

## LEEDS INSTITUTE OF MOLECULAR MEDICINE

### PHD STUDENTSHIPS

Studentships are available from October 2010 for a fixed term of 3 years. Each studentship will fund EU/Home studentship fees, a stipend at MRC rate and laboratory consumables. Non-EU applicants would be required to obtain additional funding to cover the overseas component of the registration fee.

Applicants should have, or be expecting to obtain, a relevant first degree at a standard of 2:1 or above and are encouraged to contact the appropriate supervisor to discuss the project before submitting their application for the **4th Jan 2010 closing date**.

Applicants are requested to consider the 19 projects outlined below and indicate an interest in up to 3 projects in a covering application letter.

Candidates invited to attend the interview day, on the 29th January 2010, will have the opportunity to meet supervisors and to discuss projects in more detail. Candidates will also be shown round LIMM facilities and meet staff from the different Sections. Candidates invited for interview will be informed by 15th January 2010.

Further information for LIMM Postgraduate Research Students can be found at:  
<http://www.limm.leeds.ac.uk/students.htm> and <http://www.limm.leeds.ac.uk/research.htm>

Applications should include a covering letter choosing up to 3 projects the applicant may want to be considered for, a full CV and full details of 2 academic referees.

These should be sent or emailed to: Dr Rashida Anwar, Lead Postgraduate Tutor, Leeds Institute of Molecular Medicine, Level 9, Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds LS9 7TF (tel no. 0113 2065645) email: [r.anwar@leeds.ac.uk](mailto:r.anwar@leeds.ac.uk)

### PROJECT OVERVIEWS

#### Project 1:

#### **Statistical identification and analysis of pathways involved in melanoma susceptibility and prognosis**

Dr Jenny Barrett, email: [j.h.barrett@leeds.ac.uk](mailto:j.h.barrett@leeds.ac.uk)

#### **Section of Epidemiology & Biostatistics**

Melanoma is the most serious form of skin cancer with a rising incidence and few treatment options. Recent advances in genetic technology have enabled researchers to carry out genome-wide association (GWA) studies, where genetic variation across the whole of the genome is examined to identify differences between people with disease (cases) and those without disease (controls). As part of the international melanoma genetics consortium GenoMEL ([www.genomel.org](http://www.genomel.org)) we have recently conducted a GWA study of melanoma (Bishop et al, Nature Genetics 2009, 41:920-50), testing over 300,000 genetic variants (single nucleotide polymorphisms (SNPs)) across the genome to see whether their frequencies differ between melanoma cases (n=1650) and controls (n=4336). A second phase of this study is underway using 610,000 SNPs on additional cases and controls. Several genes involved in pigmentation or in the development of moles were identified as important risk factors. Much of the genetic

variation in risk is still unexplained, and there is some evidence for the involvement of other genetic pathways (for example inflammation and DNA repair) in survival from melanoma.

Recently approaches have been proposed for interrogating GWA data to identify pathways of genes which show more evidence of association with disease than would be expected by chance. The first part of the project will be to review and develop such methods and apply them to the GWA study to identify a particular pathway or pathways on which to focus. Having identified a pathway, analyses will be conducted with the aim of better characterising the effect of genes in the pathway on disease risk. More complex models will be developed than the single-SNP association analyses conducted to date, including haplotype analyses (examining the pattern of variants along a chromosome), multiple regression and interaction analyses. Information on another type of genetic variation, copy-number variation, in the region of the relevant genes, will be analysed, and there may be scope for the analysis of sequence data (where every individual base-pair in a region is known) during the course of the project.

In addition our group is conducting studies on melanoma patients measuring gene expression (an indication of the level of activity of each gene), using both a 500-gene cancer panel and more comprehensive coverage of the whole genome, which enable us to relate expression to survival, response to therapy and to other characteristics of the patient or tumour. A similar analysis will be conducted to identify pathways important to melanoma survival, and analyses will be conducted to characterise the effect of the pathway on outcome from melanoma.

This project would suit a mathematics/statistics graduate or a biologist with an aptitude for statistics.

### **Research Group**

The genetic epidemiology group at Leeds has been in existence for over 20 years and has over thirty full-time members of staff. We have recently been involved in several genome-wide association studies such as the Wellcome Trust Case Control Consortium study (Nature, 2007, 447(7145):661-678), as well as studies of bowel and testis cancer, and we have coordinated and led the analysis of a genome-wide study of the biggest collection of melanoma patients ever conducted (Nat Genet, 2009, 41(8):920-925). More details of our research can be found at [http://www.limm.leeds.ac.uk/research\\_sections/epidemiology\\_and\\_biostatistics](http://www.limm.leeds.ac.uk/research_sections/epidemiology_and_biostatistics) and information about the Melanoma Genetics consortium we lead at <http://www.genomel.org/>. We have funding from various sources, including a major programme grant from Cancer Research UK.

### **Project 2:**

## **Identifying genetic association with disease when sampling from multiple populations**

**Dr Mark Iles, email: [m.m.iles@leeds.ac.uk](mailto:m.m.iles@leeds.ac.uk)**

### **Section of Epidemiology & Biostatistics**

In the last few years studies aiming to locate those genetic factors that influence disease risk have focussed on common genetic variants with a small effect on risk. Such variants may increase risk by only a small amount, but are of public health importance as they may be carried by more than half the population. Such studies typically require samples of several thousand cases and controls, which in turn often leads to the use of samples from multiple populations. This approach has led to the discovery of over 100 genetic variants influencing the risk of diseases such as breast cancer, melanoma, heart disease, diabetes and rheumatoid arthritis as well as variants that influence traits such as height, weight and hair colour.

Such studies have also shown there to be subtle genetic differences between even neighbouring countries (such as France and Belgium). Thus if populations are represented in different frequencies in the case and control samples, such slight genetic differences may appear to be related to disease risk. These ethnic strata may be revealed by the application of principal components analysis (PCA). Adjusting for PCs at the analysis stage apparently solves the problem of ethnic differentiation. But this may come at a price.

The aim of this project is to study the efficacy of PCA and how it is affected by different sample collection strategies. For example the degree of unevenness of sample collection across countries, the use of a large set of publically-available genetic data from a single population, the inclusion of individuals from countries with very mixed genetic backgrounds such as the USA may all impact on the power and appropriateness of such analyses. We are also interested in the effect on detection of genetic variants that we might expect to vary in frequency across Europe, such as those influencing lactose intolerance or skin cancer risk.

The PhD project requires someone with a degree in a numerate subject (such as Mathematics or Computing) and will involve a large degree of programming, although previous experience in this is not required. We also have large collections of data from across Europe, which will help to inform the project.

### **Research Group**

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### **Project 3:**

## **IKKa recruitment at NF-kappaB-dependent loci and alteration of chromatin organisation**

**Dr. Pascal Lefevre, email: [p.lefevre@leeds.ac.uk](mailto:p.lefevre@leeds.ac.uk)**

### **Section of Experimental Haematology**

The recent revolution in sequencing technologies will soon give scientists an almost unlimited access to the secrets of the genome. However such information will not be sufficient to understand how the genetic programme is implemented. Chromatin structure, superimposed upon the DNA sequence, provides an additional layer of heritable information by controlling accessibility to DNA and subsequently controlling gene expression. The dynamic interplay between nuclear components such as transcription factors and chromatin is central in this process and is modulated by multiple signals. In this respect the nuclear factor kappaB (NF-kappaB)-dependent inflammatory signals are amongst the most intensively studied in vertebrate biology, given that inflammation plays a key role in numerous diseases including arthritis, diabetes, heart disease, irritable bowel syndrome, Alzheimer's and Parkinson's diseases. In addition abundant epidemiological data show a strong correlation between inflammation and cancer incidence. IKKa (I $\kappa$ B kinase alpha), one of the proteins of the I $\kappa$ B kinase complex, which tightly controls the NF-kappaB signalling pathway, is also an essential regulator of NF-kappaB-dependent gene expression through direct control of promoter-associated histone H3 phosphorylation in response to inflammatory signals (1). A recent study unravelled the

importance of this chromatin “alteration” in the order of event mediating transcription elongation (2).

In addition our recent observations indicate that IKKa is not only initiating transcription but also interacts with chromatin along the gene in an elongation dependent manner (3). Since histone H3 phosphorylation is believed to promote release of the Heterochromatin Protein 1 (HP1) family members from chromatin, we monitored the correlation between elongation, IKKa recruitment and chromatin association with HP1. We observed accumulation of the phosphorylated form of HP1g at active NF-kappaB-dependent genes. We hypothesise that IKKa phosphorylates HP1g, which in turn will impair the HP1 dimer formation necessary to maintain condensed chromatin, therefore allowing transcription. Using complementary *in vitro* and *in vivo* experiments, the aim of this project is to study the IKKa chromatin modifier activity and in particular to validate the above hypothesis. This studentship offers an inviting opportunity to gain a wide range of expertise applied to understand mechanisms essential to the functions of eukaryotic cells.

#### **References:**

- (1) Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB. Histone H3 phosphorylation by IKK-alpha is critical for cytokine-induced gene expression. *Nature*. 2003 Jun 5; 423(6940): 655-9.
- (2) Zippo A, Serafini R, Rocchigiani M, Pennacchini S, Krepelova A, Oliviero S. Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. *Cell*. 2009 Sep 18; 138(6): 1122-36.
- (3) Lefevre P, Witham J, Lacroix CE, Cockerill PN, Bonifer C. The LPS-induced transcriptional upregulation of the chicken lysozyme locus involves CTCF eviction and noncoding RNA transcription. *Mol Cell*. 2008 Oct 10; 32(1): 129-39.

#### **Project 4:**

### **Interfering with a transcriptional master regulator to understand molecular function and validate a novel therapeutic target**

Dr Reuben Tooze, email: [r.tooze@leeds.ac.uk](mailto:r.tooze@leeds.ac.uk)

#### **Section of Experimental Haematology**

Both normal and aberrant function of the immune system whether in the context of inflammatory disease or malignancy is ultimately controlled at the level of gene expression. The transcription factor BLIMP-1 is a master regulator of gene expression in lymphocytes. In B-lymphocytes this transcription factor is essential for maturation to the terminal phase of antibody secreting plasma cells, and in mature plasma cells and plasma cell malignancies BLIMP-1 is an essential survival factor. In T-lymphocytes BLIMP-1 controls the transition between effector and memory responses, and in its absence a profound auto-inflammatory disease develops. BLIMP-1 is known to interact with several other molecules to mediate its effects, however the interactions of BLIMP-1 have not been fully characterised. Moreover BLIMP-1 represents a potential therapeutic target in plasma cell malignancies and autoimmune disease, but approaches to interfere specifically with BLIMP-1 function have not been developed.

One promising avenue for intervention into transcription factor function is the development of aptamers. These nano-tools are small protein or RNA molecules, which are designed to interact with specific parts of a target protein. Aptamers based on either RNA or peptide scaffolds provide a means for targeting complex protein surfaces. Aptamers can be used to map and disrupt protein interactions, and some aptamers have entered trials as novel therapeutics.

The aim of this project will be to build on our existing work on BLIMP-1 by evaluating a series of aptamers to target BLIMP-1. The aptamers will be evaluated for their ability to interact with specific regions of the BLIMP-1 molecule and for their ability to interfere with specific aspects of BLIMP-1 function, both using *in vitro* assays and primary human B- and T-lymphocyte

differentiation systems. The ultimate scientific goal is to provide further evidence for BLIMP-1 as a therapeutic target in malignancy and inflammatory disease.

## **Project 5:**

### **Role of Wnt signalling in the metastatic potential of ESFT**

**Professor Sue Burchill and Dr Helen Payne, email: [s.a.burchill@leeds.ac.uk](mailto:s.a.burchill@leeds.ac.uk)**

#### **Section of Experimental Oncology**

**Location of proposed research:** LImm Children's Cancer Research Group

**Overview of the group:** The overall aim of our research group is to improve outcome for patients diagnosed with Ewing's sarcoma family of tumours (ESFT). We comprise 5 post-doctoral research scientists, one research assistant, one PhD student, one clinical research fellow, 2 senior technicians and one junior technician. We identify novel molecular targets to inform the development of new therapeutics, by understanding the cellular processes involved in the induction and resistance of ESFT to cell death. We evaluate these novel targets and therapeutics in a range of pre-clinical models and clinical samples. We also identify and evaluate biomarkers to monitor patient response and disease course.

**Rationale for project:** Metastasis, relapse and drug resistance present the most difficult of challenges for the successful treatment of many people with cancer, including those with ESFT. Cancer initiating cells (CICs) are a distinct cell population thought to be responsible for this aggressive phenotype. Therefore selectively targeting and eliminating CICs should result in tumour regression, prevention of relapse and potentially patient cure. Wnt signalling plays a critical role in development and oncogenesis, and has recently been reported as a metastasis-associated pathway in ESFT (Schaeffer et al. 2008. *Eur. J. Cancer* 44:699-709). Furthermore, Wnt signalling is known to tightly regulate the self-renewal and differentiation of multipotent stem cells and is reported to be aberrant in CICs leading to malignant proliferation (Fodde and Brabletz. 2007. *Curr Opin Cell Biol* 19:150-58; Ling et al. 2009. *Gene* 433:1-7). Wnt signalling has also been linked to resistance to chemotherapeutics routinely used in the treatment of ESFT, specifically doxorubicin, ifosfamide, vincristine and etoposide. We have therefore hypothesised that this signalling pathway may be important in the development of the ESFT CIC metastatic and drug resistant phenotype, and might be a novel target for the development of more effective therapeutics. This is particularly relevant in ESFT since Wnt-1 has recently been shown to up regulate expression of the retinol binding protein receptor *stra6* (Szeto et al. 2001. *Cancer Res* 61:4197-205), which may affect the sensitivity of ESFT to fenretinide, a retinamide that induces cell death in ESFT (Myatt et al. 2005. *Clin. Cancer Res* 11:3136-3148) and is currently being evaluated for the treatment of this aggressive cancer.

**Project outline:** Initially CICs with the potential to form metastases will be isolated from a primary ESFT culture by cell sorting (CD133+ magnetic bead, Suva et al., 2009, *Cancer Res* 69; 1776-81). These isolated cells will be characterised for putative stem cell markers including OCT4, SOX2 and NANOG, and stem cell phenotype using assays that investigate the capacity for self-renewal, survival and dissemination. The expression and activity of the Wnt signalling machinery will be examined in the CICs with metastatic potential and compared to that of the non-CIC ESFT cell population by expression arrays, western blotting, qRT-PCR and FACS. Furthermore, the effects of specific Wnt protein overexpression (inducible expression vectors) and knockdown (siRNA) will be determined on the metastatic capabilities of the ESFT cell, using established migration and clonogenic assays. The expression of Wnt proteins implicated in the development of the metastatic and drug resistant phenotypes *in vitro* will be screened in primary tumours from patients by

immunohistochemical methods using Wnt protein-specific antibodies. Dependent on the findings of the above studies, we will investigate whether manipulation of specific Wnt signalling pathway proteins can be exploited to enhance the efficacy of chemotherapeutics in ESFT, identify the possible influence of Wnt signalling on the expression of the retinol binding protein receptor stra6 in ESFT and its effect on fenretinide-induced cell death. These proposed studies will closely compliment ongoing objectives of the LIMM Children's Cancer Research Group.

**Bibliography:** Myatt SS, Redfern C and Burchill SA. p38<sup>MAPK</sup>-dependent sensitivity of Ewing's sarcoma family of tumours (ESFT) to 4-hydroxy(phenyl)retinamide-induced death. *Clinical Cancer Research*, 11:3136-48, 2005.

Brownhill, S., Taylor, C., and Burchill, S. A. Chromosome 9p21 gene copy number and prognostic significance of p16 in ESFT. *British Journal of Cancer*, 96:1914-23, 2007.

Myatt, S. S. and Burchill, S. A. The sensitivity of the Ewing's sarcoma family of tumours to fenretinide-induced cell death is increased by EWS-Fli1 dependent modulation of p38MAPK activity. *Oncogene*, 27: 985-96, 2008.

Roberts, P., Burchill, S. A., Brownhill, S., Cullinane, C. J., Johnston, C., Griffiths, M. J., Dom, M., Brown, N., Morris, S. P., and Lewis, I., J. Ploidy and karyotype complexity are powerful prognostic indicators in the Ewing's sarcoma family of tumours: A study by Children's Cancer and Leukaemia Group. *Genes, Chromosomes and Cancer*, 47:207-20, 2008.

### **Project 6:**

## **Sticking peptides on protein interfaces: Developing a new, knowledge-based, computational tool to design peptides for modulating protein-protein interactions.**

**Dr Narcis Fernandez-Fuentes, email: [n.fernandez-fuentes@leeds.ac.uk](mailto:n.fernandez-fuentes@leeds.ac.uk)**

### **Section of Experimental Therapeutics**

#### **Summary**

One of the preeminent challenges in the post-genomic era is the determination of the cellular function for the numerous proteins encoded by genomes. Preventing the interaction between proteins, initially using peptide inhibitors, will lead to changes in the cell which can be observed and measured, thus providing information about the role that the protein complex plays inside the cell. On the other hand, cell growth involves an intricate set of interactions between proteins that turn on or turn off signalling pathways. When the finely tuned signals are lost, diseases such as cancer can develop. Preventing the interaction between such proteins, again using peptide inhibitors, will stop the abnormal signals, and therefore is a potential pathway towards fighting cancer. Peptides are therefore valuable tools to understand the specific roles of protein complexes inside the cell and leads for the development of new classes of therapeutic drugs aimed at disrupting disease-causing complexes.

#### **Objectives**

The overall objective of the project is to develop a novel structure-based computational tool to design peptides to modulate protein-protein interactions. The project builds upon our recent work on the computational prediction of critical, or hot-spot, residues in protein interfaces[1]. Using interface residues as anchor points, peptides extracted from known protein complexes will be modelled and assessed in the structural context of the protein surface.

The project will entail three major stages or intermediate objectives:

1. The first step will include the description of residues mediating protein-protein interactions using a newly described method[1].

2. Develop a novel knowledge-based method to model peptides on protein-protein interaction surfaces. The method will include the extraction and building of a bespoke library composed of protein fragments that mediate protein-protein interactions in protein complexes with known 3D structure and the assessment of structural fitting in the context of the new protein interface.
3. Finally, developing a novel scoring function to rank designed peptides. The first step will examine classic atomistic details of the protein-peptide interaction, while the second step will include non-mechanistic terms such as the inclusion of hot-spot residues.

[1] Assi, SA, Tanaka T, Rabbitts TH, Fernandez-Fuentes N. *PCRPI: Presaging Critical Residues in Protein interfaces*. Nucleic Acids Research, 2009.

### **Project 7:**

## **Investigation of alternative therapy targets in LMO2-dependent cancer**

**Professor Terence Rabbitts PhD FRS FMedSci, email: [t.rabbitts@leeds.ac.uk](mailto:t.rabbitts@leeds.ac.uk)**

### **Section of Experimental Therapeutics**

The *LMO2* gene is activated in T cell leukaemias as a result of chromosomal translocations and is also expressed in around 50% of T cell acute leukaemia lacking obvious chromosomal abnormalities. In addition *LMO2* is activated in around half of diffuse large B cell lymphomas and recent data shows activation in prostate and pancreatic cancer, indicating that *LMO2* is a major target in a range of human cancers. We have recently developed new technologies to isolate reagents that bind to intracellular proteins and prevent function *in vivo*. Because *LMO2* is an important therapeutic target but is also expressed in pluripotent bone marrow stem cells, we wish to evaluate *LMO2*-dependent gene expression in different tumours for possible discovery of alternative therapy targets. The project will involve **ChIP** analysis (**Chromatin Immunoprecipitation** analysis) using Next Generation Sequencing (so-called **ChIP-seq**) to determine *LMO2* DNA-binding sites in various cancers, yielding information of all sites to which the *LMO2* protein complex is located. This will elucidate two important pieces of information. First, the binding site sequences will give information on the proteins with which *LMO2* is associated at a particular DNA-binding site and allow retrospective analysis of candidate tumour-specific protein interactions. Second, the binding site sequences will define the genes to which the *LMO2* protein complex activates (or represses) in the tumour cells and which are candidates for development of therapeutics. The work will lead to translational studies in the long-term for development of drugs against tumour-specific *LMO2* protein complexes or against *LMO2*-dependent downstream processes.

### **Exemplar References**

Tanaka, T., Williams, R., & Rabbitts T.H. (2007) Tumour prevention by a single antibody domain targeting the interaction of signal transduction proteins with RAS *EMBO J.* **26**, 3250-3259

Nam, C-H., Lobato, M.N., Appert, A., Drynan, L.F., Rabbitts, T.H. (2008) An antibody inhibitor of the *LMO2*-protein complex blocks its normal and tumorigenic functions *ONCOGENE*, **27**, 4962-4968

Tanaka, T., Rabbitts, T.H. (2009) Intracellular antibody capture protocol to select intrabodies for perturbing protein function inside cells *NATURE PROTOCOLS* in press

### **Examples of students spending time in other labs (ideally non academic environments)**

Helen Sewell (my current LRF Gordon Pillar student) has spent time at the Oxford Protein Production Facility learning X-ray crystallography using her purified *LMO2* protein.

## Project 8:

### **Identification of recessive disease genes in consanguineous families**

Dr Eamonn Sheridan, email: [medesh@leeds.ac.uk](mailto:medesh@leeds.ac.uk)

#### **Section of Genetics**

Consanguineous unions are very common in the Asian community in West Yorkshire. We have collected and analysed family trees from 2500 Asian women recruited to the Born in Bradford project, in which about 52% of all recruits are of Asian origin. 46.3% of these women are in a consanguineous union, 36.2% are married to first cousins. This results in a high incidence of autosomal recessive disease in this community, for example there were 19 cases of inborn errors of metabolism in babies born to women of Asian origin compared with only 2 in the Caucasian group. The infant mortality rate is 12.9/1000 in children of Asian origin compared to 5.5/100 nationally. The causative genes for much of this burden remain unknown, however this provides us with a unique opportunity to identify novel disease genes. We have previously been very successful in this field, identifying genes for childhood cancers(1), renal disease(2) and skeletal dysplasias(3). Striking biological insights into carcinogenesis, ion transport and embryonic patterning resulted from these investigations, indicating the biological value of such studies.

We have collected a large cohort of families with multiple cases of recessive disorders, these are prime candidates for further investigation to identify new disease genes. This application is for a studentship to work on this project. We have recently obtained major funding from the Jules Thorn Trust to pursue this field, this is a prime opportunity for a PhD student to become part of this investigation.

The proposed plan of research would be:

- (a) Autozygosity mapping studies in selected families with unmapped autosomal recessive disorders, by means of genome-wide linkage analysis using SNP microarrays. The large genotype datasets that will be generated will be analyzed for regions of IBD using the in-house software packages "IBD Finder" and "AutoSNPa"
- (b) Identification of disease genes by a positional candidate strategy using both conventional sequencing and novel genome partitioning technologies and next-generation sequencing of candidate intervals. Candidate genes will also be prioritized using the "Endeavour" program on the basis of known or inferred genetic ontology, phenotypes of any animal models, and interacting proteins since the encoded protein could interact with known components in a common signal transduction pathway or multisubunit complex. We have previously characterised disease genes by identifying mutations by Sanger Sequencing. We have recently however identified a novel gene for primary ciliary dyskinesia by means of genome partitioning using the "SureSelect" Target Enrichment System for a 2.7Mb region identified in a multiplex family and subsequent sequencing of the whole region using the Illumina SOLEXA 1G Genome Analyzer. We would plan to use both approaches in the course of the project.
- (c) Functional studies of the disease gene as appropriate. The nature of these studies is dependant on the disease gene, but previously we have characterised disease genes by biochemical investigations(4), in-situ hybridisation studies and, subcellular localisation of protein products(5)

1. M. De Vos *et al.*, *Journal of the National Cancer Institute* **98**, 358 (2006).
2. D. Bockenhauer *et al.*, *The New England Journal of Medicine* **360**, 1960 (2009).
3. C. G. Woods *et al.*, *American Journal of Human Genetics* **79**, 402 (2006).
4. S. Uppal *et al.*, *Nature Genetics* **40**, 789 (2008).
5. M. R. Abdollahi *et al.*, *Am J Hum Genet.* (Nov 4, 2009).

### **Project 9:**

#### ***Helicobacter pylori* associated mutations in gastric carcinogenesis**

Professor J. E. Crabtree and Dr. P. A. Robinson, email: [j.crabtree@leeds.ac.uk](mailto:j.crabtree@leeds.ac.uk)

##### **Section of Molecular Gastroenterology**

*H. pylori* is a major risk factor for gastric carcinogenesis. The mechanisms by which *H. pylori* promotes gastric cancer are multiple. The long-term chronic inflammatory response and epithelial hyperproliferative changes are considered to increase the risk of mutational changes.

Whilst chronic inflammation is generally considered the driving force initiating gastric mutations, *H. pylori* also induces the aberrant expression of the DNA editing enzyme activation-induced cytidine deaminase (AID) in gastric epithelial cells in a *cag* PAI dependent manner. As epithelial expression of AID is upregulated in patients with chronic gastritis, aberrant expression of this DNA editing enzyme may be a key factor linking *H. pylori cag* positive strains with increased risk of developing gastric cancer. The specific role of *H. pylori* virulence factors and associated upregulation of activation induced cytidine deaminase in inducing mutations in p53 will be assessed in *in vivo* and *in vitro* cell culture systems.

The frequency of mutations in *Helicobacter* infected gastric tissue and the specific epithelial cell populations in which mutations arise will be determined. Gastric tissue from model systems exposed to parental *H. pylori* strains or isogenic mutant strains lacking key virulence genes such as *cagA* and the *cag* pathogenicity island will be examined. The use of a model system containing the bacterial transgene *lacI/lacZ* will allow *in vivo* analysis of mutational changes in DNA following *Helicobacter* infection. Immunohistological labelling will be used to identify potential stem cell populations for laser capture microscopy and analysis of gastric mutations. Mutations in the p53 gene in selected epithelial cell populations harvested using laser capture microscopy will be examined as well as proliferative and apoptotic responses of gastric epithelial cells and mucosal gene expression. In addition, sequencing of *lacI* will allow determination of the mutational frequency in different mouse epithelial cell populations.

The PhD student will gain experience in a wide range of molecular and cellular biology experimental techniques including cell and bacterial culture, mutational analysis, laser capture microscopy, immunohistochemistry and RT-PCR.

### **Project 10:**

#### **A novel strategy for therapeutic targeting of cyclooxygenase-2 gene regulation in colon cancer**

Dr Thomas Hughes and Professor Mark Hull, email: [t.hughes@leeds.ac.uk](mailto:t.hughes@leeds.ac.uk)

##### **Section of Molecular Gastroenterology**

Cyclooxygenase-2 (COX-2) is up-regulated in many cancers leading to enhanced angiogenesis and tumour cell invasiveness, and reduced tumour cell apoptosis. Inhibition of COX activity has preventative and therapeutic effects on several cancer types but generalised inhibition by drugs is associated with some serious side-effects. An alternative strategy to reduce COX-2 activity in cancer is to identify the specific molecular pathways leading to COX-2 up-regulation during carcinogenesis, and to design therapies to reverse this up-regulation. It is well established that

post-transcriptional regulation, especially at the level of mRNA stabilisation, plays a very substantial role in COX-2 up-regulation in cancer. Inhibition of COX-2 expression at post-transcriptional levels presents a currently unexplored and exciting novel therapeutic strategy. Importantly, this strategy may be generalisable for many cancer targets initiating a new class of cancer drugs.

Our aims will be to define the mechanisms of post-transcriptional regulation of COX-2 in colon cancer and to identify potential cancer therapeutics that target these mechanisms. We have already developed prototype fluorescent reporters with which we can measure post-transcriptional regulation in cancer cells. In this project we will further extend this new technology.

We will: i) Further develop novel cell-based assays for study of post-transcriptional regulation in cancer cells using fluorescent reporters; ii) Determine mechanisms of post-transcriptional regulation of COX-2 in colon cancer cells; iii) Identify small molecule drugs that inhibit COX-2 expression by targeting these mechanisms.

We expect to gain significant insights into the importance of specific molecular pathways in the regulation of COX-2 in cancer and to identify drugs that are capable of reducing COX-2 expression in cancers.

The PhD student will join a team investigating post-transcriptional gene regulation in cancer and will gain experience of a broad range of molecular and cell biology laboratory techniques applicable to cancer research

#### **Project 11:**

### **Identification of soluble and genetic biomarkers of response to conventional and biological therapy in rheumatoid arthritis.**

**Professor Ann Morgan and Dr Jenny Barrett, e-mail [a.w.morgan@leeds.ac.uk](mailto:a.w.morgan@leeds.ac.uk)**

#### **Sections of Musculoskeletal Disease and Epidemiology and Biostatistics**

Rheumatoid arthritis (RA) is a heterogeneous disease that occurs in approximately 1% of the population. Despite conventional therapy with methotrexate or sulphasalazine, one third of rheumatoid arthritis patients have evidence of joint damage (radiographic erosions) within the first year of disease, 10% develop severe functional impairment in the first 2 years and almost 50% of rheumatoid arthritis patients are unable to work after 10 years of disease. The specificity of individual prognostic factors to stratify early RA patients into benign, mild and severe destructive disease at disease onset is currently inadequate for translation into clinical practice. The treatment rationale for RA patients has undergone extensive review over recent years. There is now considered to be a 'window of opportunity' in early disease where patients should be treated more intensively, often with combinations of drugs or early biological therapy. Nevertheless, a significant proportion of RA patients fail to respond adequately to anti-TNF therapy for poorly understood reasons. The high cost of the new biologic drugs means that they are a limited resource and conventional disease modifying anti-rheumatic drugs, such as methotrexate (MTX) and sulphasalazine, are therefore likely to remain the first line treatment for RA within the framework of the NHS. There is therefore an unmet clinical need to identify the most cost-effective therapy that the patient is most likely to respond to, and not experience significant toxicity from, prior to use. This is also likely to have the added value of earlier suppression of disease activity and reduced long-term joint damage. Consequently, there is an increasing interest in the field of pharmacogenetics to determine whether genetic polymorphism within the drug transport mechanisms and enzymes within key metabolic pathways can be used to predict both response to therapy and toxicity. Biological markers that predict the response to individual therapies are at the earliest stages of evaluation in RA.

The project will assess the role of biomarkers, both soluble and genetic in predicting the response to both conventional and biological therapies in rheumatoid arthritis. Professor Morgan has established a multi-disciplinary group bringing together expertise in the area of serology, immunogenetics, pharmacogenetics and outcomes research in order to systematically evaluate the clinical utility of soluble and genetic biomarkers. This group is also working in close collaboration with industry and national partners (Wellcome Trust Case Control Consortium, UK Rheumatoid Arthritis Genetics Group, Biologics in Rheumatoid Arthritis Genetics and Genomics Study Group and the UK Psoriatic Arthritis genetics consortium) to facilitate the translation of emerging scientific discoveries into the clinical arena, specifically the incorporation of soluble biomarkers and genotyping into the clinic. There will be some flexibility for the applicant to develop their own research interests within the broad remit of this project in consultation with both their supervisors and with new discoveries within the scientific literature.

### **Project 12:**

## **Understanding the immunological contribution towards the development of early Rheumatoid Arthritis**

**Dr Frederique Ponchel, email : [mmefp@leeds.ac.uk](mailto:mmefp@leeds.ac.uk)**

### **Section of Musculoskeletal Disease**

Recent advances in therapy of Rheumatoid Arthritis (RA) demonstrate the potential to induce long-term remission with no further progression of joint damage particularly with intervention in early disease. Remission is now a realistic objective in early disease; however, RA can be difficult to identify at an early stage. A highly-specific marker for RA (presence of anti-citrullinated-protein antibodies) has recently been recognised allowing poor-prognosis patients to be identified at preclinical stages. We have identified immunological defects in RA (loss of naïve and regulatory T-cells, low serum IL-7), present in early disease. In this project, we propose to test the hypothesis that each stage in the disease continuum reflects a step further towards immunological dys-regulation. Combination of clinical and immunological data should allow the characterisation of patient's disease very early with development of effective management algorithms. We plan to recruit patients at a pre-clinical stage, follow them through the first manifestations of disease (undifferentiated arthritis), towards a fully diagnosed RA, post treatment and later in remission. Understanding how patients progress from one stage of the disease to the next is likely to define new therapeutic targets and confirm a window of opportunity in order to achieve early remission before damage become irreversible

### **Project 13:**

## **Proteomic determination of predictive biomarkers for tyrosine kinase inhibitor therapy**

**Professor Roz Banks, Dr Nav Vasudev and Professor Peter Selby  
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### **Section of Oncology & Clinical Research**

The management of patients with renal cell carcinoma (RCC), the tenth commonest cancer, is highly challenging. In recent years, a number of rationally designed, targeted agents, such as the tyrosine kinase inhibitors sunitinib and sorafenib, have been introduced and shown to be efficacious. However, these agents are expensive, carry significant toxicity and not all patients will respond. Predictive biomarkers are needed to identify those patients who would benefit, reducing

exposure to unnecessary side-effects and improving cost-effectiveness. Additionally, as traditional radiological criteria for assessment of response may not be optimal, novel markers of activity *in vivo* are needed. Using state-of-the-art proteomic technologies including liquid chromatography-tandem mass spectrometry and 2D-DIGE with both clinical fluids and cell line-based model systems, the overall aims of the project would be:

- 1) To discover novel biomarkers in tissue, serum or urine that may predict response to sunitinib and/or sorafenib
- 2) To discover novel biomarkers that may monitor response to sunitinib and/or sorafenib
- 3) To conduct initial validation of candidate markers in samples collected from patients treated with TKIs

Website: <http://www.proteomics.leeds.ac.uk>

#### **Project 14:**

### **Investigation of cancer stem cells in leukaemia and ovarian cancer**

**Dr Sandra Bell (Lead supervisor), Dr Erica de Winter and Dr Geoff Hall**  
**email: [S.M.Bell@Leeds.ac.uk](mailto:S.M.Bell@Leeds.ac.uk)**

#### **Section of Ophthalmology & Neuroscience**

Many patients have chemotherapy after surgery for cancer to reduce the possibility of it returning. Unfortunately, a number of patients have cancers that for unknown reasons are resistant to these drugs. A key cause may be the presence of 'cancer stem cells' which are resistant to chemotherapy and promote re-growth of the tumour cells. Cancer stem cells have been identified in leukaemias and in solid tumours such as breast and ovarian cancer.

The emergence of cancer stem cells has been linked to problems in the BRCA1 DNA repair pathway. Previously, we identified mutations in *MCPH1*, a DNA damage response gene, as a cause of primary microcephaly. MCPH1 regulates the expression of both the BRCA1 and BRCA2 genes, mutations in which cause familial breast cancer. Recently, we have shown that reduced MCPH1 expression in 26% of breast and 40% of ovarian cancers, is associated with increasing tumour grade. In addition we have shown that MCPH1 plays a role in resistance to chemotherapeutic agents used in the treatment of breast and ovarian cancers.

*MCPH1* patient-derived cells exhibit premature chromosomal condensation (PCC), which is a consequence of cells starting to divide before completing DNA replication. A similar phenotype has been observed in ovarian and breast cancers and is an early indicator of relapse in acute leukaemia. Therefore, the focus of the study will be to :-

1. Investigate the correlation between MCPH1 expression and the presence of cancer stem cells in acute leukaemia and ovarian cancer.
2. Determine the effect of MCPH1 expression on the response to chemotherapy in cultured cancer stem cells.

This project will involve a variety of cellular and molecular techniques including cell culture, siRNA, Real time PCR, FACS analysis, live cell imaging, immunofluorescence and confocal microscopy. Potentially the study of MCPH1 will have a significant impact on the future clinical care of cancer patients, allowing treatment regimes to be tailored to individual needs.

### **Project 15:**

#### **Does Parkin regulate mitochondrial transport in neurons?**

Dr Phil Robinson and Dr Ewan Morrison, email: [P.A.Robinson@leeds.ac.uk](mailto:P.A.Robinson@leeds.ac.uk)

#### **Section of Ophthalmology & Neuroscience**

Parkinson's disease (PD) is a debilitating neurodegenerative disorder whose incidence is increasing as our population ages. Its' causes remain unclear. Although most cases of the disease arise spontaneously, some are inherited. The characterisation of inherited PD has proven useful in identifying processes that may be affected in sporadic disease. Mutations in the *PARK2* gene cause Autosomal Recessive Juvenile Parkinsonism. The protein encoded by this gene, Parkin, is a ubiquitin-protein ligase, an enzyme involved in regulated protein degradation. An interaction between Parkin and microtubules - the "highways of the cell" - has also been identified. Furthermore, Parkin has been implicated in the disposal of damaged mitochondria in a process called autophagy. In neurons this function could involve an effect on mitochondrial movement in axons (the long, highly specialised cellular extensions that connect neurons) because damaged axonal mitochondria must be moved to the neuronal cell body for disposal. Axonal transport relies upon microtubule-based pathways of intracellular movement and is crucial for proper neuronal function. We suspect that loss of Parkin compromises the normal axonal transport of organelles, causing axons to become progressively clogged up and eventually leading to neuronal death. The neurons affected in PD possess very long and thin non-myelinated axons. As such they are likely to be more susceptible than other axons to attack from environmental agents that might cause PD. We therefore specifically wish to test whether Parkin is involved in the movement of damaged axonal mitochondria to the neuronal cell body for autophagic recycling. Interestingly, other proteins associated with the development of PD, such as  $\alpha$ -synuclein, also interact with axonal transport pathways. It therefore seems possible that disruption of these pathways could represent a common event in PD. This studentship offers a rare opportunity to gain experience in state-of-the-art molecular and cell biology techniques in a project aimed at increasing our understanding of the basic mechanisms involved in an important but poorly understood disease.

### **Project 16:**

#### **The regulation of epithelial-mesenchymal transition in gastric cancer**

Dr Phil Burns and Dr Heike Grabsch, email: [P.A.Burns@leeds.ac.uk](mailto:P.A.Burns@leeds.ac.uk)

#### **Section of Pathology & Tumour Biology**

The process of epithelial-mesenchymal transition (EMT) is an important feature of tumorigenesis in epithelial tissues, and is associated not only with the acquisition of invasiveness and metastasis, but also with response to therapy. At the molecular level EMT is characterised by a class switch from E-cadherin to N-cadherin expression, and a concomitant gain of vimentin expression. Regulators of E-cadherin expression include ZEB1&2, Snail, Slug and Twist in response to growth factors such as TGFbeta, PDGF, FGF and Wnt5A . In addition, recent studies have demonstrated an important role for the microRNA-200 family in the control of ZEB1&2 expression and the regulation of EMT. The main aim of this project will be to analyse the control of the EMT phenotype in gastric cancer cell lines and investigate our ability to induce or reverse this process. The impact of manipulation of the EMT phenotype on drug response will also be investigated. The expression of the EMT phenotype in the established cell lines will be also be compared with that seen in a collection of several hundred primary tumour samples. Techniques will include rtPCR, gene expression array, data mining, siRNA transfection, miRNA transfection,

tissue microarrays, immunohistochemistry, tissue culture and drug response assays. The project will involve the use of a panel of extremely well characterised gastric cancer cell lines in collaboration with Prof P Tan at the Genome Institute in Singapore.

**Project 17:**

**Development of an organotypic model of the normal breast: A tool to investigate breast cancer initiation and progression.**

Dr V Speirs, Dr D Holliday and Prof A Hanby, email: [v.speirs@leeds.ac.uk](mailto:v.speirs@leeds.ac.uk)

**Section of Pathology & Tumour Biology**

Breast cancer is now the commonest cancer in the UK with over 45,000 new cases diagnosed and more than 12,000 women dying of the disease every year. In order to study and understand disease progression 3D *in vitro* models of breast cancer are being developed and these are essential to study cellular function in a relevant microenvironment.

Our previous work has established a reproducible *in vitro* model of pre-invasive breast cancer, the first model of its kind to culture in 3D the 3 major cellular components of the breast: luminal epithelial cells, myoepithelial cells and fibroblasts (Breast Cancer Research. 2009;11:R3). This model is a valuable tool to investigate the role of the microenvironment in breast cancer progression. To date there is no fully characterized equivalent model to look at normal breast biology. Such a model is not only essential for dissecting normal breast function but has tremendous potential to study specific gene changes involved in cancer initiation in a human system within a well defined *in vitro* setting.

The project will involve cell culture (both primary and established cell lines), development and validation of multicellular 3D models, confocal microscopy and state of the art 3D computer reconstruction of the models using digital pathology. The effect of manipulating expression of genes associated with cancer initiation (e.g. p53/Ras/PI3K) in normal breast epithelial using RNA interference techniques will also be explored.

You will be working within The Breast Research Group which consists of academic staff, postdocs and postgraduates students with strong and diverse areas of expertise.

This is an exciting new project in the further development and validation of 3D culture models of normal breast and breast cancer, the importance of which is becoming increasingly recognized in cancer biology.

**Project 18:**

**An *in vitro* study of the effect of anaesthetic agents on macrophage function: implications for cancer recurrence.**

Dr Simon Howell, email: [s.howell@leeds.ac.uk](mailto:s.howell@leeds.ac.uk)

**Section of Translational Anaesthetic & Surgical Sciences**

Although surgery for primary tumour removal offers the best prognosis in cancer treatment, recurrence can occur through micrometastatic development. Within micrometastases, tumour cells can subvert macrophage function through local cytokine-mediated paracrine dialogue, facilitating escape from immunosurveillance, enhancing angiogenesis and increasing tumour cell invasiveness. Different anaesthetics may predispose to recurrence by perturbing immune function, although the impact of these agents in the tumour microenvironment has been largely

overlooked. This pilot study will assess the impact of isoflurane, sevoflurane and propofol on endometrial cancer cell and macrophage phenotype, behaviour and interactions *in vitro*, assessed by flow cytometry and cytokine, prostaglandin E<sub>2</sub> and nitric oxide production. Macrophage-cancer cell interactions will be investigated in co-culture, where gene expression changes (microarray), angiogenic signalling (HUVEC bioassay) and tumour cell invasiveness (Matrigel invasion assay) will be determined. These findings will clarify the impact of general anaesthetics on the tumour microenvironment, informing the anaesthetic choice in cancer surgery.

### **Project 19:**

## **The PDH/PDK system: a metabolic target for cancer therapy**

Mr David Jayne, email: [d.g.jayne@leeds.ac.uk](mailto:d.g.jayne@leeds.ac.uk)

### **Section of Translational Anaesthetic & Surgical Sciences**

The majority of solid cancers show a preference for aerobic glycolysis rather than oxidative phosphorylation as a means of deriving energy from glucose. This is irrespective of the prevailing tissue oxygen tension, and a characteristic known as the Warburg effect. Although aerobic glycolysis is inferior to oxidative phosphorylation in terms of the amount of ATP generated per molecule of glucose, it is believed to bestow certain advantages to cancer cells allowing them to survive in the local tumour microenvironment. Aerobic glycolysis is a means of rapid ATP generation, which may be beneficial to the rapidly proliferating cancer cell. Lactic acid is produced as an end-product of glycolysis, which is exported out of the cell resulting in an acidic extracellular milieu that may be favourable for invasion but detrimental to the host immune response. The switch away from oxidative phosphorylation may also help to prevent excessive free radical formation in the presence of diminished oxygen supply, and so prevent cancer cell apoptosis.

Current research is focused on the pyruvate dehydrogenase (PDH) enzyme system, which controls the switch between aerobic glycolysis and oxidative phosphorylation in normal and cancer cells. PDH catalyses the conversion of Pyruvate to Acetyl CoA, which subsequently enters the citric acid cycle and fuels oxidative phosphorylation. Down-regulation or inhibition of PDH favours aerobic glycolysis. Tissue microarray techniques have confirmed that aerobic glycolysis is up-regulated in colorectal cancer and that this is probably brought about by differential up-regulation of pyruvate dehydrogenase kinases (PDK1-4), the natural inhibitors of PDH. *In vitro* work using a variety of colorectal cancer cell lines has shown that inhibition of the PDK1-4 by Dichloroacetate results in up-regulation of PDH activity with a switch to oxidative phosphorylation and accompanying decreased cell proliferation, cell viability, G2 cell cycle arrest, and increased apoptosis. The PDH/PDK system would therefore seem to be a suitable metabolic target for cancer therapy.

A studentship is offered to pursue research into the role of the PDH/PDK system as a therapeutic target for cancer. Future work will concentrate on confirming the potential of the PDH/PDK system as a therapeutic target, assessing the effects of PDK1-4 siRNA knock down on cancer cell function, and developing an animal model for evaluating the *in vivo* effects of PDK1-4 knock-down/inhibition.