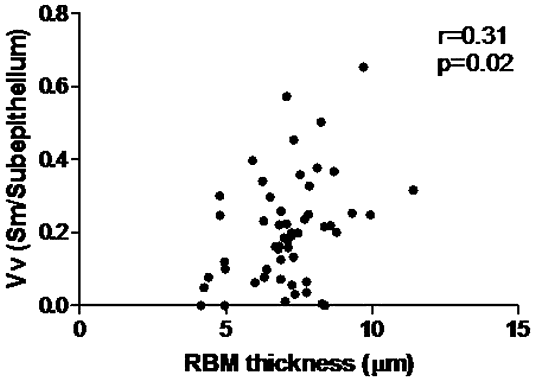

Abstract S87 Figure 1 Correlation between RBM and volume fraction of airway smooth muscle in asthma.

(bronchoscoped for upper airway symptoms) were included. All underwent fibreoptic bronchoscopy with endobronchial biopsies (EB). EB were processed to paraffin, and 5 µm sections were cut and stained with haematoxylin and eosin and used to quantify RBM thickness, epithelial shedding and volume fraction (Vv) of subepithelial smooth muscle indexed to submucosa.

Results Epithelial shedding was increased in atopic but not asthmatic subjects, (p=0.02 and p=0.37, respectively), and in children with asthma was correlated with exhaled nitric oxide (r=0.4, p=0.005). RBM thickness was increased in severe asthmatics compared to controls (p<0.0001), but a trend only to increased thickness was seen in mild asthmatics compared to controls (median (range) values: 6 (4.4–8.4) and 4 (3.1–7.5) µm, respectively; p=0.06). The Vv of subepithelial airway smooth muscle was only increased in severe asthmatics compared to controls (0.20 (0–0.65) and 0.09 (0–0.16), respectively; p=0.002). Interestingly, there was a positive relationship between RBM thickness and smooth muscle Vv fraction in asthmatics, but not in controls (r=0.31, p=0.02 and r=0.5, p=0.07, respectively) (Abstract S87 Figure 1).

Discussion We report for the first time a direct relationship between RBM thickness and airway smooth muscle mass in paediatric asthma. It is unknown if the relationship is causal, or both are driven by a common underlying process. Combinations of components of airway remodelling, rather than single factors, may prove to be more informative when phenotyping children with severe asthma.

S88 MAST CELL MYOSITIS IS ASSOCIATED WITH PERSISTENT AIRFLOW LIMITATION (PAL) IN CHILDHOOD SEVERE ASTHMA (SA)

doi:10.1136/thx.2010.150938.39

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Background Studies of airway inflammation and remodelling may help us to understand the pathophysiology of SA. Adult studies have shown mast cell inflammation within smooth muscle is specific to asthma and is associated with airway hyperresponsiveness (AHR). However, this has not been studied in childhood disease.

Hypothesis Children with SA have increased submucosal eosinophils and mast cells within smooth muscle compared to age-matched mild asthmatics and non-asthmatic controls.

Methods 75 children, mean age 11.8 (5.6–17.3) years, 53 with SA, 7 with mild/moderate asthma (MA) and 15 non-asthmatic controls (bronchoscoped for upper airway symptoms) were included. All underwent spirometry and bronchodilator reversibility, fractional exhaled nitric oxide (FeNO) measurement, fibreoptic bronchoscopy with bronchoalveolar lavage (BAL) and endobronchial biopsy (EB). EB were stained for: eosinophils (congo red), neutrophils (neutrophil elastase), mast cells (mast cell tryptase); and reticular basement membrane (RBM) thickness, epithelial shedding and volume fraction (Vv) of smooth muscle.

Results **See Abstract S88 Table 1.** Children with SA had significantly increased BAL and submucosal eosinophils compared to controls. There were no significant group differences in submucosal mast cells, but the presence of mast cells within smooth muscle exhibited a non-significant trend to be increased in SA and MA. Children with mast cells within smooth muscle were more likely to have PAL (post bronchodilator, post steroid trial FEV_1<80% predicted) (p<0.05). The Vv of subepithelial tissue occupied by airway smooth muscle (ASM) was only increased in SA.

Abstract S88 Table 1 Airway inflammation and remodelling in severe, mild/moderate asthma and non asthmatic control subjects

	Severe asthma (n=53)	Mild/moderate asthma (n=7)	Control (n=15)	p
BAL eosinophils %	**2.7 (1–51)**	**0.7 (0–27.7)**	**0 (0–5.7)**	**<0.001**
BAL neutrophils %	3.3 (0.3–73.7)	1.7 (0–7.3)	2.7 (0.6–14)	NS
Mucosal eosinophils (/mm²)	**11.2 (0–209.3)**	**3.7 (0–14.5)**	**0 (0–25.1)**	**0.01**
Mucosal neutrophils (/mm²)	9.8 (0–125.6)	11.4 (0–22.2)	1.2 (0–58.3)	NS
Mucosal mast cells (/mm²)	45.7 (0–185)	63.1 (9.2–79.7)	60.5 (0–165.6)	NS
Muscle mast cells (/mm²)	12.3 (0–299)	18.3 (0–72.8)	0 (0–50)	NS
Vv (sm/subepithelium)	**0.20 (0–0.65)**	**0.06 (0–0.3)**	**0.09 (0–0.16)**	**0.002**

Values are median (range).
All highlighted p values denote difference between severe asthma and controls.
BAL, bronchoalveolarlavage; RBM, reticular basement membrane; sm, smooth muscle; Vv, volume fraction of airway smooth muscle indexed to subepithelium.

Conclusions Children with SA have increased luminal and submucosal eosinophilia. However, in contrast to reports in adults of AHR being associated with mast cell myositis, we have found severe asthmatic children with mast cell myositis were more likely to have PAL. Mast cell myositis may be a feature of severe asthma in children.

Basic mechanisms in lung cancer

S89 BIMODAL IRON OXIDE NANOPARTICLES FOR HYPERTHERMIA THERAPY AND MR IMAGING IN CANCER

doi:10.1136/thx.2010.150938.40

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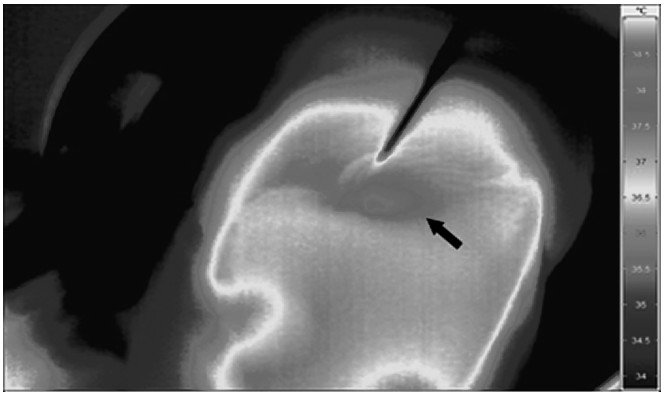
Introduction Super paramagnetic iron oxide nanoparticles (SPION) offer attractive possibilities in biomedicine. Hyperthermia treatment of cancer involves introducing SPION into tumours and applying an alternating magnetic field (AMF). The AMF causes the SPION to heat, resulting in cell death. It has been shown previously that mesenchymal stem cells (MSCs) can be labelled with SPION, with no effect on cell survival, and that they will migrate to and integrate into lung metastases in vivo, following systemic administration. Furthermore, SPION can be used to follow the fate of labelled cells in the body as they cause a marked shortening in T2* on MRI. Therefore, MSCs labelled with SPION offer a promising delivery mechanism for treating lung metastases with hyperthermia therapy. In this preliminary study, the distribution of SPION labelled MSCs and the anti-tumour effect of hyperthermia treatment was evaluated in vitro and in a subcutaneous murine tumour model.

Methods

- MSCs were obtained from Tulane University, New Orleans. Cells were incubated overnight in 0.5 mg/ml of the SPION



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

Ferucarbotranfor 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 9.4Thor-izontal 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 HOMOCYSTINE LEVELS IN NON-SMALL CELL LUNG CANCER

doi:10.1136/thx.2010.150938.41

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Plasma homocystine level is the most sensitive marker of sub-clinical folate deficiency. Elevated levels of homocystine 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 homocystine 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 homocystine is ≤15 μmol/l. 43% of patients had elevated plasma levels of homocystine at diagnosis. In 35% the level was between 15.1 and 25 μmol/l, and in 8% the homocystine level was greater than 25 μmol/l. Patients with elevated plasma homocystine 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 homcystine 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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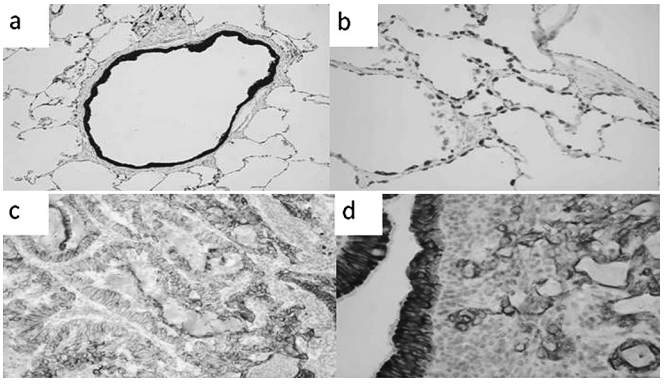
Introduction Vitamin D deficiency has been associated with many cancers, although little is known about its role in lung cancer. This is biologically plausible since vitamin D influences apoptosis and inhibits cell proliferation. Vitamin D receptor (VDR) polymorphisms have also been suggested to play 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.