GROWTH FACTOR SIGNALLING AND SQUAMOUS CANCERS

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The transforming growth factor beta (TGFβ) superfamily can act as potent tumour promoters and tumour suppressors and their signalling pathways are frequently dysregulated in cancer. Work in our laboratory seeks to understand the molecular basis of how, when and where TGFβ superfamily signalling can act to both promote and inhibit tumour progression. Dysregulation of TGFβ signalling is particularly prevalent in squamous cell cancers (SCC) and we are investigating the molecular landscape and drivers of disease progression in cutaneous SCC (cSCC), Recessive dystrophic epidermolysis bullosa (RDEB) associated cSCC and Head and Neck SCC (HNSCC) using systems biology and biological functional approaches.

TGFβ signalling in cancer

TGFβ superfamily ligands are produced by a myriad of cell types and signal in autocrine, paracrine and systemic fashions to regulate a panoply of cell fate and biological processes during development, tissue homeostasis and in pathophysiological situations. TGFβ exerts its effects by activation of signal transduction pathways emanating from a heterotetrameric complex of TGFBR2 and TGFBR1 receptors whose formation is facilitated by ligand binding. TGFBR2 activates the kinase activity of TGFBR1 and this in turn phosphorylates SMAD2 and SMAD3, which then form hetero-oligomeric complexes with SMAD4, and regulate expression of hundreds of target genes that in turn mediate the biological effects of growth factor exposure. Inactivation of the potent tumour cell autonomous tumour suppressive effects of TGFβ signalling frequently occurs via genetic and epigenetic silencing of canonical signalling components whereas modulation of the pathway and its downstream target genes may enable autocrine, paracrine and systemic tumour promoting activity. Through transcriptome wide studies we identified *C1orf106* as a novel SMAD3 dependent TGFβ target gene that promotes clonogenicity in murine breast cancer cell lines and correlates with poor prognosis in human breast cancer (Figure 1, Strathearn *et al.*, 2024, *Cells*) and we are currently investigating the roles of other potential modulators and mediators of TGFβ tumour promoting activity.

TGFβ signalling in squamous cell carcinomas

In collaboration with Owen Sansom's and Irene Leigh's group (Queen Mary University of London) we have shown that TGFβ receptors are inactivated in 30% of sporadic cSCC and that TGFβ signalling can have potent tumour suppressive effects in the face of other mutational events *in vivo*. Despite TGFβ's powerful tumour suppressive effects in cSCC,

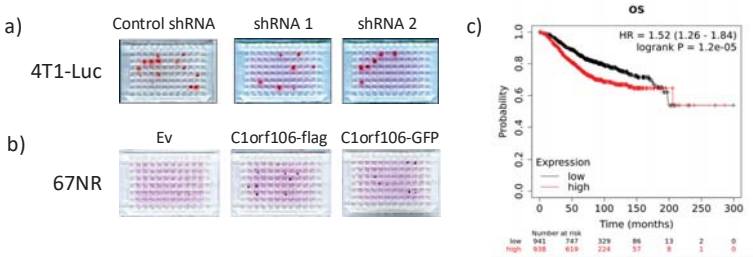

Figure 1. The TGFβ target gene C1orf106 promotes clonogenicity and correlates with poor prognosis in breast cancer. a. Knockdown of c1orf106 expression in metastatic 4T1-luc cells with two independent shRNAs inhibits colony formation. **b.** Overexpression expression of FLAg or GFP tagged c1orf106 in non-metatstic 67NR cells promotes colony formation. **c.** Kaplan-Meier survival analysis on human breast cancer patients split based on median *C1orf106* mRNA expression. OS= overall survival. (Adapted from Strathearn *et al.*, 2024, *Cells*).

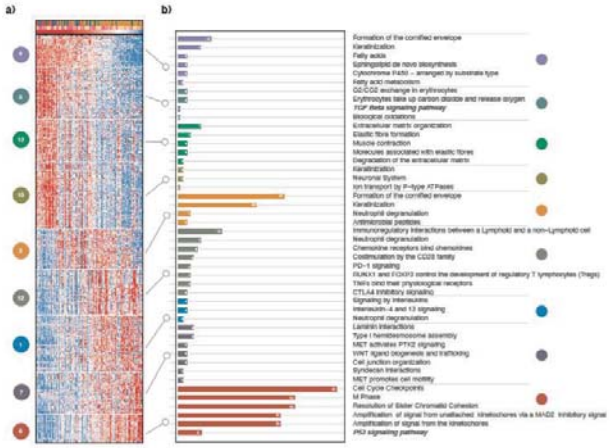

Figure 2. Pathways and processes associated with disease progression of the cSCC disease continuum. a. Heatmap of gene expression levels of genes in 9 core co-expressed gene clusters ordered by differentiated to progenitor like status (left to right). **b** GSEA analysis showing significantly enriched molecular pathways and/or processes in the 9 core co-expressed gene clusters. (Adapted from Bailey *et al.*, 2023, *Nat Commun*).

70% of tumours display no obvious inactivation of the canonical signalling pathway. Similarly, analysis of publically available HNSCC data sets indicate potential tumour suppressor roles of TGFβ signalling (loss/downregulation of canonical signalling components) in ~30% of tumour samples whilst the remaining ~70% of tumours show overexpression of TGFβ1 and many tumours upregulate TGFBR1 expression relative to normal tissue indicative of potential tumour promoting roles. Taken together, these observations indicate that TGFβ signalling may also act to promote tumour progression in both cSCC and HNSCC and we are focusing our initial efforts into understanding the potential tumour promoting effects of TGFβ signalling in cSCC and HNSCC in a panel of patient derived cell lines (PDCLs).

cSCC is a life-threatening complication for patients who suffer from recessive dystrophic epidermolysis bullosa (RDEB), a skin blistering disease caused by germline mutations in collagen VII, the anchoring fibril component in the skin. Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ receptors (Cho *et al.*, 2018, *Sci Transl Med*) pointing to a potential tumour promotion role in these cancers. Intriguingly, we have found that exogenous TGFβ stimulation inhibited proliferation of all RDEB cSCC PDCLs but that endogenous TGFβ signalling drove proliferation, clonogenicity, migration and invasion in the majority but not all of these cell lines (Dayal *et al.*, 2021, *BJD*). Targeting TGFBR1 kinase activity may have therapeutic benefit for patients with these tumours but in some it maintains tumour suppressive activity. Working in partnership with DEBRA we are building new models of RDEB cSCC, investigating the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours and striving to identify novel therapeutic susceptibilities of these aggressive cancers.

Deciphering drivers of SCC disease progression

The incidence of keratinocyte skin cancers represents a rising global health burden. Driven by UV mediated DNA damage, development of primary cSCC tumours can be preceded by pre-malignant Actinic Keratosis (AK). In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC. Metastasis occurs in ~5% of cases, and there are few effective treatments for advanced cSCC, with five-year mortality rates of ~30% for metastatic disease. In collaboration with Irene Leigh, Catherine Harwood, Jun Wang (QMUL and Barts Cancer Institute), Charlotte Proby (University of Dundee), David Adams (Sanger Institute) and Peter Bailey (University of Glasgow), Crispin Miller and John Le Quesne we are carrying out a detailed characterisation of cSCC disease progression using a variety of next generation sequencing approaches coupled with spatial analysis of protein and RNA expression. Using whole exome sequencing (WES) we have previously demonstrated that both pre-malignant (Thomson *et al.*, 2021, *J Invest Dermatol*) and primary tumours possess remarkably similar complex genetic landscapes (Inman *et al.*, 2018, *Nat Commun*). Using bulk RNASeq transcriptomic profiling of 110 patient samples representing normal sun exposed skin, AK, primary and metastatic cSCC we have found that cSCC disease progression manifests as a disease continuum from a differentiated to a progenitor-like state (Bailey *et al.*, 2023, *Nat Commun*). K-Means clustering coupled with gene set enrichment analysis (GSEA) demonstrated that progression of cSCC is associated with the orchestrated modulation of key pathways and processes and reveals potential targets for therapeutic intervention (Figure 2). We are now investigating the transcriptional and genetic landscape of an independent cohort of primary cSCC tumours that did and did not metastasise and their matched metastases with a view to identifying mediators of metastasis.

In collaboration with the Glasgow Head and Neck Cancer group (GLAHNC,) and the Northern Head and Neck alliance we are seeking to understand the molecular basis of chemo-radiotherapy resistance, disease recurrence, lymph node metastasis and distant metastatic spread of HNSCC. Initial collaborative clinical studies are revealing the risk factors for developing HNSCC (Smith *et al.*, 2024, *Head and Neck*), changes in incidence rates (Smith *et al.*, 2024, *BJC Rep*) and the survival outcomes of laryngeal cancer patients (Rajgor *et al.*, 2024, *Clin Otolaryngol*). We are building on these studies and undertaking multiomic molecular profiling of clinically annotated patient samples from local site-specific cohorts and clinical trials and are developing pre-clinical experimental models.

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