

TITLE OF PROJECT: cDNA Expression Profiling In Multiple Endocrine Neoplasia Type 1

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INTRODUCTION

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the combined occurrence of parathyroid, pancreatic islet, anterior pituitary and adrenocortical tumours, for which effective treatments are not available^{2,3}. The MEN1 gene^{4,5,6} is located on chromosome 11q13 and encodes a 610 amino acid protein, MENIN^{1,6}. 1336 germline mutations have been reported⁷. Somatic mutations in MEN1 and sporadic (non-MEN1) endocrine tumours are consistent with Knudson's 'two-hit' hypothesis⁸. Professor Thakker's group has successfully generated an MEN1 knockout mouse model. The heterozygous (+/-) mice develop the expected tumours of the parathyroids, anterior pituitary, pancreatic islet cells and adrenal cortex. The functional role of MENIN in tumourigenesis has not been fully elucidated. However, MENIN acts as a tumour suppressor via transcriptional regulation and genome stability⁹. Thus, I intend to identify factors involved in MENIN tumourigenicity using the technique of microarray analysis of mRNA obtained from MEN1 tumours.

AIM

The main purpose of this research is to determine pathways to be targeted to suppress the tumourigenicity of MEN1-associated tumours. The results of this project together with downstream *in vivo* studies in mice will establish proof of principal to plan a clinical trial in patients with MEN1 tumours. I applied for a RCPSCG Travelling Fellowship to enable this work to be carried out at the Max-Delbrueck-Center for Molecular Medicine (MDC) in Berlin, Germany, where they have developed a specialism in microarray expression profiling.

METHODS

I collected five pituitary tumours from affected heterozygous knockout mice, and five pituitaries from wild type littermates. Total RNA was extracted and purified. cDNA was synthesized and subjected to *in vitro* transcription, to yield antisense biotinylated cRNA. This was fragmented and used as a target to hybridize to mouse oligonucleotide probe arrays (n=45102) on an Affymetrix Mouse 430 2.0 array. The hybridized probe array was then stained with the light sensitive substrate streptavidin phycoerythrin conjugate and scanned electronically to measure photon emission. The amount of light emitted at 570nm is proportional to the amount of bound target at each location on the array.

The raw data generated was assessed for quality, using internal controls (β -actin and GAPDH). Then data was normalised across the arrays to create a 'robust multichip average'. Finally I identified those probesets that correspond to over or under expressed genes in the pituitaries of mice with tumours, compared to the littermate controls. These genes represent candidates that may be involved in tumourigenic pathways, which could be therapeutic targets in the clinical treatment of MEN1.

RESULTS

Four tumours, and five wild type pituitaries yielded good quality RNA which was suitable for hybridization. One tumour dataset was subsequently removed from the microarray analysis, due to poor quality, and therefore the robust multichip average of three pituitary tumours could be compared to that of five controls. Gene expression profiling of Men1 pituitary tumours identified differential expression in pathways involved in cell metabolism, tumour development and growth factor signalling.

DISCUSSION

The molecular pathways identified mediate functions that include the cell cycle, apoptosis, gene expression, proliferation and tumourigenesis. These are likely to be involved in the mechanism of action of MENIN in Multiple Endocrine Neoplasia Type 1. There are several advantages to using the cDNA expression profiling approach. Firstly, it is an efficient use of animal resources and contributes to our targets for the reduction, refinement and replacement of their use. Furthermore, it is likely to provide additional information related to the molecular and physiological pathways involved in endocrine tumourigenesis, which will be of general use in studying tumour biology and genetics. I gratefully acknowledge the Royal College of Physicians and Surgeons of Glasgow in providing a Travelling Fellowship in support of this project.

References (*Publications from Professor Thakker's group)

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March 2008