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 EPITHELIAL MESENCHYMAL TRANSITION OCCURS EARLIER THAN PREVIOUSLY THOUGHT IN THE DEVELOPMENT OF SQUAMOUS CELL CARCINOMA OF THE LUNG

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[1]N M Cummings, [2]D M Rassl, [2]H C Boobier, [3]J R Gosney, [4]A G Nicholson, [5]L D Sharples, [1]R C Rintoul. *[1]Department of Thoracic Oncology, Papworth Hospital, Cambridge, UK; [2]Department of Pathology, Papworth Hospital, Cambridge, UK; [3]Department of Histopathology, Royal Liverpool University Hospital, Liverpool, UK; [4]Department of Histopathology, Royal Brompton Hospital, London, UK; [5]MRC Biostatistics Unit, Cambridge, UK*

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 β-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, β-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?

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[1]M H Lawson, [2]D M Rassl, [1]S L Vowler, [2]N M Cummings, [1]W J Howat, [1]G Murphy, [2]R C Rintoul. *[1]CRUK Cambridge Research Institute, Cambridge, UK; [2]Papworth Hospital NHS Foundation Trust, Cambridge, UK*

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 formal in 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

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[1]M H Lawson, [2]N M Cummings, [2]D M Rassl, [1]R Russell, [1]J D Brenton, [2]R C Rintoul, [1]G Murphy. *[1]CRUK Cambridge Research Institute, Cambridge, UK; [2]Papworth Hospital NHS Foundation Trust, Cambridge, UK*

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