGF- β regulates actin-cytoskeletal dynamics, focal adhesions and cell morphology in rodent fibroblasts. Immunofluorescence analysis for vinculin (green, focal adhesions) and the actin cytoskeleton (red) of serum-starved REF52 cells with and without (control) 15 minutes treatment with the ALK5 inhibitor SB-431542.

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