Progress Report

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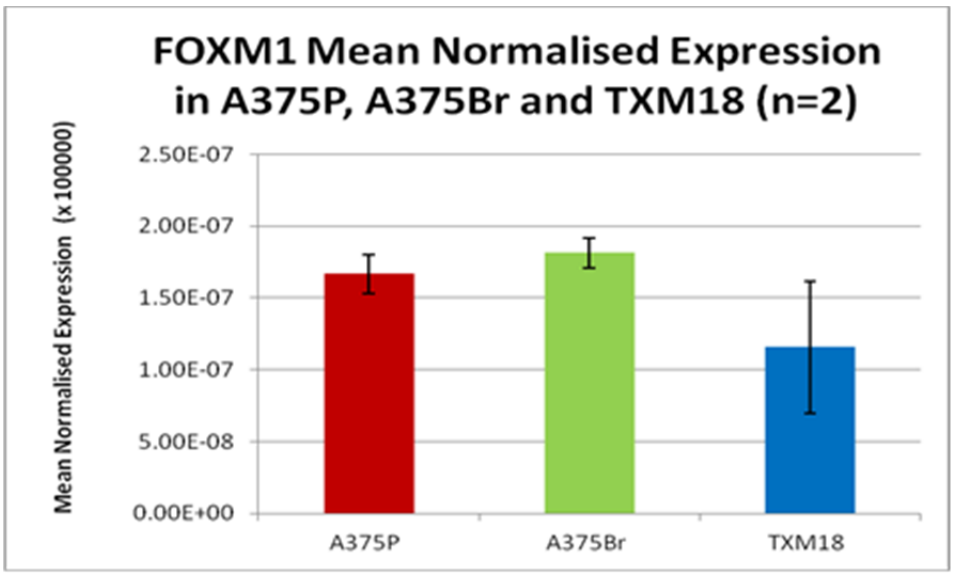
Title of Project: FoxM1 and melanoma cerebral metastases

Summary:
Our project aims to identify the role of a novel proto-oncogene FOXM1 in the development of metastatic phenotypes in different human melanoma cell lines in vitro. After obtaining three different melanoma cells lines (A375P, A375Br and TXM-18) from overseas we began by evaluating the expression of FOXM1 in these cell lines using quantitative polymerase chain reaction (qPCR) and Western blotting. A375P and A375Br cell lines were isolates of lymph node metastases and TXM-18 from brain metastases.

For qPCR assays, RNA was extracted from the cells according to protocols (the RNeasy Mini Kit protocol, QIAGEN, USA) and cDNA was then prepared from this RNA in a ThermoCycler. PCR amplication was then performed using FOXM1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (the later serves as a housekeeping gene). We were successful in detecting the expression of FOXM1 mRNA in all three cells lines. These real-time q-PCR RNA levels were normalized to human GAPDH mRNA levels and despite the difference in their origin, the normalised values did not differ significantly between the three tumour cell lines. This suggests that FOXM1 expression may be an early event in malignant transformation.



To verify FOXM1 protein expression, Western blotting was then performed using cell lysates extracted from these melanoma cell lines. Immunodetection was performed using two different commercially available anti-FOXM1 antibodies (Santa Cruz and Abcam). However, we found that despite optimisation of the experimental protocol, FOXM1 proteins were not detected or detected at very low levels from lysates of the tumour cell lines.

To troubleshoot this we sought to obtain positive controls with the use of human breast adenocarcinoma (MCF7) whole cell lysate and glandular cells from a rat adrenal gland, which are known to express FOXM1. However, while positive results were obtained from adrenal gland lysates, the other runs had remained negative.

We repeated the same set of experiments several times to confirm this but unfortunately the results had remained inconclusive. It was felt that the problems may either lie in having a faulty batch of antibodies or cell lines or both.

In order to clarify this, we sought to repeat the experiments using other melanoma cells lines and antibodies. With the generous support from the melanoma research group at Macquarie University, we have recently obtained nine new human melanoma cell line samples and have also obtained a new batch of anti-FOXM1 antibodies. We are commencing on a new set of Western blot experiments and in order to advance our project in a more expeditious fashion, following these, we are planning to evaluate expression of FOXM1 with live cell fluorescence microscopy. We will attempt fluorescent labelling of whole cultured cells with FOXM1 antibodies and perform live cell imaging. We will then evaluate the effects of different chemotherapeutic agents on FOXM1 expression using multiwell chambers for efficient screening. We will then perform various assays for metastatic behaviour as planned to verify the biological significance of such treatment.

What these research outcomes mean

Currently despite recent advances in immunotherapy (anti-cytotoxic T cell antigen 4) and targeted therapy (BRAF inhibitors) for advanced melanoma, the survival for patients harbouring melanoma brain metastases remains at about 6 months. Prioritised focus in conducting translational research for melanoma brain metastases is much needed for improving patient survival. FOXM1 is a novel anti-cancer treatment target that is overexpressed in a multitude of human solid tumours but not expressed in normal mature cells. The goal of our project is to examine whether anti-FOXM1 treatment would inhibit melanoma cell proliferation in vitro and thus potentially provide a potent anti-melanoma therapy with minimal toxicity to normal tissue. The outcomes from our project will be the first step in testing the hypothesis.

Please submit this report as a PDF using the following naming convention:

Lastname Firstname – Simplified Project Title

For example: Smith Jane – The anatomy of the Brain.PDF