Biochemical Society Summer Vacation Studentship Report
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Investigating the role of Integrin in c-Met signalling.

Introduction

C-Met is a receptor tyrosine kinase (RTK) found exclusively in vertebrates. It is essential for embryological development and for physiological repair and defence mechanisms during life. Activation of c-Met is by Hepatocyte Growth Factor (HGF) and gives rise to characteristic intracellular responses resulting in proliferation, scattering and regulation of cell motility. Dysregulation of c-Met, due to the met oncogene, has a well established and extensively described role in tumorigenesis and metastasis (1).

Integrins are cell surface receptors, which facilitate both mechanical and chemical activities. From a mechanical perspective they are essential for the adhesion of cells to extracellular matrix (ECM) components (e.g. laminins, fibronectins, collagens) and organisation of the cytoskeleton (2). Integrins also activate intracellular signalling pathways, promoting cell survival and proliferation; it is in this role that their interaction with RTK's is emerging as a critical step for tumour invasion and metastases. Mammals express 24 different integrins, each with an α and β subunit, 18 α and 8β subunits have been described. Cells may express varying numbers and subtypes of integrins depending on their type and degree of differentiation (3).

Aims

The aim of this project was to investigate the potential role of a specific integrin in c-met signalling. Work already has been done to demonstrate that integrin $\alpha 6\beta 4$ associates with c-met and plays a role in c-met signalling (4). Neoplastic cells have demonstrated changes in integrin expression with upregulation of those that facilitate tumour progression and down regulation of those that don't. These changes may also differ according to the stage of disease (3).

For these reasons, it is essential that the normal roles of all subtypes of integrins are established, and to elucidate their mechanisms of action in relation to oncogenes. It is only once this has been achieved that it will be possible to develop drugs that can exploit specific integrin activity and halt tumour progression.

Experimental work

Cell model

The work was performed on three different cell line systems that I routinely cultured:

1) HeLa cells – immortal human cervical cancer cells widely used in cancer research. These were used as a control for qualitative assessment of the integrin primary antibodies. Cells were cultured in Eagle's 4 medium containing 10% foetal bovine serum (FBS).
2) HEK 293 (Human Embryonic Kidney cells), chosen to establish the role of the integrin in normal cells. These cells are widely available for research and have been shown to express significant levels of integrins (5). The cells are also transfected with a GFP (Green Fluorescent Protein)-c-Met construct, which expression is inducible by stimulation with tetracycline. GFP-c-met is induced so that any movement (internalisation/trafficking) of the receptor, can be visualised using immunofluorescence.
3) Murine cells which have been established from mouse knock-outs for the integrin and the same cells re-expressing the integrin. Cells were cultured in Eagles 4 medium containing 10% foetal bovine serum (FBS) and 10µg/ml of puromycin.

All cells were maintained at 37°C in an humidified atmosphere in CO_2 8%.

Experiments

The role of the integrin in c-Met signalling was monitored by comparing the activation upon HGF stimulation of several signalling pathways in cells expressing, or not, the integrin. Down-regulation of the integrin in 293-HEK cells was obtained by RNA inteference. For this, I have performed several experiments consisting of a time-course of HGF stimulation (from 0 to 3 hours), protein harvesting and Western blotting with a panel of antibodies: phospho-c-Met, c-Met protein, phospho-Shc, phospho-JNK, JNK protein, phospho p44/42 MAPK; these are all protein kinases which indicate, when phosphorylated upon HGF stimulation, activation of the c-Met receptor and resulting downstream activity within the cell (6).

Results

My results indicated that the integrin plays a role in the activation of c-Met dependent signalling pathways. These results are original and this work was important as it replicated results that Dr. Kermorgant had produced in earlier work.

Future directions

Most of my results were obtained in the murine cells and these were consistent. I obtained the same results in the 293-HEK cells although this needs to be confirmed since I have performed only one experiment. Moreover, the detection of the integrin in 293-HEK cells was difficult. However, the integrin expression was well detected in HeLa cells, indicating that the primary antibody worked well. The experiment should be repeated using larger amounts of protein from 293-HEK cells for the Western blot because if we cannot prove the presence and knockdown of integrin then there is no point taking the experiment further. Once the presence/knockdown of the integrin is established in theses cells, a series of Western blots looking at the signalling proteins should be done. Experiments should also be carried out on HeLa cells to compare the role of the integrin in neoplastic cells. The mechanisms involved in the cooperation between integrin and c-Met need to be investigated in the future. For example, co-immunoprecipitation studies will reveal whether they can be found in a protein complex.

Conclusions

Shortly before my arrival, one of the post-doctoral researchers had started the project outlined on my application. Unfortunately, the approach was unsuccessful. It was therefore felt that my time would be better spent looking at the role of integrins. This is also an area of research for the group and my Western blot analysis of the murine cells was essential to Dr. Kermorgant's work.

The studentship gave me a valuable insight into the workings of a research laboratory, the frustrations when things don't go according to plan, problem solving to find out what went wrong followed by elation when, on repeating the experiment, wonderful results are obtained! I have gained valuable experience in tissue culture techniques, electrophoresis and Western blotting. I also have developed a greater understanding of the molecular biology of tumorigenesis and metastasis. This has provided me not only with food for thought about other possible directions in which research could be taken, but also an insight into the complexities inherent in trying to ultimately find cures for cancer.

The knowledge I have gained and the thought processes that this experience has uncovered will stand me in good stead for my future studies. It has given my a deeper understanding of the mechanisms of disease at a cellular level and also will be invaluable to me when reading and critiquing research papers. I now understand more the relevance of some of the experimental techniques described in such papers.

A really worthwhile experience, definitely tempting me towards a career in research!

References

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