



Te Whare Wānanga o Ōtago

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Research Report
Canterbury Medical Research Foundation
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Title: An International Collaborative study to validate biological markers for Hepatoblastoma: recovery of biomarkers (DNA and RNA) from paraffin embedded tissue and development of tissue arrays

Research Report

We are delighted to report the outcome of research funded by a generous grant from the Canterbury Medical Research Foundation (06/09). Funding from the Foundation has supported the establishment of an international collaborative study to identify new biological markers for the diagnosis and treatment of hepatoblastoma.

Hepatoblastoma is a relatively rare childhood liver cancer. Until 20 years ago the outcome from this cancer was poor with an overall survival of less than 30%. Commencing in 1990, members of the International Paediatric Oncology Society (SIOP) established a clinical trials consortium (SIOPEL) to run a series of international clinical trials to improve the overall survival for children with hepatoblastoma and hepatocellular carcinoma. The first clinical trial, SIOPEL 1, was a non randomised trial of preoperative chemotherapy, it commenced in 1991 and ran to 1994. This study was followed by SIOPEL 2, a pilot study of two treatment strategies for hepatoblastoma which ran from 1994-1998. The most recent study, SIOPEL 3, was a large multicentre randomised study of preoperative chemotherapy with either single agent Cisplatin, or Cisplatin in combination with Doxorubicin. These trials have led to an impressive improvement in overall outcome and long term survival for this childhood cancer. SIOPEL 3 recruited over 500 patients from 36 countries and over 120 treatment centres. We recently reported the outcome of SIOPEL 3 (Perilongo et al NEJM 2009) and showed single agent Cisplatin given preoperatively can achieve a 92% overall survival for standard risk hepatoblastoma.

In 2005, our Children's Cancer Research Group proposed a study to identify novel biological markers for diagnosis and to new pathways for targeted therapy.

With the support of the SIOPEL group and under their auspices, we sought tumour material to commence a series of biological studies. Being a relatively rare tumour, many treatment centres had not kept fresh tumour tissue for future biological studies.

To overcome this barrier, we proposed applying new laboratory methods to extract biological material (DNA and RNA) from the standard diagnostic histology blocks prepared by each treatment centre at the time of diagnosis.

We then proposed the development of a tumour tissue array, a relatively new method for the rapid analysis of many tumours for candidate biological markers, or new therapeutic targets.

Our specific research aims in our 2006 application to the Foundation were:

Specific Aims

- 1. To apply new methods for recovery of nucleic acid (RNA and DNA) from paraffin embedded tumour tissue**
- 2. To prepare tumour tissue arrays for collaborative studies of prognostic markers**
- 3. To validate candidate gene and protein markers from tumours of patients enrolled in the SIOPEL 2 and 3 Hepatoblastoma studies**

Aim 1: To apply new methods for recovery of nucleic acid (RNA and DNA) from paraffin embedded tumour tissue

This aim was supported by the SIOPEL central clinical trials office (Margaret Childs, Leicester University, UK) and the central statistical centre (Rudolf Maibach, SIAK, Bern, Switzerland). We obtained diagnostic tumour blocks with paraffin embedded tumour material from 98 patients prospectively enrolled on the SIOPEL 3 clinical trial. In collaboration with our anatomical pathologist (Dr Carina Miles), the paraffin blocks were marked to identify representative tumour and adjacent normal liver tissue. Using a tissue sampler, Ms Purcell took several punch biopsies from each tumour block. From these punch biopsies, samples were extracted for RNA and DNA, and additional punches were used to prepare the tissue array. Quality analysis revealed the RNA extracted from tumour and normal tissue was suitable for gene expression studies and allowed us to extract a unique fraction of RNA, termed microRNA, for microarray expression analysis.

Aim 2: To prepare tumour tissue arrays for collaborative studies of prognostic markers

Duplicate punch biopsies (1 x 5 mm) from tumour and normal liver were inserted into a secondary block in a gridded array. A total of 98 hepatoblastomas and 40 adjacent normal liver samples were arrayed. This is the largest tissue array of its type prepared for this tumour.

The tissue array has permitted us to do multiple high throughput experiments testing different candidate biomarkers for diagnosis and possible treatment.

Aim 3: To validate candidate gene and protein markers from tumours of patients enrolled in the SIOPEL 3 Hepatoblastoma study

We have now analysed a portfolio of candidate biomarkers, including Beta-catenin, E-cadherin, Cyclin D1, and c-Met looking for associations between marker expression and the risk of relapse. We have evidence to show expression of E-cadherin is associated with a higher risk of relapse in standard risk tumours, but the number of relapse events in this tumour set are low and an enriched set of relapsed tumours will need to be analysed to validate this result. This work will be submitted for publication in the near future.

Additional Research Results:

Funding from the CMRF has been crucial for the establishment of our longterm hepatoblastoma study. Without the CMRF support we would not have been able to obtain the tumour material and establish the tissue array for our subsequent research. In achieving these funded research aims, which are now completed, we now have established a research platform for several subsequent research studies.

1. HGF/c-Met related activation of β -catenin in Hepatoblastoma identifies a new therapeutic target

Like many other more common cancers, Hepatoblastoma shows activation of a crucial developmental pathway (Wnt/ β -catenin) that regulates the growth and development of tissues. Previous research has shown that approximately 75% of hepatoblastoma tumours have tumour related activity of the β -catenin protein. However, gene mutation analysis of the β -catenin, in several small series of tumours, has only been able to account for 15-60% of β -catenin related activity.

In our research, Ms Purcell has screened all 98 hepatoblastoma tumours by RNA mutation analysis and identified mutation in the β -catenin gene in approximately 20% of cases, but we show, as others have, that over 75% of cases have active β -catenin protein.

To address this disparity we have sought other mechanisms for the activation of the Wnt/ β -catenin pathway in these tumours. We now have evidence to show that the c-Met protein pathway is involved in activation of β -catenin, bypassing activation of the Wnt pathway.

This result is important for two reasons. Firstly it accounts for a disparity in the observed difference between β -catenin mutations and β -catenin protein activity. But more importantly, the c-Met pathway, which is active in many other cancers, can be targeted by new therapeutic drugs.

We recently submitted this research to *Cancer Research*, the leading journal of the American Association for Cancer Research (copy attached) with acknowledgement of the CMRF. We are waiting the outcome of peer review.

2. c-Met activation and inhibition in Hepatoblastoma

Following our observation of c-Met pathway activation in Hepatoblastoma, we have now started experiments to analyse the effect of different c-Met pathway inhibitors on the growth of hepatoblastoma cell lines. This work is being done by Dr Lucia Alonso-Gonzalez, who recently joined our research group. We anticipate confirming the potential for inhibition of this pathway to be included in the next SIOPEL clinical trial for high risk and relapsed hepatoblastoma.

3. microRNAs in the pathogenesis of hepatoblastoma

MicroRNAs are a novel class of RNA molecule involved in the regulation of many genetic pathways. Recent research has shown that specific patterns of microRNA expression can predict tumour behaviour.

Ms Purcell has recently developed the method for extraction of microRNA from paraffin embedded tumour material and we now have microRNA from over 60 tumours. We are now analysing these microRNAs by a new microarray method, permitting the analysis of several hundred microRNAs in a single experiment. We anticipate this work will be completed by the end of July 2010 and submitted for publication in the near future.

A/Professor Michael Sullivan
Director of Research
June 2010

Rachel Purcell, Arthur Zimmermann, Margaret Childs, Rudolf M Carina Miles⁴, Clinton Turner⁴, Michael Sullivan **HGF/c-Met related activation of β -catenin in Hepatoblastoma identifies a new therapeutic target, *Cancer Research* 2010, submitted**

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