S110 DYSREGULATED IRON METABOLISM MEDIATED BY IRP2 MAY INFLUENCE LUNG CANCER PROGRESSION, PARTICULARLY IN THE CONTEXT OF CIGARETTE SMOKE EXPOSURE

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Background Iron is required for cell growth, and various cancers have been shown to proliferate more readily when iron replete. We have shown this previously in lung cancer and further demonstrated that this was reduced by either iron chelation or knockdown of *IREB2*, an iron regulatory gene. [1] Differences in iron content of bronchoalveolar lavage (BAL) fluid have been reported in smokers compared to non-smokers, [2] so we hypothesised that iron dysregulation might be an active mechanism of cancer progression in smokers.
Methods Two lung cancer cell lines were cultured with either ferrous (Fe2+) or ferric (Fe3+) forms of iron, or with cigarette smoke extract (CSE). Proliferation, apoptosis, necrosis and migration were assessed by BRDU assay, FACS and scratch wound assay respectively. Iron regulation was assessed by means of gene expression and Western blot for *IREB2* (protein product IRP2), ferritin and transferrin receptor.

Resected lung cancers (n = 78) were stained for iron regulatory protein 2 (IRP2) and staining related to clinical features such as tumour size and survival.
Results Cancer cells proliferated more in the presence of ferrous iron or 5% CSE (p

Cancers staining positive for IRP2 tended to be larger (p = 0.045) and survival poorer (p = 0.079).
Conclusions Proliferation of cancer cells driven by iron dysregulation may be a clinically relevant mechanism in lung cancer, particularly in smokers.

REFERENCES

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S111 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.
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

S112 MMP12 AND LMO7 ARE KEY GENES INVOLVED IN THE EARLY PATHOGENESIS OF SQUAMOUS CELL CARCINOMA OF THE LUNG

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Lung cancer is the most lethal cancer type worldwide. In order to improve patient survival it is important to enhance our understanding of the early changes associated with lung cancer progression. UCLH has a unique cohort of patients with pre-invasive lung squamous cell carcinoma (SCC) lesions. Within this cohort there is a discrepancy between the prevalence of pre-invasive lesions and the incidence of invasive lung cancer, which suggests that not all pre-invasive lesions progress to invasive carcinomas. The aim of this study was to identify and characterise key genes involved in the early pathogenesis of lung SCC.

We performed genome-wide gene expression Illumina Whole-Genome DASL® 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 cancer 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

related cancer pathways including chromosome instability, p53 signalling and Wnt/β-catenin signalling. MMP12 and LMO7 were found within the highest significantly differently expressed genes and were therefore chosen to pursue studies focused on understanding the potential mechanisms leading to the development of lung SCC. In agreement with the gene expression data the expression of MMP12 and LMO7 proteins were up-regulated and down-regulated, respectively, in progressive when compared with regressive lesions. Inhibiting MMP12 by MMP12 knockdown significantly reduced the migration and invasion of different squamous cell carcinoma cell lines (A431, H357 and H376). We also established HBECs knockdown targeting LMO7. We observed a significant increase in the migration and invasion of HBECs cells in the LMO7 shRNA knockdown compared to control.

Our results suggest that MMP12 and LMO7 may be potential therapeutic markers for lung cancer at early stage.

Infection of the pleural space in disease and on purpose

S113 PREDICTORS OF BACTERIAL 'LOAD' IN PLEURAL INFECTION

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Pleural infection is usually defined using pleural fluid biochemical characteristics, given that only ~30% of cases are culture positive, but the relationship between these characteristics and pleural space bacterial concentration is unclear.

We developed an assay to estimate bacterial 'load' using quantitative polymerase chain reaction (PCR) to determine 16S rRNA gene copy number in pleural fluid samples (this gene is present in all bacteria). This enabled us to explore the relationship between patient characteristics and pleural fluid bacterial 'load'.

Methods Pleural infection samples were obtained from the Second Multicentre Intrapleural Sepsis randomised controlled Trial (MIST2), REC no. 04/MRE5/53. DNA was extracted using the FastDNA SPIN Kit. Quantitative PCR (qPCR) of the 16S rRNA gene was undertaken using the ultra-pure Power SYBR Green PCR reagent and primers that amplified the 467 nt V3–4 region of the 16S rRNA gene. A 3-step thermal cycling profile was empirically determined to give optimal results. Ten-fold dilutions of *Acidothermus cellulolyticus* DNA were used to estimate sample 16S rRNA gene concentration. All PCRs were performed in duplicate. Melt-curve analyses and agarose gel electrophoresis of qPCR amplicons were used to ensure absence of non-specific PCR products.

Results 172 pleural fluid samples were analysed. Pleural fluid pH, culture status, appearance, LDH and glucose were all predictive of bacterial load (see Table). Patient C-reactive protein (CRP) and white cell count (WCC) were not significantly associated with bacterial load.

Conclusions Bacterial 'load' was associated with acknowledged predictors for pleural infection. Such findings add further support to the utility of pH, glucose and LDH values as proxies for pleural infection, in the correct clinical context. Patient WCC and CRP were not significantly associated with bacterial 'load'.

This assay is limited in that it assesses total bacterial DNA (from viable and dead bacteria), rather than quantifying viable

Abstract S113 Table 1 Relationships between copies of 16S rRNA gene (base 10 logarithmic values) and characteristics of patients and pleural fluid (PF) samples

		Copies of 16S rRNA gene, measured by qPCR				
	Number	% change per unit[1]	95% CI	Geometric mean	95% CI	*p* value
PF pH	97	-89.6	-97.6, -55.5			0.003
PF culture status						
Negative	110			2.8×10^5	1.9×10^5, 4.0×10^5	
Positive	44			2.2×10^6	1.2×10^6, 4.0×10^6	<0.0001
PF appearance						
Non-purulent	74			1.9×10^5	1.2×10^5, 2.9×10^5	
Purulent	76			1.4×10^6	8.8×10^5, 2.2×10^6	<0.0001
PF LDH						
≤1000	26			1.4×10^5	6.9×10^4, 2.8×10^5	
1000–5000	41			4.1×10^5	2.3×10^5, 7.1×10^5	
>5000	36			6.2×10^5	3.4×10^5, 1.1×10^6	0.007
PF glucose						
≤1.0	43			5.9×10^5	3.4×10^5, 1.0×10^6	
1.0–2.2	14			2.9×10^5	1.1×10^5, 7.3×10^5	
>2.2	35			1.5×10^5	8.5×10^4, 2.8×10^5	0.007
Patient CRP						
<100	28			3.5×10^5	1.6×10^5, 7.5×10^5	
100–160	27			3.5×10^5	1.6×10^5, 7.7×10^5	
≥160	87			5.8×10^5	3.7×10^5, 8.9×10^5	0.372
Patient WCC						
≤11.0	41			3.7×10^5	1.9×10^5, 7.0×10^5	
11.0–16.5	58			4.6×10^5	2.7×10^5, 7.9×10^5	
>16.5	53			7.7×10^5	4.4×10^5, 1.4×10^6	0.215

[1]% change in 16S rRNA gene copies number per unit increase in the specified variable. CI = confidence interval. *p* values for tests of linear trend (continuous variables) and for tests of heterogeneity (categorical variables). LDH units – IU/L; glucose units – mmol/L; CRP units – mg/L; WCC units – x 10^9/L