CRUK Cambridge Centre Clinical PhD project

PhD Project Title	Subversion of quiescence mechanisms in lung squamous cell cancers
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Project Outline	**Aims and objectives** A recent surprise in airway biology is that there are multiple signalling mechanisms that promote quiescence in homeostatic adult airways. How are these quiescence signaling mechanisms subverted during the initiation of squamous lung cancers? Can they be manipulated to inhibit cancer progression? Our work shows that basal cells are the key stem cells in the steady-state mouse airways. Moreover, we have identified a network of interacting Receptor Tyrosine Kinases (RTK) which precisely control steady-state airway stem cell proliferation in mice (1,2). One control point downstream of the RTKs is levels of the transcription factor SOX2. SOX2 is necessary for stem cell proliferation and increased levels are a driver of squamous cell lung cancer and increased pAKT signalling (3). There is ad-hoc evidence for post-translational changes in RTK signalling in human squamous cell cancers. In addition, FGF Receptors have been shown to be useful combinatorial targets in KRAS-driven lung adenocarcinomas (4). **The overall objective** of this research is to test if manipulating RTK signalling levels to restore cellular quiescence could be of therapeutic benefit in squamous cell lung cancers. **Aims:** 1. Test the hypothesis that the RTK networks which limit airway stem cell proliferation in mice are conserved in the normal human airways and determine the mechanism by which oncogenic SOX2 impinges on FGFR signalling. 2. Test the hypothesis that reproducible post-transcriptional re-wiring of RTK signalling modules occurs in primary human tumours and that these are potential therapeutic targets.
Experimental plan	1. Establish organoid cultures from human airway basal cells using established methods in routine use in the lab. Test the ability of specific signalling ligands and RTK inhibitors to modulate proliferation, colony forming ability and differentiation of organoids. Test the effects of knocking-out the key pathway modulators *SPRY1* and *SPRY2* using Crispr-Cas9 technology. Use an established human organotypic culture system to induce airway dysplasia by SOX2 over-expression and investigate the effects on FGFR signalling in the presence/absence of a common SOX2/RTK target, ETV4. 2. Use custom antibody arrays to determine protein levels and phosphorylation status of RTK pathways in primary squamous cell samples. Tumour samples will be split such that a part of each sample will be cultured as a tumour spheroid/organoid and frozen to allow future analysis. The effects of manipulating RTK pathways will be tested using in vitro organoid assays and

	ultimately in patient-derived xenografts.
Main Techniques	• organotypic and organoid cultures of primary and transformed human airway basal cells • microscopy-based proliferation and differentiation assays • qRT-PCR • western blots/antibody arrays • CRISPR • tumour xenografts
Key References	**1. Balasooriya, G.I., Johnson, J.A., Basson, M.A., Rawlins, E.L.,** *2016. An FGFR1-SPRY2 Signaling Axis Limits Basal Cell Proliferation in the Steady-State Airway Epithelium. Dev Cell 37, 85-97.* **2. Balasooriya, G., Goschorska, M., Piddini, E., Rawlins, E.L.,** *2017. FGFR2 is required for airway basal cell self-renewal and terminal differentiation. Development.* **3. Correia, L.L., Johnson, J.A., McErlean, P., Bauer, J., Farah, H., Rassl, D.M., Rintoul, R.C., Sethi, T., Lavender, P., Rawlins, E.L., Littlewood, T.D., Evan, G.I., McCaughan, F.M.,** *2017. SOX2 Drives Bronchial Dysplasia in a Novel Organotypic Model of Early Human Squamous Lung Cancer. Am J Respir Crit Care Med.* **4. Manchado, E., Weissmueller, S., Morris, J.P.t., Chen, C.C., Wullenkord, R., Lujambio, A., de Stanchina, E., Poirier, J.T., Gainor, J.F., Corcoran, R.B., Engelman, J.A., Rudin, C.M., Rosen, N., Lowe, S.W.,** *2016. A combinatorial strategy for treating KRAS-mutant lung cancer. Nature 534, 647-651.*