

Abstract S89 Figure 1 Infra-red thermal imaging of coil apparatus with mouse in situ receiving hyperthermia treatment (arrow indicates tumour).

Ferucarbotran for labelling. Subcutaneous tumours were induced by co-injecting 5 million OVCAR cells and 0.5 million SPION-labelled MSCs.

- ▶ Images of cells within mice were obtained on a 9.4T horizontal bore Varian NMR system.
- ▶ A 10 mT 1.05 MHz AMF was generated using a copper solenoid coil, diameter 3 cm. Mice were placed inside the coil under anaesthesia and each hyperthermia session lasted 20 min.
- ▶ Post-heating histology was obtained.

**Results** Hyperthermia treatment caused a rise in temperature up to 60°C *in vitro* and a rise in tumour temperature of up to 4°C above body temperature *in vivo* (Abstract S89 Figure 1), detected by thermal imaging and with fibre optic probes. Iron was confirmed in the tumour using MR imaging and histology.

**Discussion** This study demonstrates the potential of SPION to act as an imaging agent and cancer therapy. SPION-labelled MSCs can be imaged *in vivo* in very low cell numbers. We have demonstrated that the application of an alternating magnetic field causes a temperature rise in these cells, both *in vitro* and *in vivo*. In the future, we will optimise SPION as an imaging and hyperthermia agent, for the targeted treatment of lung metastases.

**Acknowledgements** We are grateful for the support of the Medical Research Council.

## S90 HOMOCYSTEINE LEVELS IN NON-SMALL CELL LUNG CANCER

doi:10.1136/thx.2010.150938.41

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Plasma homocysteine level is the most sensitive marker of sub-clinical folate deficiency. Elevated levels of homocysteine have been associated with several pathologies, particularly vascular and cardiac disease. Patients with lung cancer are frequently elderly and may have insufficient intake of folate. Sub-clinical folate deficiency may lead to increased morbidity during chemotherapy and radiotherapy, and possibly reduced response rates to both treatment modalities. We have measured plasma homocysteine levels in 460 patients with a diagnosis of non-small cell lung cancer who were referred to our lung cancer unit between 2005 and 2009. Two hundred and fifty-six patients were male, 204 were female. The median age was 71. 22% of patients were PS 0-1, 35% PS2 and 43% PS3. The local laboratory reference range for homocysteine is  $\leq 15 \mu\text{mol/l}$ . 43% of patients had elevated plasma levels of homocysteine at diagnosis. In 35% the level was between 15.1 and 25  $\mu\text{mol/l}$ , and in 8% the homocysteine level

was greater than 25  $\mu\text{mol/l}$ . Patients with elevated plasma homocysteine levels had inferior median and 1 year survival rates, both for stage III disease (464 vs 356 days ms, 1 year survival 65% vs 47%) and stage IV disease (212 vs 190 days ms, 1 year 30% vs 21%). The finding of elevated homocysteine levels in 43% of newly diagnosed patients with non-small cell lung cancer is of potentially profound significance, as this implies that there could be a possible therapeutic benefit from folate and B12 supplementation before and during treatment for this group of patients with non-small cell lung cancer.

## S91 THE ROLE OF THE VITAMIN D AXIS IN LUNG CANCER

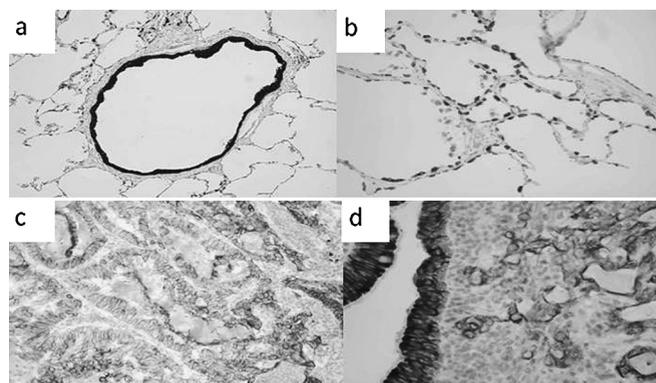
doi:10.1136/thx.2010.150938.42

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**Introduction** Vitamin D deficiency has been associated with many cancers, although little is known about its role in lung cancer. This is biologically plausible since vitamin D influences apoptosis and inhibits cell proliferation. Vitamin D receptor (VDR) polymorphisms have also been suggested to play a role in genetic susceptibility, their direction being consistent with inability to respond to vitamin D. Finally, vitamin D binding protein (DBP) indirectly activates alveolar macrophages, a higher number of which within a tumour links to better prognosis in lung cancer.

**Methods** 37 patients with a diagnosis of lung cancer were studied in the first phase, together with 18 healthy controls. Circulating vitamin D level was measured at initial presentation by tandem mass spectrometry, and DBP by ELISA. Vitamin D and DBP level was compared between groups, and against other clinical features. VDR was quantified in normal lung and tumour tissue by immunohistochemistry, and compared between the two. In the second phase the lung cancer patients were genotyped for four functional SNPs in the vitamin D pathway, and their genotype frequencies compared to 484 healthy controls.

**Results** Vitamin D levels were lower in lung cancer than controls, after adjustment for season of collection (21.8 vs 15.5 ng/ml,  $p=0.018$ ), as was DBP (28.2 vs 51.7 mg/dl). DBP was higher in women ( $p=0.001$ ) and correlated directly with vitamin D ( $r=0.446$ ,  $p=0.013$ ). VDR was expressed primarily in bronchial epithelium, and to a lesser extent in pneumocytes, but was generally expressed



Abstract S91 Figure 1 VDR stains brown in (A) bronchial epithelium (B) less strongly in pneumocytes and (C&D) adenocarcinoma. Tissue in all bar (D) is from the same individual. (D) shows both epithelium and cancer tissue. Similar results were seen in two patients with squamous carcinomas.

less in tumour tissue (Abstract S91 Figure 1). Of the four SNPs studied two were associated with lung cancer; the associated allele of rs4588 is known to reduce macrophage activation and conferred an odds ratio (OR) of 3.04, whilst that of rs1544410 produces less stable VDR mRNA and conferred an OR of 4.10 of disease.

**Conclusions** Vitamin D deficiency is common in lung cancer, but reduced VDR in tumour tissue suggests that it is unlikely to be useful as an adjuvant treatment, as tumour tissue will not be able to respond to it. We have also confirmed the role of VDR polymorphisms in lung cancer, similar to other malignancies.

## S92 EPIHELIAL MESENCHYMAL TRANSITION OCCURS EARLIER THAN PREVIOUSLY THOUGHT IN THE DEVELOPMENT OF SQUAMOUS CELL CARCINOMA OF THE LUNG

doi:10.1136/thx.2010.150938.43

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**Introduction** Squamous cell carcinoma is believed to develop in a step-wise fashion from squamous metaplasia (SqMet), through low- and high-grade dysplasia (LGDys; HGDys) and carcinoma-in-situ (CIS) to invasive disease (InvSCC). Epithelial mesenchymal transition (EMT) is an important process by which epithelial cells shed their differentiated characteristics and acquire mesenchymal, fibroblast-like properties including increased motility and invasiveness. There is now convincing phenotypic, genetic and functional evidence that EMT plays a central role in carcinogenesis. To date, EMT is believed to occur during transition from pre-invasive disease to invasive disease. A hallmark of EMT is the down-regulation of E-cadherin, a cell adhesion molecule present in the plasma membrane of epithelial cells. We have examined the role of EMT during the progression of bronchial dysplasia.

**Methods** Using immunohistochemistry, 170 formalin-fixed paraffin-embedded blocks from lung cancer biopsies and resection specimens were stained for epithelial markers E-cadherin and MNF116 and the mesenchymal marker S100A4. In each sample, areas of SqMet, LGDys, HGDys (incorporating CIS) and InvSCC were identified. Up to three representative areas were assessed for each lesion type present and an aggregate score assigned which took into account strength and extent of staining. Specimens were also stained for  $\beta$ -catenin which translocates from the membrane to the nucleus during EMT. One hundred cells per high power field were counted and the proportion of membranous, cytoplasmic and nuclear staining calculated.

**Results** There was a progressive loss of epithelial markers and a concurrent gain in S100A4 with increasing dysplasia. The switch from expression of epithelial to mesenchymal markers began as early as SqMet and reached significance for all three markers between LGDys and HGDys. Likewise,  $\beta$ -catenin showed translocation from membranous to cytoplasmic expression; this also reached significance between LGDys and HGDys. The difference in nuclear expression between groups did not reach significance.

**Conclusions** In order to improve lung cancer survival there is increasing interest in identifying early stage disease. The mechanisms underlying progression of bronchial dysplasia are poorly understood. Our results suggest that EMT is occurring much earlier in bronchial dysplasia than previously appreciated and this may have implications for the surveillance and treatment of pre-invasive disease.

## S93 IS BCL-2 IMPORTANT IN SMALL CELL LUNG CANCER?

doi:10.1136/thx.2010.150938.44

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**Introduction** Bcl-2 is an anti-apoptotic protein that has previously been associated with increased cell survival in Small Cell Lung Cancer (SCLC) *in vitro*. However, several immunohistochemical studies of Bcl-2 expression and survival using patient samples have produced conflicting results. We aimed to determine if Bcl-2 expression has prognostic relevance in SCLC using a unique dedicated SCLC tissue microarray (TMA).

**Methods** The TMA was constructed using formalin fixed, paraffin embedded diagnostic biopsy samples (endobronchial biopsies, TBNA, CT-guided needle biopsies) from 203 patients diagnosed at Papworth Hospital (Cambridge, UK) between 1998 and 2005. 189 cases had cores of tissue on the completed TMA. There was some attrition due to the small size of individual biopsies. The TMA was stained for Bcl-2 using a standard antibody (DAKO clone 124) and the slides were scored by two readers for both extent and intensity of tumour cell staining. Data from the TMA were then correlated with clinical data. The results obtained were combined with those of previous studies in a meta-analysis.

**Results** 140 cases had tumour tissue on the TMA that could be evaluated for Bcl-2 staining. Patients with low intensity staining had better overall survival than those with high intensity staining in a Cox regression analysis (HR 0.55, 95% CI 0.33 to 0.94,  $p=0.03$ ,  $n=117$ ). The meta-analysis included 510 deaths in 673 cases and showed no significant effect of Bcl-2 on survival (HR 0.91, 95% CI 0.74 to 1.09).

**Conclusion** This study equals the largest published study of Bcl-2 expression in SCLC. Our data showed improved survival in SCLC patients with lower Bcl-2 expression, consistent with *in vitro* data and the rationale behind current on-going trials of Bcl-2 antagonists in SCLC. However, our meta-analysis showed no overall effect and this may be due to differences in staining and scoring methods between the studies.

## S94 TWO NOVEL DETERMINANTS OF ETOPOSIDE RESISTANCE IN SMALL CELL LUNG CANCER

doi:10.1136/thx.2010.150938.45

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**Introduction** Small Cell Lung Cancer (SCLC) typically responds well to initial chemotherapy with Etoposide and a platinum-containing agent. However survival is poor due to invariable relapse with chemoresistant disease. We used a unique series of SCLC cell lines (GLC-14, 16, 19), derived from a single patient at various time-points during her treatment, to identify genes involved in Etoposide resistance. We then attempted to determine their functional role and validate their importance using patient specimens.

**Methods** The relationship of the cell lines to each other was confirmed using genomic methods. Genes whose expression pattern could explain the relative response of the cell lines to treatment with Etoposide were identified using cDNA microarray. These candidate Etoposide response genes were cloned from the cell line in which they were expressed at the highest level and transiently over-expressed in the cell line in which they were naturally expressed at the lowest level to determine whether this altered Etoposide resistance. Using