ROLE OF CADM1 IN SQUAMOUS CELL CARCINOMA

Iron chelation reduces lung cancer proliferation in vitro

We investigate the role of a tumour suppressor gene, first identified in lung cancer, tumour suppressor in lung cancer 1 (TSLC1) or cell adhesion molecule 1 (Cadm1) in regulating squamous cell carcinoma (SqCC) growth and metastases.

**Methods**

Cadm1 expression levels were examined using q-PCR analysis on human pre-invasive airway and normal lung tissue collected as part of an on-going UCL/CRUK longitudinal-tracking study (Lung-Surveillance and Lung-SEARCH trials). Cadm1 was introduced into an established SqCC cell line (A431) and *in vitro* functional assays performed to investigate its effect on tumour growth, progression and invasion. Pre-clinical mice models were used to study the effect of Cadm1 expression in tumour growth and metastatic potential.

**Results**

q-PCR analyses demonstrated that loss of Cadm1 expression is a frequent early event in pre-invasive human airway compared to normal tissue (p = 0.001). Functional assays using A431, with Cadm1 reintroduced, showed Cadm1 expression levels directly associated with a significant decrease in cell proliferation (p = 0.001) over 10 days and significant reduction in invasion (p = 0.001) over 72 hours compared to control A431 cells without Cadm1. Pre-clinical xenograft tumourigenicity experiments in mice showed that Cadm1 expression significantly inhibited tumour growth (p = 0.01) together with a significant reduction in the number of metastases observed (p = 0.01) when compared with the control group.

**Conclusion**

These data suggest that restoration of Cadm1 expression in human squamous cell carcinomas play an important role in regulation of tumour growth and metastasis. Understanding the mechanism through which Cadm1 expression is able to modulate cancer progression may be therapeutically beneficial.

**Introduction**

There is growing evidence that iron plays an important role within the lung cancer, the leading cause of cancer-related mortality worldwide. As a result of this, iron homeostasis has potential as a new avenue for targeting and treatment of lung cancer. In this study, the effect of iron loading on cellular proliferation and iron homeostasis gene expression was investigated. In addition, the effect of the chelator deferasirox on cellular iron levels and proliferation rates was studied.

**Methods**

Cellular proliferation was assessed by the BrdU assay and cellular iron levels were assessed using the ferrozine assay. Manipulation of *IREB2* gene expression was achieved using short interfering RNA (siRNA) and subsequent expression of this and other iron homeostasis genes was assessed using real time PCR. All experiments were carried out on both the A549 adenocarcinoma and QG56 squamous cell carcinoma cell lines in triplicate. Primary bronchial epithelia cells (PBEC) were used as reference of normal behaviour.

**Results**

A dose of 150uM of iron was seen to cause a significant increase in proliferation in both the A549 (50% increase) and QG56 (40% increase) cell lines (P = 0.002 and 0.03 respectively) whilst no change was seen in the PBECs. A corresponding increase in cellular iron was also seen. When the cancer cell lines were treated with deferasirox, cellular iron loading decreased by roughly 25% in each cell line (P = 0.001 and 0.01 respectively).
and cellular proliferation decreased below levels seen in unstimulated cells. Deferasirox was also seen to effect unstimulated cancer cells, reducing their proliferation by 50% (P = 0.02 and 0.03 respectively).

Conclusion Iron exposure was shown to have a significant effect on cellular proliferation within lung cancer cell lines, although the underlying mechanism is not yet fully understood. This iron mediated cellular proliferation could be reversed using the chelator deferasirox. Down-regulated expression of IREB2 may cause the cancer cell lines to exhibit similar behaviour to the PBECs when stimulated with iron. These finding show that iron may provide a potential new target and deferasirox a potential new therapeutic agent for lung cancer.

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Abstract S131 Figure 1. The dotted line shows that the increasing concentration of FeSO4 has a statistically significant effect at 100 μM (M = 1.33, SD = 0.19, P = 0.04), however, 150 μM shows an even more significant increase in proliferation (M = 1.59, SD = 0.12, P = 0.002). A dose of 200 μM of FeSO4 shows a return to baseline and no significant difference in cellular proliferation. The solid line shows that deferasirox causes a decrease in proliferation when applied to cells after incubation with 150 μM of FeSO4. This is statistically significant at 50 μM (M = 0.86, SD = 0.03, P = 0.04), 100 μM (M = 0.62, SD = 0.08, P = 0.01) and 200 μM (M = 0.60, SD = 0.08, P = 0.0004) of deferasirox and the greater the dose of deferasirox, the greater the decrease in proliferation. The dashed line indicates the effects of FeSO4 incubation on PBECs. There is no statistical significance seen in proliferation rates for any concentration of FeSO4.

Outcomes post critical care

S133 OBSERVATIONAL COHORT STUDY OF OUTCOME OF PATIENTS REFERRED TO A REGIONAL WEANING CENTRE

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Introduction Data on outcome of the patients referred to weaning and rehabilitation centres are limited. In this observational cohort study, we report the outcomes of patients referred to a specialist complex home ventilation, weaning and rehabilitation centre.

Methods Data from the LFRU database from February 2005 to February 2011 were analysed. The primary diagnosis causing prolonged mechanical ventilation (MV) were classified into five groups: (1) neuromuscular and chest wall disease (NMD-CWD); (2) chronic obstructive pulmonary disease (COPD); (3) post-surgical patients; (4) obesity related respiratory failure (ORRF); and (5) other causes. The principal outcomes measured were weaning success, hospital mortality, 1-year and 2-year survival following discharge.

Results A total of 369 patients were referred over the 6 year period. Of these, 194 (52.6%) were admitted. The commonest outcome was total liberation from all forms of MV (45%). The remainder were shown to (1) require nocturnal non-invasive ventilation (NIV) (22%); (2) require nocturnal and intermittent daytime NIV (1%); (3) require long-term tracheostomy ventilation (24%); and (4) died in hospital (8%). Post-surgical and COPD patients had the highest rate of total liberation from mechanical ventilation at 60% and 54%, respectively. The median time from admission to tracheostomy decannulation was 18 days (9–33). NMD-CWD patients had the lowest hospital mortality (7%), whereas COPD patients had the highest hospital mortality (29%). The overall survival at 12 and 24 months was 60% and 50%, respectively. 25% of the COPD patients were alive and 59% of the NMD-CWD patients were alive at 24 months (Figure 1).

Conclusions The majority of patients with weaning failure were successfully liberated from mechanical ventilation. The weaning