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GROWTH FACTOR SIGNALLING AND SQUAMOUS CANCERS

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The transforming growth factor beta (TGFβ) superfamily comprises approximately forty related dimeric polypeptide cytokines, including the bone morphogenetic proteins (BMPs), the growth and differentiation factors (GDFs), activin, nodal and the TGFβs (TGFβ1, TGFβ2, TGFβ3). TGFβ family members 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, Head and Neck SCC and other squamous tumour subtypes using systems biology and biological functional approaches.

TGFβ signalling in cutaneous squamous cell carcinoma

TGFβ exerts its biological 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, accumulate in the nucleus and regulate expression of hundreds of target genes. In collaboration with Owen Sansom's group, we have previously shown that both TGFBR1 and TGFBR2 are mutationally inactivated in ~ 30% of human cutaneous squamous cell carcinomas (cSCC) and that combined deletion of TGFBR1 coupled with activation of the MAPK pathway is sufficient to drive rapid invasive cSCC formation from the Lgr5+ve hair follicle bulge stem cells in the mouse (Cammareri *et al.*, Nat Commun. 2016; 7: 12493). We are currently investigating how driver gene combinations act in concert with loss of TGFβ signalling to influence cSCC progression both *in vitro* and *in vivo*. As well as possessing potent tumour suppressor activity, members of the TGFβ superfamily can also act as potent tumour promoters. Our analysis of sporadic cSCC indicates that TGFβ signalling is maintained in ~70% of tumours, and we are investigating if this may drive tumour progression and represent a potential therapeutic target.

cSCC is a significant 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 which is responsible for maintaining normal dermal–epidermal junctional architecture in the skin (Figure 1). Unlike in sporadic cSCC, RDEB SCC tumours do not contain inactivating mutations in TGFβ receptors (Cho et al., Sci Transl Med. 2018; 10. pii: eaai7795), and our studies indicate that they exhibit elevated canonical TGFβ signalling activity. We are investigating the potential tumour-promoting role of TGFβ signalling in RDEB cSCC in collaboration with Dr Andrew South (Thomas Jefferson University,

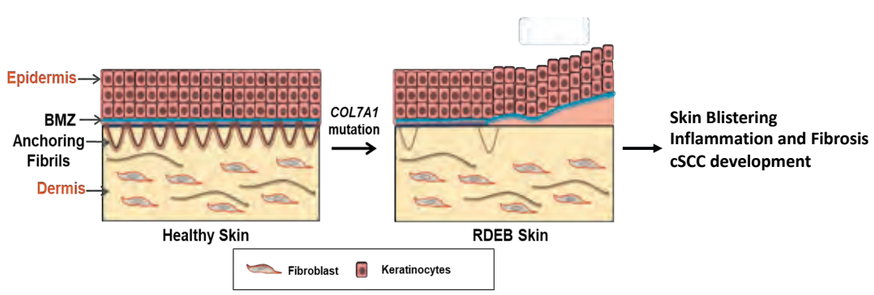

Figure 1. Loss of type VII collagen promotes skin blistering in RDEB patients. RDEB patients have loss-of-function mutations in the collagen VII gene, which results in separation of the epidermis from the underlying dermis as a result of mild friction/trauma. This results in severe blistering and wounding of the skin, leading to inflammation, fibrosis and cSCC development.

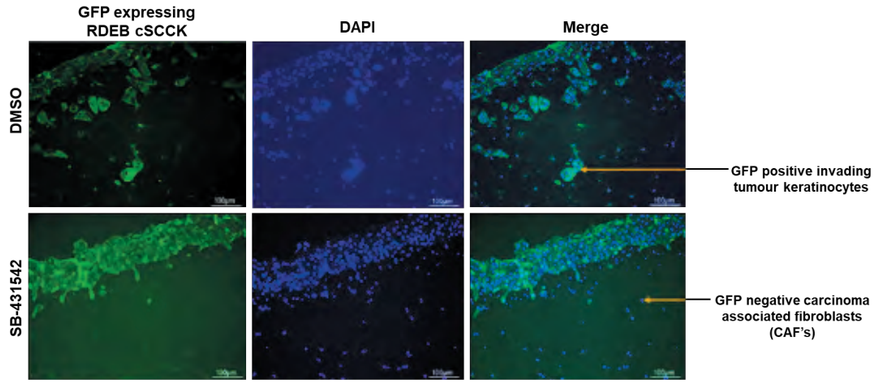

Figure 2 Organotypic assays indicate endogenous TGFβ signalling promotes invasion of RDEB cSCC tumour cells. 3D organotypic assays using RDEB cancer-associated fibroblasts embedded in type 1 collagen-matrigel gels forming a dermal component to test the invasive potential of GFP-positive RDEB cSCC tumour 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.

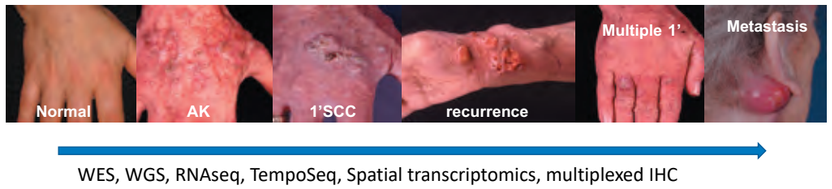

Figure 3 Disease progression of human cSCC. Images illustrating disease progression(Courtesy of Professor Charlotte Proby, University of Dundee). We are performing molecular profiling of human disease progression using next-generation sequencing approaches, immunohistochemistry and spatial transcriptomics.

Philadelphia, Pennsylvania). Our studies so far indicate that whilst stimulation with exogenous TGFβ ligand can inhibit proliferation of all RDEB cSCC patient-derived cell lines (PDCLs) they also exhibit heterogenous TGFβ addiction to endogenous TGFβ signalling. Inhibition of endogenous TGFβ signalling can markedly inhibit the proliferation, clonogenicity, migration and invasion in organtotypic culture (Figure 2) of the majority of but not all RDEB PDCLs. Targeting TGFBR1 kinase activity may have therapeutic benefit for patients with these tumours, but in some it maintains tumour suppressive activity. Our efforts are focusing on developing biomarkers for TGFβ tumour promotion and in understanding the molecular processes by which TGFβ signalling acts to drive proliferation, migration and invasion in these tumours.

The Molecular Landscape of cSCC

The incidence of keratinocyte skin cancers in white-skinned populations currently exceeds that of all other cancers combined and is increasing year on year in our ageing population. In the case of squamous cell carcinoma, development of primary tumours may be preceded by the development of pre-malignant actinic keratosis (Figure 3). In contrast to most other epithelial malignancies, more than a third of patients develop multiple primary cSCC. This is especially true in immunosuppressed individuals, with evidence in organ transplant recipients of a more than 100-fold increased risk of developing cSCC. 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., Acta Derm Venereol. 2016: 96, 3-16,). 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) and Peter Bailey (University of Glasgow) we are embarking on a detailed molecular characterisation of cSCC disease progression using a variety of state-of-the-art next-generation sequencing approaches coupled with spatial analysis of protein and RNA expression. Our initial whole-exome sequencing analysis of primary tumours confirmed the high mutational load of cSCC, with tumours exhibiting an average of 50 mutations per megabase of DNA. (Inman et al., Nat Commun. 2018 Sep 10;9(1):3667). We are now analysing whole-genome and bulk RNA-seq profiles of human and murine cSCC samples derived from genetically engineered mouse models (in collaboration with Owen Sansom). 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 actionable susceptibilities for future therapeutic intervention.

Squamous tumours from other primary sites such as the head and neck, oesophagus, lung and the squamous subtype of pancreatic ductal adenocarcinoma (PDAC) share many common molecular features with cSCC, with prominent dysregulation of TGFβ superfamily signalling. We are assembling panels of PDCLs from these tumour types, and in collaboration with Jen Morton, Peter Bailey and Claire Paterson (NHS Greater Glasgow and Clyde) we are investigating mechanisms of therapy resistance and disease progression in HNSCC and PDAC both *in vitro* and *in vivo* with an initial focus on TGFβ superfamily signalling.

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