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 using systems biology and biological functional approaches.

TGFβ signalling in squamous cell carcinomas

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. In collaboration with Owen Sansom's and Irene Leigh's group (Queen Mary University of London) we have shown that TGFβ receptors were inactivated in 30% of sporadic cSCC and that TGFβ signalling could have potent tumour suppressive effects in the face of other mutational events *in vivo*. We are currently investigating how driver gene combinations act in concert with loss of TGFβ signalling to influence cSCC progression. Despite TGFβ's powerful tumour suppressive effects in cSCC, 70% of tumours displayed no obvious inactivation of the canonical signalling pathway. Analysis of the TCGA head and neck squamous carcinoma (HNSCC) data set revealed a similar potential loss/downregulation of canonical signalling components in ~30% of tumour samples with downregulation of TGFBR2 and SMAD4 being particularly prevalent (Figure 1). Strikingly ~70% of tumours showed overexpression of TGFβ1 and many tumours upregulated TGFBR1 expression relative to normal tissue. Taken together, these observations indicated that TGFβ signalling might 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*)(Figure 2). Targeting TGFBR1 kinase activity might have therapeutic benefit for patients with these tumours but in some it maintains tumour suppressive activity. Our efforts are focusing on both understanding the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours and on identifying novel therapeutic susceptibilities of these aggressive cSCCs.

The Molecular Landscape of cSCC and HNSCC

The incidence of keratinocyte skin cancers in white-skinned populations represents a rising global health burden. In SCC, development of primary tumours may be preceded by pre-malignant Actinic Keratosis. In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC.

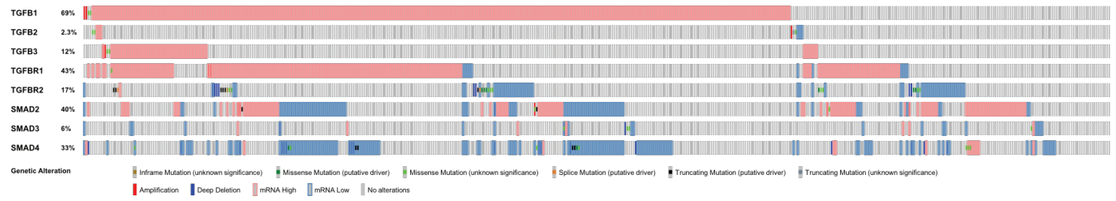

Figure 1
Oncoprint analysis of TGFβ canonical signalling components in HNSCC
Cbioportal (Cerami *et al.,* Cancer Discov. 2012, and Gao *et al.,* Sci. Signal. 2013) analysis of HNSCC (TCGA, Pancancer Atlas) reveals frequent mutational alteration and downregulation of mRNA expression of TGFBR2 and SMAD4 but overexpression of TGFB1 and TGFBR1 compared to normal samples pointing to potential tumour suppressor and tumour promoter roles of TGFβ signalling.

Metastasis occurs in ~5% of cases, and there are few effective treatments for advanced cSCC, with five-year survival of less than 30% reported for metastatic disease (Harwood *et al.,* 2016, *Acta Derm Venereol*). Cutaneous SCC is poorly understood at a molecular level. 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 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. Our whole exome-sequencing analysis of Actinic Keratosis has revealed remarkably similar complex genetic landscapes of both pre-malignant (Thomson *et al.,* 2021, *J Invest Dermatol*) and primary tumours. (Inman *et al.,* 2018, *Nat Commun*). We are now analysing whole genome, exome and bulk RNAseq profiles of human and murine cSCC samples derived from genetically engineered mouse models (in collaboration with Owen Sansom and Karen Blyth). Using systems biology approaches (driven by Peter Bailey) we are integrating these datasets and interrogating the biological pathways, processes and driver genes required for disease progression with a view to identifying therapeutic intervention approaches.

In collaboration with the Glasgow Head and Neck Cancer group (GLAHNC) we are seeking to understand the molecular basis of chemo-radiotherapy resistance, disease recurrence, lymph node metastasis and distant metastatic spread of HNSCC. Our efforts are initially focusing on molecular profiling of clinically annotated patient samples from local site specific cohorts and clinical trials coupled with the development of pre-clinical experimental models.

Publications listed on page 105

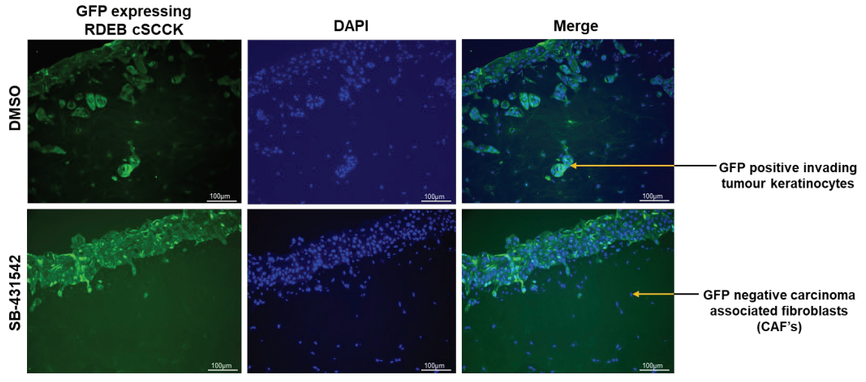

Figure 2
Organotypic assays indicate endogenous TGFβ signalling promotes invasion of RDEB cSCC tumour cells
3D organotypic assays using RDEB cancer associated fibroblasts embedded in type 1 Collagen-Matrigel gels forming a dermal component to test the invasive potential of GFP positive RDEB cSCC tumour keratinocytes. Gels containing SB-431542, a TGFBR1 kinase inhibitor, can inhibit the invasive potential of a subset of RDEB skin tumour cells compared to the DMSO control.