RESEARCH REPORT - JULY TO SEPTEMBER 2009

Progress report July 2009 to September 2009 ovarian cancer biomarker laboratory

Executive Summary - research progress to September 2009

The Ovarian Cancer Biomarker Laboratory continues to make good progress in the application of proteomics technologies to identify potential new markers for early stage ovarian cancer detection. Whilst analysis of existing DIGE data by Associate Professor David Robertson is ongoing, the research team continues to develop and implement additional strategies aimed at identifying novel cancer markers of early stage disease. To this end four additional, inter-dependant project areas have been added to the research program. These include the use of iTRAQ technology to analyse immunodepleted plasma; the use of novel nanoparticle technology, developed at Prince Henry's Institute, to analyse small and low abundance proteins from plasma and urine; and the analysis of circulating immune complexes found in plasma as novel sources of early cancer markers.

Over the last quarter the team has made significant progress, including;

♣ Optimized the manufacture and application of SEA nanoparticles to isolate proteins from clinical samples of both plasma and urine;

♣ Identified over 200 peptides and protein fragments present in the urine of cancer patients, including three that have never been reported in urine from ovarian cancer patients;

♣ Established SOP's for the iTRAQ labelling procedure, located a suitable facility for data collection and generated preliminary data enabling analysis to commence;

♣ Expanded the library of clinical samples available for research to include over 400 patients, as well as 40 control women; The team has also submitted an amended manuscript to a peer reviewed, scientific journal describing the optimized nanoparticle-based protein capture system established.

Nanoparticle-based purification and protein analysis

The use of the research team's nanoparticle-based technology has now been optimized for the capture of proteins from plasma, urine and uterine washings. Nanoparticles are now routinely manufactured in-house, with a diameter of ~900nm and a negatively charged core surrounded by a porous shell. The buffering system and procedure for protein capture have now been optimized for use with plasma, urine and uterine washings; A manuscript describing the optimized procedure has been submitted to a peer-reviewed scientific journal, and we are awaiting a response from the editor. This nanoparticle purification system has been applied to capture protein fragments and peptides from patient's urine samples. We have identified 213 different proteins / protein fragments in patient's urine, including low abundance proteins such as receptors and growth factors; Three proteins have been identified that have previously not been associated with ovarian cancer, but that play known roles in oncogenesis. Antibodies against these proteins have been obtained, and are being used to validate the presence of these proteins in patient urine samples; SELDI TOF MS analysis of 6 patient groups (control, benign adenoma, benign adenofibroma, stage I/II EOC, stage III EOC, internal standard) detected over 80 protein peaks; statistical analysis shows that these peaks are able to differentiate between sample groups. Attempts to identify the peaks located by SELDI TOF MS have thus far been unsuccessful. We are now working on obtaining suitable amount of protein for use in iTRAQ labelling experiments to obtain quantitative information for identified peptides.

ITRAQ Labeling and analysis of proteins

Stable isotope labelling using iTRAQ reagents is a new technology ideal for application in the context of cancer biomarker discovery. This approach allows up to 8 different samples to be combined and quantitatively analysed, providing both comparison of the amounts of protein present as well as protein identifications. We are applying iTRAQ labelling to proteins in plasma and urine to identify new candidate cancer markers. To date, the team has; Identified a suitable facility at the Institute for Molecular Biology, University of Queensland, to collect and process iTRAQ data; Demonstrated correct reporting of the iTRAQ system on protein ratios from a complex plasma sample, and the ability to identify the proteins involved; Labelled (a) an immunodepleted plasma sample, and (b) a nanoparticle-captured plasma protein sample with iTRAQ reagents, and sent these for analysis.

Samples of urine proteins are also being prepared for iTRAQ labelling (as detailed above). We are currently awaiting the return of iTRAQ data for analysis.

Cancer-specific proteins generating an auto-immune response

Isolation of auto-antigens from cancer patients remains an area of interest for the team. Progress has slowed in this portion of the project with the absence of Dr Andrew Stephens during the month of September. Progress in this project area is expected to re-commence immediately.

Other issues

Access to suitable instrumentation to perform the required analyses remains a key issue for the research team. Whilst we have identified facilities to perform iTRAQ analyse, we have yet to identify a suitably equipped laboratory enabling us to perform top-down analysis – the identification of intact peptides and small proteins – necessary to match SELDI MS data with protein identities. This may lead to future delays as we seek to identify unknown proteins for further development.

Clinical collection program

The clinical collection program continues to move forwards, with approximately 20 samples per month collected at each of the Melbourne and Sydney sites. Professor Andreas Obermair has recently indicated his willingness to commence collection activities in Brisbane, and Ms Nicole Fairweather is working with him to assist in ethics submission and co-ordination of activities. To date; over 400 patient samples have been collected from sites in Melbourne and Sydney; 40 samples from healthy, post-menopausal women have also been collected in Melbourne to contribute towards a control group; ethics approval has now been obtained to establish a similar recruitment program in Sydney; Collection activities are being reviewed by Ms Fairweather, with a view to increasing productivity and streamlining data management across the three sites. It is also anticipated that additional -80OC freezers will be required in Melbourne and Sydney in 2009 to accommodate the growing sample collections.