S7 MOUSE LUNG ADENOCARCINOMA CELL LINES REVEAL PRL2C2 AS A NOVEL LUNG TUMOUR PROMOTER

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Background Carcinogen-inflicted human cancers, including lung tumours harbour thousands of mutations per genome, most of which are unknown (Garraway, LA *et al*, *Cell* 2013;153:17–37).

Aim To develop a faithful mouse model of human tobacco carcinogen-induced lung adenocarcinoma suitable for the identification of novel oncogenic genes and pathways.

Methods We repeatedly managed to obtain several murine lung adenocarcinoma cell lines (MLA) by chronically exposing various mouse strains to different tobacco carcinogens. MLA were characterised for cancer stemness and oncogenes, as well as global gene expression.

Results To date, 12 MLA cell lines have been derived from *Wt* and transgenic mice on the *FVB*, *Balb/c*, and *C57BL/6* strains by means of urethane or diethylnitrosamine exposure. All MLA were immortal, phenotypically stable, and indefinitely passaged *in vitro* over a period of over 18 months and/or 60 passages. In addition, all cell lines were oncogenic, transplantable, metastatic, and uniformly lethal *in vivo*. Interestingly, MLA displayed *Kras* mutations in codon 61, mono- or bi-allelic *Trp53* loss, and expression of lung cancer stemness factors *Itgb3* and *Lgr6*, in amazing similarity to human lung cancers. Microarray revealed that all MLA cell lines heavily overexpressed *Prl2c2*, encoding proliferin, in comparison to the native lungs. *Prl2c2* silencing diminished MLA proliferation and stemness, to a degree comparable with *Itgb3* interference.

Conclusions MLA are faithful models of human lung adenocarcinoma that led to the discovery of *Prl2c2* as a candidate lung tumour promoter.

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S8 OSTEOPONTIN AS AN AIRWAY EPITHELIAL TUMOUR PROMOTER

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Osteopontin (secreted phosphoprotein 1; SPP1) expression has been identified in human lung cancer and has been linked with

enhanced tumour progression. To examine its functional role, we induced lung tumours by repetitive urethane or MCA/BHT lung carcinogens in *C57BL/6* mice lacking both (*Spp1*^{-/-}), one (*Spp1*^{+/-}), or no (*Spp1*^{+/+}) copy of the endogenous *Spp1* gene. Primary end-points were lung tumour number and size; secondary end-points were SPP1 expression, epithelial cell survival, carcinogen-induced inflammation, and angiogenesis. Data are presented as mean ± SD.

Compared with *Spp1*^{+/+} mice (n = 22), *Spp1*^{-/-} mice (n = 25) developed dramatically fewer and significantly smaller lung tumours in response to urethane, while *Spp1*[±] mice (n = 12) behaved similar to *Spp1*^{-/-} mice (number/diameter of lung tumours in *Spp1*^{+/+}, *Spp1*^{+/-}, and *Spp1*^{-/-} mice, respectively: 16.1 ± 12.7/1.2 ± 0.3 mm, 2.4 ± 2.3/0.9 ± 0.2 mm, and 1.3 ± 1.6/0.7 ± 0.2 mm; P < 0.05 for comparison of *Spp1*^{+/+} with *Spp1*^{-/-} and *Spp1*^{+/-} mice). *Spp1*^{-/-} mice were also protected from two-hit MCA/BHT-oncogenesis compared with *Spp1*^{+/+} controls. *Spp1*^{-/-} mice displayed decreased epithelial cell survival and reduced numbers of airspace macrophages early after urethane, and enhanced tumour cell apoptosis and limited tumour angiogenesis at late stages of lung tumour progression. SPP1 was expressed in the naïve lung by non-ciliated airway epithelial cells and alveolar macrophages and was significantly up-regulated during multi-stage lung carcinogenesis.

Our data indicate that SPP1 is functionally involved in airway epithelial carcinogenesis and may present a target for lung cancer treatment and prevention.

S9 THE ROLE OF LRIG1-DEPENDENT EGFR SIGNALLING IN AIRWAY HOMOEOSTASIS AND SQUAMOUS CELL LUNG CANCER DEVELOPMENT

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Background Aberrations of EGFR signalling drive cancer development. In squamous cell lung cancer (SqCLC), EGFR is overexpressed. LRIG1 is a negative regulator of EGFR and patient pre-invasive SqCLC samples show LRIG1 loss, suggesting involvement in early disease pathogenesis. In skin and gut homeostasis, LRIG1 regulates stem cells. In the upper airway, basal cells act as stem cells and are the putative origin of SqCLC. We hypothesise LRIG1 has a key role in airway homeostasis and its loss promotes pre-invasive SqCLC development.

Methods *Lrig1* EGFP-ires-CreERT2 mice were used to delineate airway LRIG1 expression. Flow sorted LRIG1-positive and -negative murine basal cells were used in 2D and 3D colony-forming, spheroid and proliferation assays. A murine SqCLC model was set up through application of N-Nitrosotris-(2-chloroethyl)urea (NTCU). Pre-invasive lesions and tumour development were compared between wild-type (WT), heterozygous and LRIG1-knockout (KO) animals. Human basal cells obtained from bronchoscopy were sorted according to LRIG1 expression and used directly in colony-forming assays or maintained in primary culture to assess the effect of shRNA knockdown of LRIG1. LRIG1-knockdown cells were assessed in colony-forming and proliferation assays, and differentiation and invasion were assessed using organotypic models.

Results LRIG1 is expressed by 40% of airway basal cells. LRIG1-expressing murine basal cells exhibit increased colony-forming capacity (p = 0.0286), spheroid formation (p = 0.0043) and

proliferation ($p = 0.0043$) compared with LRIG1-negative cells. Similarly, LRIG1-expressing human airway basal cells isolated from endobronchial brush biopsy samples exhibit increased colony-forming capacity ($p = 0.0469$). Topical application of NTCU to mice recapitulates the development of human pre-invasive and SCLC lesions after 23 weeks. Results show lesions in LRIG1-KO mice to be larger than those of WT animals. Knock down of LRIG1 in cultured human airway basal cells alters cell phenotype, leading to an increased colony-forming efficiency and greater proliferation at cell confluence.

Conclusions LRIG1 has an important role in stem cell homeostasis of the human and murine airway epithelium. Loss of LRIG1 promotes pre-cancerous lesion development in a murine SqCLC mouse model and behaviour of human epithelial cells in culture, indicating a potential target for chemoprevention of SqCLC in humans.

S10 INVESTIGATION OF VESSEL STRUCTURE IN THE VICINITY OF LUNG TUMOURS

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Lung cancer is considered a major cause of cancer death.¹ We are currently developing methods for detection of vessels in lung CT images. The aim of this study was to investigate the number of vessels in areas of unilateral lung tumours and compare with the equivalent contralateral lung with no tumour. Lung CT images were downloaded from the Cancer Imaging Archive wiki.cancerimagingarchive.net/display/Public/LungCT-Diagnosis.² Software was written in MATLAB (The MathWorks Inc., USA) in order to display and analyse the DICOM images. Windowing was performed manually in order to clearly display the tumours as well as surrounding vessel like structures. Using the software eight sets of images were analysed; the number of clearly defined vessel like structures directly attached to the tumour were counted and compared with the corresponding region in the contralateral lung with no evidence of tumour; small vessel like structure and branches were not included. The area of the tumour was manually delineated and calculated in terms of pixels. For each set of CT images, one image was used where the tumour size was greatest. In all eight cases the number of clear vessel like structures in the immediate vicinity of the tumour was greater than that in the corresponding area on the contralateral side, mean (standard deviation) of the difference 5 (1.6), there was a significant difference $p < 0.001$ (one sample t test). In addition vessel like structures often appeared brighter on the side of the tumour. The results of this pilot study suggest that the number of clear bright vessel like structures in the immediate vicinity of a lung tumour may be higher than in the corresponding area on the contralateral side. We feel this research merits further study in order to investigate if this approach may help enable early detection of lung tumours.

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Progress in the ITU

S11 DECREASED ANTI-INFLAMMATORY POTENTIAL OF MESENCHYMAL STEM CELLS AFTER PROLONGED IN VITRO EXPANSION WILL IMPACT ON THEIR USE AS A THERAPY FOR ACUTE RESPIRATORY DISTRESS SYNDROME

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Introduction and objectives Mesenchymal stem cells (MSC) have well-established anti-inflammatory properties and could potentially be used therapeutically in Acute Respiratory Distress Syndrome (ARDS).¹ MSC conditioned medium (MSC-CM) has been found to reproduce the beneficial effects of MSCs.² However, the impact of expansion on MSC secretome remains elusive. In the present study we assessed the expression of four potent MSC paracrine factors after prolonged *in vitro* culture, while investigating the effects of passaging on the *in vitro* properties of MSCs.

Methods Human Bone marrow-derived MSCs were expanded *in vitro* in α MEM with 16% FBS. Conditioned medium was collected from each passage and stored at -40°C . Scratch assays were undertaken using A549 cells treated with MSC-CM and control media. Proliferation of A549 cells was assessed via BrdU assay. Gene expression of expanded MSCs was assessed by RT-qPCR. VEGF and Angiopoietin in the MSC-CM were quantified by ELISA.

Results RT-qPCR revealed that MSCs express the anti-inflammatory genes *PTGES2*, *FGF7* and *ANGPT1*. The expression of these genes doubled after 8 days in culture and subsequently decreased. ($P < 0.0005$, one way Anova, $n = 2$). The secretion of Angiopoietin and VEGF decreased with prolonged expansion (Figure 1). MSC-CM obtained after 8 days of culture induced more efficient wound healing compared to MSC-CM obtained following prolonged expansion ($P < 0.005$, one way Anova, $n = 2$). However, MSC-CM failed to affect the proliferation of A549 cells.

Conclusion We conclude that MSCs of early passages are more potent angiogenic inducers, while promoting wound healing in

Abstract S11 Figure 1 Levels of secreted VEGF decrease after prolonged *in vitro* culture (* $P < 0.05$, *** $P < 0.0005$, $n = 2$)