DYSREGULATED IRON METABOLISM MEDIATED BY IRP2 MAY INFLUENCE LUNG CANCER PROGRESSION, PARTICULARLY IN THE CONTEXT OF CIGARETTE SMOKE EXPOSURE

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Methods Conventional enzymatic digestion of the murine upper respiratory epithelium involves an overnight pronase incubation. Using flow cytometry, we compared this strategy to other reported methods: a dispase/trypsin digest,1 collagenase incubation and a combination of these.

A method allowing selection of a pure basal cell population in the mouse trachea was subsequently translated to human airways to assess its efficacy.

Results Following pronase digestion, only 2% of epithelial cells were basal cells, probably as a result of enzymatic epitope removal. Optimal extraction of murine basal cells involved removal of the epithelium through a dispase/trypsin incubation followed by incubation of tracheal remnants in collagenase to release the remaining basal cells from submucosal glands. We identified a well-defined basal cell population representing 30% of the airway epithelium, consistent with known airway basal cell frequency, which can be isolated by fluorescence-activated cell sorting.

Application of this strategy to digest human airways revealed a comparable population of basal cells and allowed sorting of a viable cell population.

Conclusions We optimised a method to facilitate the extraction of basal epithelial cells from both mouse and human airways. This strategy allows sorting of a pure, viable basal cell population for use in further assays.

REFERENCE
1 Hegab AE et al. Isolation of basal cells and submucosal gland duct cells from mouse trachea. JOVE 2012;67

METHODS TO ISOLATE BASAL CELLS FROM THE RESPIRATORY EPITHELIUM

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Introduction and objectives Maintenance of a healthy respiratory epithelium is essential in the prevention of airway disease. Perturbations in airway homeostasis have been linked to the pathogenesis of airway disease including asthma, fibrosis and lung cancer. The ‘stem cell hypothesis’ describes how a change within cells responsible for airway maintenance and repair can lead to development of cancer. Basal stem/progenitor cells in the upper airways are suggested to represent the cell of origin in squamous cell carcinoma and therefore are of research interest.

Isolation of this cell type has been hampered because established airway enzymatic digestion methods destroy epitopes of interest on the surface of basal cells. We sought an optimised method of digestion for the isolation of viable basal cells from murine, and then human, airway epithelium.

MMP12 AND LMO7 ARE KEY GENES INVOLVED IN THE EARLY PATHOGENESIS OF SQUAMOUS CELL CARCINOMA OF THE LUNG

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Introduction and objectives Early events associated with lung cancer progression are poorly understood. We performed genome-wide gene expression Illumina Whole-Genome DASIL® arrays in 19 regressive and 20 progressive pre-invasive lung SCC lesions. The expression of matrix metallopeptidase 12 (MMP12) and LIM domain 7 (LMO7) was also determined in the 39 pre-invasive lung SCC lesions by immunostaining analysis. The functional role of MMP12 and LMO7 in cell migration and invasion was demonstrated by MMP12 and LMO7 knockdown in different squamous cell carcinoma cell lines and human bronchial epithelial cells (HBECs), respectively.

We found 939 genes significantly differently expressed between the progressive and the regressive pre-invasive lung SCC lesions. We identified a remarkably elevated expression of a spectrum of genes in the progressive lung SCC lesions involved in different