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

[1]NI Kanellakis, [2]A Giannou, [3]M Pepe, [2]T Agalioti, [2]D Zazara, [3]M Vreka, [2]I Lilis, [2]I Giopanou, [2]M Spella, [2]A Marazioti, [1]N Rahman, [4]I Pavord, [1]I Psallidas, [3]GT Stathopoulos. *[1]Laboratory of Pleural Translational Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [2]Laboratory Molecular Respiratory Carcinogenesis, University of Patras, Patras, Greece; [3]Lung Carcinogenesis Laboratory, Comprehensive PneumologyCenter (CPC), University Hospital, Ludwig-Maximilians University and Helmholtz ZentrumMünchen, Munich, Germany; [4]Respiratory Medicine Laboratory, Nuffield Department of Medicine, University of Oxford, Oxford, UK*

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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

[1]I Psallidas, [1]N Kanellakis, [2]M Vreka, [3]A Giannou, [3]L Maniatis, [4]C Moschos, [3]I Giopanou, [3]T Agalioti, [3]I Lilis, [4]S Magkouta, [4]I Kalomenidis, [1]NM Rahman, [1]I Pavord, [2]GT Stathopoulos. *[1]Laboratory of Pleural Translational Research, Nuffield Department of Medicine, Oxford Centre for Respiratory Medicine, Oxford University Hospitals Trust, Oxford, UK; [2]Comprehensive Pneumology Centre (CPC) and Institute for Lung Biology and Disease (iLBD), Ludwig-Maximilians-Universität, Asklepios Fachkliniken München-Gauting und Helmholtz Zentrum, Munich, Germany; [3]Laboratory for Molecular Respiratory Carcinogenesis, Department of Physiology, Faculty of Medicine, Rio, Greece; [4]First Department of Critical Care and Pulmonary Medicine, University of Athens School of Medicine, General Hospital Evangelismos, Athens, Greece*

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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

L Succony, KHC Gowers, RE Hynds, R Thakrar, A Giangreco, D Davies, S Janes. *University College London, London, UK*

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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