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Both Honours (2) and PhD/Masters projects available. Projects will be modified accordingly.

Project title: Identification of cell surface membrane proteins as biomarkers for colon tumour cells and potential therapeutic targets

Our group developed a technique (immunobead RT-PCR) for capturing circulating tumour cells (CTCs) from blood or peritoneal lavage fluid using immunomagnetic beads (Epithelial Enrich, Dynal) with RT-PCR of the target markers (mRNA) to detect the captured cells. A survival study of 125 early stage colorectal cancer (CRC) patients showed that detection of CTCs in post-resection peritoneal lavage fluid correlated with reduced disease-free survival ($P < 0.0001$) and this was independent of other established risk factors; hazard ratio 6.2, 95% CI 1.9-19.6, $P = 0.002^1$. Currently, immunobead capture relies on EpCAM labelled magnetic beads, an antibody specific to epithelial-derived tumour cells as well as normal epithelial cells. Although EpCAM is the antibody used in recently developed commercial kits for detection of CTCs in blood (CellSearch™, Veridex, NJ, USA), it has been reported that EpCAM expression was some 10-fold lower on CTCs compared to primary and metastatic tissues, suggesting that EpCAM expression is dependent on the local microenvironment and is down-regulated in disseminated cells². Thus there is an explicit need for a more effective antibody panel to increase the specificity of immunobead capture of tumour cells. Further, surface membrane colon tumour biomarkers are needed for specific targeting of tumour cells by novel therapeutics such as antibody-coated nanoparticles encapsulating RNA interference (RNAi) molecules or small molecule inhibitors.

Aims:

1. To develop a protocol for cell surface membrane protein preparation, enriching for glycoproteins
2. To develop a quantitative mass spectrometry technique to measure differential expression of tumour cell surface biomarkers.
3. To validate differential expression between colon tumours and matched normal mucosal cells.
4. Use of such biomarkers to select (or make) antibodies to label immunobeads for tumour cell capture from blood or other body fluids.
5. Use of antibodies for molecular targeting e.g. in 'proof of principle' experiments for therapeutic applications.

Techniques include proteomics, quantitative mass spectrometry, cell culture, flow cytometry, RNAi, immunobead isolation of tumour cells, RT-PCR, Western blotting, Bioplex assays, ELISA.

1. JM Lloyd, CM McIver, Stephenson SA, PJ Hewett, N Rieger, JE Hardingham. *Clin Cancer Res* 2006, 12:417-23.
2. Rao CG Chianese D, Doyle GV et al. *Int J Oncol* 2005, 27(1):49-57.

Project title: Determination of biomarkers of resistance to monoclonal antibody therapies in colorectal cancer

Cetuximab is a monoclonal antibody in clinical use for advanced colorectal cancer (CRC). It targets the epidermal growth factor receptor (EGFR) by binding to the extracellular binding domain preventing activation of tyrosine kinase within the cytoplasmic domain, inhibiting auto-phosphorylation and downstream signalling via the Ras-Raf-ERK pathway. When *KRAS* is mutated it is constitutively active and signals to the nucleus independently of EGFR activity. *KRAS* mutant tumours have been found to be resistant to the effect of EGFR inhibitors, including cetuximab. However, in one study there were 26% of non-responders that were wild type for *KRAS* ¹. Similarly, another study found that although there were no mutations in *KRAS* in any of the tumours that responded to cetuximab therapy, there were also no mutations found in 6/19 non-responder patients ². Thus other mechanisms are involved in this non-response to cetuximab, possibly via constitutive activation of downstream signalling molecules such as Stat3 ³ or Akt ⁴ which would circumvent the inhibition of EGFR tyrosine kinase activity, rendering tumour cells resistant to EGFR antagonists.

Hypothesis

Other biomarkers for resistance to EGFR inhibitors (cetuximab) exist apart from *K-ras* mutation status, likely to be constitutively activated downstream signalling molecules in the PI3K-AKT-mTOR pathway or the Ras-Raf-ERK pathway.

Aims:

1. To establish cell lines either resistant or sensitive to cetuximab treatment in culture.
2. To determine differentially expressed biomarkers within the supernatant or in the cells using 2D DIGE (difference gel electrophoresis) or quantitative mass spectrometry (iTRAQ) and to validate differential expression.
3. To validate the use of such biomarkers by correlating expression in patients' tumours with clinical outcome after cetuximab therapy.

Techniques include cell culture, proteomics (2D DIGE, mass spectrometry), protein and RNA isolation, ELISA, Western blotting, Bioplex assays, qRT-PCR.

1. Khambata-Ford, S. *et al.* Expression of Epiregulin and Amphiregulin and K-ras Mutation Status Predict Disease Control in Metastatic Colorectal Cancer Patients Treated With Cetuximab. *J Clin Oncol* **25**, 3230-3237 (2007).
2. Lievre, A. *et al.* KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer. *Cancer Res* **66**, 3992-3995 (2006).
3. Dae Joon Kim, *et al.* Signal transducer and activator of transcription 3 (Stat3) in epithelial carcinogenesis. *Mol Carcinog* **46**, 725-731 (2007).
4. Vivanco, I. & Sawyers, C.L. The phosphatidylinositol 3-Kinase-AKT pathway in human cancer. *Nat Rev Cancer* **2**, 489-501 (2002).