The effect of HGF on Lung Cancer Cell Tight Junction Proteins (TAMPs) Occludin, MARVEL-D3, Tricellulin, migration, and cell adhesion

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Background

Lung cancer represents the most common cause of cancer death within the UK (1). Accounting for more than 35,000 deaths each year (2), evidence suggests that approximately 90% of all lung cancer deaths occur as a result of metastasis as opposed to primary tumours (3). Recent studies have been focusing on understanding and identifying the role of Tight Junction (TJ) proteins within tumour cell invasion and distant metastasis (4-6). Tight junctions have a crucial role in maintaining cell to cell adhesion and tissue integrity (5). Tumour invasion and cancer metastasis can occur as a consequence of cohesion loss within Tight Junction proteins (6).

Of particular interest are Tight Junction-Associated MARVEL (MAL and related proteins for vesicle trafficking and membrane link) Proteins (TAMPs). These have recently been shown to play a key role in cancer spread and progression (7). However, current evidence regarding the specific role and regulation of MARVEL proteins in normal epithelial and lung cancer cells is scarce. TJ proteins have been previously shown to be regulated by the Hepatocyte Growth Factor (HGF), a cytokine secreted by stromal cells. This was previously found to modulate the expression and function of TJ molecules in human breast, and prostate cancer cell lines (8,9).

Aim

This study aimed to investigate the changes in expression of Tight Junction proteins in adenocarcinomic human alveolar basal epithelial cells in response to the Hepatocyte Growth Factor at different time intervals.

Materials and Methods

Cell lines and cell culture

Adenocarcinomic human alveolar basal epithelial cells (A549) were used. These are squamous cells responsible for the diffusion of substances across the alveoli of the lungs. The origin of the cell line is human Caucasian lung carcinoma. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with supplements as prescribed by the manufacturer, and incubated in a humidified chamber at 37 °C with 5% CO2.

HGF Treatment

Cells were seeded into two, six-wells plates. HGF treatment at 50ng/ml was added to one six-well plate over a period of 24h at the following times: 24h, 4h, 2h, 1h, 30min, 0h. (4h, 2h, 1h, 0h). After the 24h course, the cells were lysed, and prepared for Western Blotting.

Further cells were seeded into two eight-wells chamber slides and treated with HGF over a 4h period (2h, 1h, 30min, 0h). After the 4h course, the cells were fixed in ethanol and prepared for Immunofluorescence.

Proteins and molecules investigated

Immunofluorescence, Western Blotting and PCR was used to investigate changes in expression of the following molecules:

- F-actin (Phalloidin)
- Occludin
- ZO-1
- MARVEL D3
- Tricellulin
- Claudin 1
Results

Figure 1. Immunofluorescence results. TJ proteins stained green, Actin stained red, and cell nuclei in blue. A difference can be seen after the application of HGF over the time course at 1h in particular. By 4h, the location of both TJ proteins (green) was re-established at the cell peripheries.

Figure 2. Western Blotting Raw Data. Immunoblot for the proteins of interest in the lysate of A549 cells treated over 24 hours with the HGF. Occludin was used as the protein loading control. First band Ladder, Sample 1 (HGF added at 24h), Sample 2 (HGF added at 4h), Sample 3 (HGF added at 2h), Sample 4 (HGF added at 1h), Sample 5 (HGF added at 30 min), Sample 6 (0h).

Conclusion

Although a change in the modulation of Tight Junction proteins can be noted on Immunofluorescence, further research is needed to ensure accuracy of results. Further understanding of the underlying molecular mechanisms of TJ metastasis is required to develop novel strategies for preventing and treating lung cancer.

Self-reflection

Spending a month and a half at the Cardiff-China Cancer Research Collaborative has been one of the most rewarding and eye opening experiences I have been involved into so far. This experience has motivated me to consider an intercalated degree in science. Nevertheless, I feel very motivated and empowered to take part in further research in the future. The team I was part of was made of surgeons, consultants, research assistants, clinicians, and many others. It is amazing to see how everyone has one thing in common: A passion for expanding horizons and for exploring previously unknown avenues.

As a medical student, I feel grateful for having been given this amazing opportunity. The INSPIRE research scheme has made this wonderful experience possible and I would like to thank the whole team for the support provided. I would have not been able to organise this placement without the INSPIRE scholarship. During my time in Cardiff, I have been part of a bigger project. My supervisor, Dr Tracey Martin, has taken good care of me and has involved me in the write up of the bigger project. Hopefully, I can one day go back and continue my work and perhaps publish the findings in a journal. This placement has made me value the work done behind the scenes, at the molecular level in labs and has taught me the essential skills required to carry out lab-based research. I am very much looking forward to using these skills again in during intercalating and perhaps later on as an academic FY1 doctor.

Thank you very much for all the support – I truly recommend this scholarship to peers from all over the UK!

Best wishes, Bogdan Chiva Giurca, BMBS Y3, Exeter Medical School