Our Travel Fellowships, co-sponsored by the Association for International Cancer Research, are designed to assist student and early-career researchers to further their research through learning and collaboration.

Tamás Garay
2nd Department of Pathology, Semmelweis University, Budapest, Hungary
Host institute: Department of Tumour Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
Dates of visit: August – September 2012

Malignant melanoma has - despite all efforts - one of the worst prognoses among solid tumours and its incidence is increasing worldwide, notably in Northern Europe as well. BRAF is the most common oncogenic mutation in malignant melanoma. This finding - together with the demonstration that these tumours are dependent on the activation of the RAF/MEK/ERK pathway - led to the development of BRAF inhibitors. There are no recently introduced BRAF inhibitors that are selective to mutant BRAF (e.g. vemurafenib). Both drugs are potent molecules capable of inhibiting the downstream effects of mutant BRAF. However, it is now clear that a tumour resistance often emerges and its mechanism is not yet understood. In my PhD project I am investigating the role of NRAS/BRAF oncogenic signalling in melanoma progression. Prof. Øystein Fosdadt and the Department of Tumour Biology have a long track record in the field of melanoma research, and are among others working on investigating the resistance mechanism of melanoma against BRAF inhibitors.

Our investigations targeted to clarify the molecular mechanism behind the resistance against BRAF inhibitors in melanoma cells especially through the examination of changes in the AKT pathway. We used for our experiments melanoma cell lines, consisting of one sensitive and one resistant cell line, obtained from metastases of the same patient before and after the treatment with the BRAF inhibitor vemurafenib. We characterised the difference in proliferation and wound healing ability in the scratch assay (i.e. migration) between the cell lines in presence or absence of kinase inhibitors. Using viral vectors to transduce cells with short hairpin RNA members of the AKT pathway were silenced in BRAF inhibitor resistant cell lines for further analysis of their role in the mechanism of inhibitor resistance. Altogether 5 transduced sub-cell lines could be grown to stable clones using puromycin as selecting agent. Whole cell lysates of sensitive and resistant cells were examined with a tyrosine kinase array to screen for differences between sensitive and resistant cells. Differences observed between sensitive and resistant cells will provide basis for further analyses and most relevant findings are going to be verified Western-blot measurements.

Herein I would like to thank the European Association for Cancer Research for the travel fellowship that gave me the opportunity to acquire not only new laboratory skills such as performing live content imaging, scratch assay, in vivo staining and transducing of cells but the possibility to learn data management from high throughput investigation methods such as kinase arrays or microarrays, and to top it all I could attend the Nordic Melanoma Meeting in Oslo where I have met leading experts of the field. I would also like to thank Prof. Øystein Fosdadt and members of his department, especially Dr. Karianne Riesberg for the excellent support throughout the project and for their kind hospitality during my stay in Oslo.

Deborah Hill
The Institute of Cancer Research, UK
Host Institute: University of California, San Francisco (UCSF), USA
Dates of visit: October 1 2011 - March 31 2012

Dynamic Nuclear Polarisation (DNP) is a technique that has revolutionised 13C Magnetic Resonance Spectroscopy (MRS) by generating over ten thousand times signal enhancement to 13C spectra, thus allowing the measurement of real-time metabolic flux both in vitro and in vivo. My PhD project focuses on the application of hyperpolarised 13C MRS as a tool to develop early biomarkers of treatment response in cancer by characterising metabolic processes. I am particularly interested in understanding the kinetic properties of enzymes involved in cancer energy metabolism and how we can exploit them as targets for determining response to therapy.

"It is incredibly exciting to be contributing to such a quickly progressing field."

To date, my research has focused on investigating the properties of the enzyme lactate dehydrogenase (LDH), the expression of which is often upregulated in cancers. LDH mediates pyruvate-lactate exchange and is a key enzyme involved in the cellular energy metabolism. By hyperpolarising 13C-labelled pyruvate, the apparent rate of the enzyme can be measured both in vitro and in vivo via MRS, and changes in this rate have been used as early markers of treatment response.

I am incredibly grateful to the EACR for awarding me a travel fellowship, which helped facilitate a highly productive six months with Professor John Kuraniewicz's group at the University of California, San Francisco, who are pioneering research in the field of hyperpolarised MR. My research project at UCSF was predominantly focused on developing a platform to interrogate LDH enzyme kinetics in a controlled manner using hyperpolarised 13C MRS and a bioreactor system. In doing this I learned the technique of encapsulating isolated LDH enzyme within alginate microspheres. The microspheres mimic a cellular environment, whilst removing compounding factors to the enzyme kinetics such as metabolite transport into cells, and allowing control over other parameters, such as enzyme cofactor concentrations. My task was then to characterise the LDH microspheres for LDH leakage, reproducibility of size and shape, and finally to determine the reproducibility of hyperpolarised 13C pyruvate kinetics. The ultimate goal of this project is to model a cellular environment under controlled conditions by matching concentrations of intracellular metabolites to probe cellular properties, such as the effect of membrane transport. All of the hyperpolarised experiments were carried out using an MR-compatible bioreactor system, which allows continuous perfusion of the microspheres and mimics an in vivo environment. My home institution is interested in developing a bioreactor system, and so learning what a powerful piece of equipment it is, as well as how to use it, is incredibly valuable. The work that I conducted in UCSF on the microsphere/bioreactor system was accepted for a presentation at the 20th annual meeting of the International Society for Magnetic Resonance in Medicine, and received the Magna Cum Laude award for merit. Although my time at UCSF has come to an end, the model I developed will continue to be expanded upon collaboratively between our institutions.

In addition to being welcomed into such an exciting research group, I was also encouraged to take part in many of the opportunities that UCSF has to offer. I was invited to attend a student Bioengineering conference along side the University of Berkeley at beautiful Lake Tahoe, where I learned about much of the diverse and exciting research that is carried out across the campuses. Another highlight of my stay was witnessing the completion of the first phase I clinical trial of hyperpolarised pyruvate in prostate cancer, led by the UCSF Department of Radiology. It is incredibly exciting to be contributing to such a quickly progressing field; the remarkable results obtained from pre-clinical studies and the fact that similar results have been yielded from the clinical trial, pose great possibilities for the future of DNP and its application to the clinic.

Overall, the project was immensely beneficial both for my home and host institutions, and for myself. I learned a huge amount, much of which I will implement in further studies that I will carry out at the ICR. This project wouldn’t have been possible without the financial assistance from the EACR travel fellowship, which I am extremely grateful to have been awarded.

Tamás Garay
Deborah Hill
Deborah using the bioreactor system
Cum Laude award for merit.
Molecular mechanisms of recombiningonic activity induced by chemical compounds: PARP response on DNA damage caused by minor groove binding ligands

Kirill Kirsanov
Institute of Cartogenezis, Blohkin Cancer Research Center RAMS, Moscow, Russia
Host institute: Fox Chase Cancer Center, Philadelphia, PA, USA
Dates of visit: February 1 - April 30 2012

Molecular mechanisms of recombinogenic activity induced by chemical compounds: PARP response on DNA damage caused by minor groove binding ligands

Efficient and accurate repair of DNA double-strand breaks (DSB) is crucial for cell survival. Homologous recombination (HR) provides an accurate repair of DSB. However, when non-sister chromatids are used as templates, HR may lead to a Loss of Heterozygosity (LOH). Causing manifestation of recessive mutant alleles accumulated during lifetime, it represents frequent DNA rearrangements in cancer. Moreover it has been shown that LOH is the main cause for inherited retinoblastoma and cancer in patients with Li-Fraumeni syndromes.

Recently we revealed for the first time a group of chemical compounds, which induce LOH at high frequency whilst point mutations and chromosome deletions at the insignificant level. These compounds are minor groove binding ligands (MGBLs), in particular bisbenzimidazoles Hoechst 33342, Hoechst 33258 and their derivatives. Evaluation of their genotoxicity is complex due to the influence of MGBLs on PARP activity in vitro. In vivo and in vitro.

The first series of experiments was devoted to investigation of changes in PARP activity after MGBL treatment in vivo. By means of Western Blot we found that after 48 hrs treatment PARP was excluded from the nuclear fraction in MMTV-PyMT primary tumours. Furthermore, the activity of PARP was decreased in comparison with untreated ones. These data demonstrated that MGBLs inhibited PARP1 in vivo.

At the next step using confocal microscopy we compared PARP localisation in salivary gland polytene chromosomes in treated and untreated larvae. We demonstrated that after MGBL 48 hours treatment PARP was excluded from the regions of heterochromatin. Previously Alexei Tulin demonstrated that PARP protein is required for silencing of heterochromatic repetitive sequences. Also it is known that mammalian PARP1 binds specific sequence GGTTGG, which is evolutionary conserved flanking sequence for many retrotransposons elements. We proposed that Hoechst bound this sequence and abolished targeting PARP to heterochromatin and this led to the desilencing of retrotransposable elements. Moreover, using ChIP we found that MGBL treatment resulted in diminished PARP1 binding to retrotransposable elements. The third series of experiments was performed to study the influence of MGBLs on PARP activity in vitro. In this study we revealed that Hoechst inhibited PARP activity in dose-dependent manner.

Our findings demonstrate PARP involvement in cell response on MGBL genotoxic treatment and it opens new directions for our further investigations.

I would like to thank EACR for the wonderful opportunity to perform this project in the Laboratory of Prof. Tulin. Besides having mastered new laboratory technique, I thoroughly enjoyed many inspiring discussions with everyone in the Prof. Tulin Laboratory. These discussions about science in general and MGBL role in cancer in particular gave me a strong basis for my future career. I would really like to thank Prof. Tulin and all the members of his team for welcoming me, for their help and guidance.

Yaiza del Pozo Martin
Dates of visit: April 29 - May 9, 2012.

Searching for a self-renewal signature in metastatic breast CSCs

Tumours resemble normal organs in that only a small fraction of cells, termed Cancer Stem Cells (CSCs), retain the potential of sustaining long-term growth. According to this idea, similar to the growth in normal proliferating tissues, tumour growth is driven by a limited number of stem cells that are capable of self-renewal. CSCs are functionally defined in vivo by their capacity to initiate primary tumours and metastasis upon transplantation. Metastatic development in distant organs is the major cause of cancer mortality. Therefore, it is essential to determine which pathways are responsible for the CSCs phenotype in order to develop treatments targeting specifically this population.

We have identified several key pathways underlying CSCs maintenance. Using the MMTV-PyMT breast cancer mouse model that metastasises to the lungs, active signalling concentrates in the CSC population, enabling them to successfully metastasise. Also, we know that when using spheres cultures, a stem cell expansion assay, these pathways are active (indicated by reporter activity) in such CSC-enriched cultures.

Therefore, one of the questions of my PhD project is addressing the role of these pathways in defining the cancer stem cell phenotype. Particularly, we aim to examine its role in CSC self-renewal, a key mechanism defining CSCs functionality. To this end, CHIP-Seq (chromatin immunoprecipitation paired with sequencing) for the main nuclear factors of these pathways is being performed in MMTV-PyMT primary tumour cultures cultured as spheres that allow the CSCs to grow, self-renew and propagate indefinitely. The experimental setting consists of comparing the target genes in CSC-enriched sphere conditions and adherent cultures containing CSCs committed to differentiation. As a result, we aim to identify a specific signature responsible for CSC self-renewal.

Huelsken’s lab has a broad experience in the cancer stem cell field and in performing the CHIP-Seq technique on stem cells in vivo and in vitro. Therefore, the visit enabled me to learn this multi-stage elaborated technique that I will set up in the Tumour-Host Interaction Laboratory, and meant a great step forward in my PhD project. Specifically, during my visit to Huelsken’s lab, I had the chance to work with Dr. Anja Irmisch, the postdoc who has established this specific technique in Huelsken’s lab. She helped me to role out the appropriate conditions for CHIP in MMTV-PyMT primary cells and to collect the first round of immunoprecipitated chromatin for sequencing.

Moreover, receiving the EACR Travel Fellowship allowed me to meet Huelsken’s lab members, and facilitated the ongoing collaboration between the two laboratories. Undoubtedly the visit was a highly enriching experience that provided me with new scientific skills that will be of great value for the rest of my scientific career. I am truly grateful to EACR for supporting the development of my PhD project with this fellowship. Finally, I would like to thank Professor Huelsken for hosting me in his laboratory and providing thoughtful discussion on the experimental results, and the rest of the members of the Cancer Stem Cell Laboratory, especially Dr. Anja Irmisch for helping with the design of the experimental approach and providing invaluable scientific input into this project.
Danielle Park  
Lowy Cancer Research Centre, Sydney, Australia  
Host institute: the Molecular Imaging Laboratory Leiden (MILL), The Netherlands  
Dates of visit: February - May 2012  
Tumour cell death is the primary aim of most chemotherapeutics, anti-hormonal agents and radiotherapies. Despite this there are currently no specific measures of cell death in vivo to assess tumour responsiveness and guide the treatment programme.

We have synthesised an organoarsenical, GSAO, that rapidly concentrates in dead and dying tumour cells. Upon fluorescent or radioactive labelling, GSAO serves as a novel and effective imager of tumour cell apoptosis and necrosis.

Labelled GSAO could determine a tumour’s response to therapy within hours of the initial treatment, enabling physicians to customise the treatment programme to the individual. Time wasted on ineffectual therapies would be minimised, not only improving the efficiency of the treatment programme, but also sparing the patient unnecessary side effects.

We recently elucidated the mechanism of action of our cell death imaging agent. We have shown that the probe enters the cell during the late stages of cell death when the plasma membrane integrity is compromised. It is then retained in the cytosol by interacting with cysteine thiols in Hsp90.

Hsp90 is the most abundant chaperone of the eukaryotic cytoplasm and plays a central role mediating the folding, stabilization, activation, and assembly of various client proteins. Notably, tumour cells are highly dependent on Hsp90, which assists in the folding/stabilisation of mutant or over-expressed oncoproteins. The abundance of this cytosolic target lends to pooling of the imaging agent in dying tumour cells and thus superior detection and resolution of cell death.

The agent is likely to have many applications in patient management and in various experimental settings, however if it is to progress to the clinic we must now demonstrate the in vivo utility of the probe. It was the aim of the EACR Travel Fellowship to conduct these studies in collaboration with Professor Lowik at the Leiden University Medical Centre, Netherlands.

A near infra-red conjugate of GSAO was trialed in a mouse model of breast cancer subjected to chemotherapy. Nude mice bearing orthotopic 4T1 breast cancers were treated with cyclophosphamide and their response monitored non-invasively using our cell death probe and small animal fluorescence imaging.

Professor Lowik’s laboratory has extensive experience with this model and is ideally situated to carry out these studies. Professor Lowik is the President of the European Society for Molecular Imaging and heads the Molecular Imaging Laboratory in Leiden (MILL), The Netherlands. The facility houses state-of-the art small animal optical, CT and MR imaging equipment including the Pearl Impulse Infrared small animal imager, Maestro CRI, IVIS-100, IVIS Spectrum, IVIS-3D system, and MX3 life-time time domain imager. It is one of the few centres in the world to offer such a comprehensive and integrated approach to small animal imaging, and allowed us to explore multiple facets of tumour cell death simultaneously.

The particular mouse model used in Professor Lowik’s laboratory also offered a number of advantages. Firstly, introduction of the tumour into the mammary fat pad ensured that the tumour microenvironment is accurately represented. This is particularly important in the context of imaging studies as GSAO is administered intravenously, and variation in tumour vascularity affects dissemination of the probe. Secondly, orthotopic inoculation also results in the spontaneous generation of lung, liver, bone and brain metastasis, mimicking progression of the disease in humans. The model therefore offered a clinically relevant system in which to test our agent.

We were able to successfully demonstrate application of near infra-red GSAO to this model of tumour cell death. Tumours treated with cyclophosphamide had a significantly greater uptake of tagged GSAO than untreated tumours. This places us in an ideal position to pursue the application of radiolabelled GSAO in vivo and advance the agent to the clinic.

I would like to thank the EACR for the opportunity to travel to The Netherlands to perform this work. The visit allowed me to develop a range of skills in whole body fluorescence imaging and has facilitated ongoing collaboration between our two labs. It was also exceptionally personally rewarding to live and work in a foreign country and experience such a unique culture. I would also like to thank the excellent team under Professor Lowik who devoted their time and energy the project, Bangwen Xie, Vicky Blankervoort, Ermond Van Beek, Eric Kajziel, Alan Chan and Ivo Que. It was a pleasure to work with such a wonderful group of scientists.

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Dissecting the functional role of putative breast cancer risk SNPs in normal breast and breast carcinomas

I am a post-doctoral fellow of the Norwegian Cancer Society in the Cancer Genome Variation group at the department of Genetics, Institute for Cancer Research at Oslo University Hospital, Norway. I, i.e. my family of four, spent two months in Amsterdam where I worked in Professor Lodewyk Wessels’ group at the Bioinformatics and Statistics Department, the Netherlands Cancer Institute.

Breast cancer (BC) in general is a complex and heterogeneous disease where gene expression phenotypes can serve as a proxy for tumour classification, dividing the carcinomas into five groups with different clinical outcomes. Recent Genome Wide Association Studies (GWAS) have revealed common variants as risk factors for developing sporadic breast cancer. We have reported genes harbouring these variants to be differentially expressed between BC subtypes, and correlated them in cis to germline genetic variation, suggesting that the observed genes are associated with different breast cancer subtypes, and may exert their effect through their expression in the tumours.

“Several new, international collaborations have been established based on the work done”

To generate a more complete picture of breast cancer as a heterogeneous disease, it is vital to understand the genetics behind the process a healthy individual undergoes during the development of breast cancer. To address this aim, we generated both genotype and expression data on approximately 200,000 SNPs, referred to as the iCOGS breast cancer data set, offering a unique opportunity for comparison.

A stay abroad in the group of Lodewyk Wessels, a leading Professor in the research field of statistics and Informatics, provided me not only with the means to combine our data sets, but also access to the TCGA breast cancer data set, offering a unique opportunity for comparison.

Several new, international collaborations have been established based on the work done while in Lodewyk Wessels’ group in Amsterdam, with multiple papers in the pipeline. Further, the results obtained from our analyses will be strong candidates for further functional analysis and verification, with the goal of developing new genetic markers for breast cancer risk. I sincerely hope that this study will contribute to that matter, and this knowledge will hopefully benefit breast cancer patients of the future.

Not only did this stay provide insight into genetic variants influencing gene expression in breast carcinomas, but it also gave me personally a unique opportunity to broaden my horizons, an occasion to gain experience in analytical techniques and to strengthen international relationships. Privately, it was a fantastic opportunity for me and family to experience something new together. I am very grateful to the EACR for this Travel Fellowship, and would like to thank the committee for supporting our project! I also want to express my gratitude to Professor Wessels for admitting me into his group, and to him and Sander Canisius for spending so much of their time working with me, and the fun we had!