EACR Travel Fellowship Awards

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.

Alexander Henke

Medical Research Council, Centre for Reproductive Health with the University of Edinburgh, Edinburgh, Scotland, UK

Host Institute: Prof. Simon Hayward, Vanderbilt University, Medical Center, Department of Urology, Nashville, Tennessee, USA

Dates of visit: 12th - 23rd September 2011

Inhibiting the activity of cancer-associated fibroblasts in vitro and in vivo

Prostate cancer as well as other solid cancers are embedded in and supported by the surrounding tumour microenvironment, which usually consists of fibroblasts, smooth muscle cells, endothelial cells and immune cells. In the prostate, fibroblasts and smooth muscle cells represent the bulk of the stroma in both normal and malignant conditions. In carcinomas, there are also pro-tumourigenic cancer-associated fibroblasts (CAF, aka tumour-associated fibroblasts), which secrete growth factors and cytokines as well as enzymes that remodel the extracellular matrix. In addition, they can potentially become mobile and create paths in the extracellular matrix (ECM), which could be used for epithelial cells for invasion. Therefore CAF represent a clinically important target across a range of malignancies but also in fibrotic diseases as well.

The aim of my project is to identify CAF inhibitors that could potentially blunt the activity of activated fibroblasts and hence tumour support.

In vitro model: preparation of fibroblasts from tissue of prostate cancer patients, who underwent transurethral resection of the prostate (TURP). The cultivated primary fibroblasts were used to build a bank of 20 CAF lines across the high end of the spectrum of Gleason Scores 6 to 10.

After having tested numerous compounds in a 3D in vitro assay, I identified a few compounds that inhibited CAF activity as well as some that enhanced it. Since the enzyme lysyl oxidase (LOX) and its family members are well known to be crucial for pro-tumourigenic effects in the tumour microenvironment, I used a LOX inhibitor as a positive control for inhibitory effects on CAF in my assay.

I wished to test the most promising candidate compound (low cytotoxicity, IC50 in nM range) in vivo as well. A derivative of the compound with high in vivo stability was kindly provided by the pharmaceutical company Daiichi Sankyo, Tokyo, Japan, who originally developed this compound for a different purpose than CAF inhibition.

“"The EACR Travel Fellowship is a great programme to support young researchers in the progression of both their science and their careers."

Simon Hayward, a world expert in urological xenograft animal models, kindly agreed to collaborate on this project. He co-developed an animal model, in which immortalised and initiated but non-malignant prostatic epithelial cells (BPH1) are mixed with primary fibroblasts in a collagen matrix and then transplanted under the kidney capsule of severe combined immunodeficiency (SCID) mice. Compared with other mouse models, it takes a relatively long time to form prostate cancers in this mouse model: 3 months. This also reflects the decade-long time required of clinically detectable prostate cancers in humans. Only when both CAF and epithelial cells are transplanted together do tumours form but not when BPH1 cells or fibroblasts are transplanted on their own.

Since the kidney is an extremely well vascularised organ, there is an instant supply of oxygen and nutrients so that, on the one hand, grafts have an extremely good implantation rate and, on the other hand, tumour growth is independent of angiogenic influences and aspects in this model, so that effects of
tumour growth and invasion can be traced to the influence of CAF.

My aim was to learn how to make the grafts and how to do the procedure in this mouse model, so that I could establish it back in Edinburgh.

As a preparation and prerequisite, I sent frozen aliquots of CAF cells from my cell bank to Simon and Omar for grafting experiments. Furthermore, I studied for and passed online exams on animal handling & laws and received local IACUC approval to allow me to work with animals. Once I arrived in the lab, I was very warmly and wholeheartedly welcomed by all lab members. Omar Franco (MD, PHD) kindly taught me how to do the graft preparation in vitro and then the subsequent surgery. He taught me quickly and so well that in my second week I could actively contribute to ongoing surgeries and perform it myself as well. In addition, I learned further dissection skills and some histological techniques.

Apart from the lab work, I had the privilege of great scientific discussions with all members of the lab as well as with other scientists on the campus, which enhanced my general knowledge in the field.

The collaboration will definitely enhance the quality of my project and its results will be part of the manuscript - once the project is completed and all data evaluated.

The lab visit not only broadened my repertoire of skills but also helped me to establish connections and deepen existing collaborations. Therefore I would sincerely say a huge “thank you very much” to Simon, Omar and the other lab members, and of course the EACR for providing this fantastic opportunity.

I feel honoured to have been chosen for a travel fellowship and of course will acknowledge EACR funding in the manuscript. The EACR travel fellowship is a great programme to support young researchers in the progression of both their science and their careers.

Mijiana Tanic
Human Cancer Genetics Group, CNIO, Madrid, Spain
Host Institute: Institute for Cancer Genetics, HICCC, Columbia University, New York, NY, USA
Dates of visit: 1st June - 31st August 2011

Breast cancer is one of the most common causes of death in women. Despite extensive research in the field, the exact causes of breast cancer are not fully understood. Hereditary breast cancer comprises 5-10% of all breast cancers. Germline mutations in two high susceptibility genes BRCA1 and BRCA2, along with mutations in other susceptibility genes can account for only up to 30% of familial cases. Thus, the large majority of breast cancer cases that arise in families with strong familial aggregation are not explained by mutations in any known breast cancer susceptibility gene, and are designated as BRCAX-type tumours. The identification of novel susceptibility genes responsible for hereditary breast cancer is of great interest. Likewise, new markers for diagnosis and prognosis could be of great benefit for better classification and management of breast cancer patients.

As a 3rd year PhD student in the laboratory of Dr. Javier Benitez at Spanish National Cancer Research Centre (CNIO) in Spain, I am studying the role of miRNAs in hereditary breast tumours using high-throughput genetic approaches, as well as basic cell biology and genetics.

By miRNA microarray expression profiling in a large cohort of hereditary breast tumours, we have identified miRNAs deregulated in hereditary breast tumours compared to normal breast tissue, and we have established miRNA signatures associated with specific subtypes, BRCA1-mutated, BRCA2-mutated and BRCAX that could provide us with better diagnostic and prognostic markers and could represent novel targets for therapy. Given that BRCA1 gene deregulation has a central role in the pathogenesis of a subset of both hereditary and sporadic tumours, we are interested in identifying miRNAs that regulate this important gene, and how they are expressed in breast tumours. This knowledge could provide us with new insights into the biology of breast cancer, and could potentially represent a novel mechanism of susceptibility to breast cancer.

Thanks to the EACR Travel Fellowship I was able to spend 3 months in the laboratory of our
collaborator Dr. Jose Silva in the Institute for Cancer Genetics at Columbia University in New York, USA. The work performed by Dr. Silva’s lab involves the use of high-throughput genetic screens using shRNA/miRNA viral libraries.

“The stay enabled me to learn invaluable techniques and greatly facilitated the ongoing collaboration between our groups.”

The aim of the study was to experimentally identify all miRNAs that are regulating BRCA1 gene expression by performing an unbiased high-throughput screen using a lentiviral miRNA library representing more than 600 human miRNAs.

During my three month stay I have constructed retroviral reporters and I have established stable reporter cell lines expressing GFP-labeled CDS and whole 3’UTR of the BRCA1 gene. Reporter cell lines were sorted by FACS for a narrow range of GFP expression and subsequently infected with a lentiviral pool of miRNAs. The cell population with reduced GFP expression will be selected by FACS and miRNAs infected will be identified by sequencing. For validated miRNAs we will perform one-by-one functional studies to examine the effects of these miRNAs. Furthermore, we will analyse the correlation of already available miRNA expression microarray data and BRCA1 expression in hereditary breast tumours.

Finally, I would like to thank EACR for their continuous support to young investigators! It has been a great pleasure to work with Jose Silva and his group in Columbia University. The stay enabled me to learn invaluable techniques and high-throughput genetic screening methodology which I will implement at my home institute, and greatly facilitated the ongoing collaboration between our groups.

Federica Cappuccini

Institute of Cell Biology (Cancer Research), University Hospital Essen, Essen, Germany

Host Institution: Centre for Cancer and Inflammation, Barts and The London School of Medicine and Dentistry (Queen Mary, University of London), London, United Kingdom

Dates of visit: May-June 2011, October-November 2011

Molecular mechanisms of radiation-induced normal tissue damage

My PhD project focuses on pneumonitis and fibrosis as side effects of thorax or total body irradiation. Lung sensitivity to ionizing radiation constitutes a dose-limiting factor when radiotherapy is used to treat cancer patients. In vivo investigations suggest that inflammatory responses of the lung tissue involve a complex set of interactions between different resident cell populations, the extracellular matrix and infiltrating immune cells. As observed for other fibrotic diseases, an impaired balance between inflammation and repair may be causative for extensive remodelling processes and finally fibrosis. However, it is still controversial whether the inflammatory reaction is a prerequisite for progression to lung fibrosis. Considering that the network of pathologic events leading to tissue inflammation and fibrosis is not completely understood, it is important to characterise changes in the behaviour of resident cells and immune cells in the lung. The use of genetically modified mouse strains helps to analyse the role of signalling molecules suggested to be involved in these processes, such as the purinergic CD73/adenosine system. An improved comprehension of the mechanisms linking the initial tissue injury, inflammatory response, and fibrosis development would be a prerequisite for developing effective radioprotective strategies.

For this purpose I use a murine model of radiation-induced pneumopathy. C57BL/6 or specific genetically modified mice (e.g. CD73−/−) receive 15 Gray (Gy) of thoracic irradiation in a single dose. I collected lung tissue over 30 weeks after irradiation to cover all the critical phases of radiation response. Interestingly, as an early result, I was able to show radiation-induced changes in the morphology of resident cells; in particular, irradiation triggered a time-dependent formation of lipid-loaded macrophages. This observation is reminiscent of inflammation-associated changes in macrophages during atherosclerotic processes.

Dr. Thorsten Hagemann is a specialist in haematology and oncology and greatly experienced on macrophages phenotyping, particularly on tumour associated macrophages. Therefore, the purpose of my visit in his lab was to shed light on the role of the
alveolar macrophages in irradiated lungs, and to analyse whether an altered polarisation of these cells could influence the development of fibrosis in our mouse model. In the first visit I was able to generate tissue microarray from my complete collection of paraffin-embedded lung tissues and to optimise immunofluorescence staining for the most prominent markers of macrophage activation. This enabled me to be introduced to confocal microscopy. In addition, the flexibility of this cooperation gave me the opportunity to subdivide my visit in two turns, allowing me to irradiate new groups of mice in Essen. Thus, during the second visit, I investigated macrophages via flow cytometry and cell sorting from freshly isolated lung tissue, from 3 different time points after irradiation. The sorting strategy was crucial to separate alveolar macrophages from infiltrating macrophages, and RNA purified from these different populations will give me the possibility to describe the pattern of differential gene expression over time as a consequence of radiation response.

Thanks to this stimulating experience, I made an important breakthrough in my PhD project. Moreover, I deepened my general knowledge on macrophages and acquired new experience on macrophages activation. At the same time, the possibility to learn new techniques, such as the preparation and in vitro polarisation of mouse bone marrow derived macrophages, allows me to introduce new methods in my lab and to share them with my colleagues.

I am sincerely grateful to EACR for supporting me with this fellowship. The effort and care to promote young scientists’ research and fruitful collaborations is remarkable. My special thanks go to Dr. Hagemann, to all the members of C&I and to the Flow Cytometry lab for the excellent support and for the extremely inspiring and friendly atmosphere; the constant availability to dialogue and the open exchange of ideas was totally impressive. They all made my stay there absolutely unforgettable.

Judit Berta

Department of Experimental Pharmacology, National Institute of Oncology, Budapest, Hungary

Host Institute: Institute of Cancer Research, Medical University of Vienna, Vienna, Austria

Dates of visit: 1st April-31st May; 1st July-31st August 2011

Apelin, initially isolated from bovine stomach homogenates, has been recognised as the endogenous ligand of the human orphan G-protein-coupled receptor APJ, a member of the seven-transmembrane-receptor family. Evidence has accumulated during the past few years that APJ is expressed by various cells/tissues. An increasing number of studies show, for example, that APJ signalling plays an important role in blood capillary formation. Our group showed for the first time the presence and important prognostic role of apelin as an angiogenic factor that increases the aggressive behaviour of human non-small cell lung cancer. Interestingly, however, no studies have addressed apelin’s effects on lymphangiogenesis. The lymphatic system participates in the pathogenesis of several diseases of direct and indirect lymphatic dysfunction. However, contrary to angiogenesis, lymphangiogenesis and its role in the pathogenesis of these diseases has only recently become a focal point of biomedical research. Therefore, our understanding of lymphangiogenesis is still rather limited. Therefore, we planned to clarify the exact role of apelin in the lymphatic system.

During my visit to the Institute of Cancer Research in Vienna we have studied in vitro models of lymphangiogenesis and analysed APJ signalling in lymphatic endothelium. We used human skin derived lymphatic endothelial cells. The cells were separated by magnetic sorting with an anti-podoplanin serum. We found that lymphatic endothelial cells expressed APJ both at the mRNA and protein levels. Exogenous apelin-13 stimulated the cell motility. However, the APJ receptor antagonist, [Gln,Ala]-apelin-13 did not influence the migration significantly. Apelin also stimulated the Erk and PI3-K/Akt signal transduction pathways in the lymphatic endothelial cells. Based on our results we think that apelin probably also has lymphangiogenic potential.

I would like to thank the EACR for the award of a travel fellowship, which has allowed me to establish a bilateral Austrian-Hungarian research project on the apelin molecule. This award has also developed my own career in basic/translational lymphangiogenic research. Finally I wish to thank Prof. Walter Berger for inviting me to spend time working in his team.

Reference

Zita Carvalho Santos

Instituto Gulbenkian de Ciência, Oeiras, Portugal


I am currently a Post-Doc in the Laboratory of Cell Cycle Regulation in the Instituto Gulbenkian de Ciências, in Oeiras Portugal, headed by Dr Mónica Bettencourt-Dias. My work focuses on the study of the evolution and biogenesis of centrioles that compose the major microtubule organiser in the cell, and cilia/flagella, structures that protrude from the cell and work both as antennae and as propelling cells during movement. These structures have been shown to be aberrant in a variety of human diseases such as cancer or male infertility. EACR co-funded my attendance in the Marine Biology Summer Course "Physiology: Modern Biology Using Microscopic, Biochemical and Computational approaches" which was held at the MBL in Woods Hole, Massachusetts, from June 11 - July 31, 2011.

Every day during the whole course, except on Sundays, our day would start with a lecture at 9 am. These lectures were always exceptional with really outstanding scientists and they were mostly directed to cell biology and modelling. At 10:30 am, we would have the chance to discuss with the seminar speaker and ask additional questions. These questions were both scientific and personal which gave us the opportunity to also discuss themes such as scientific career, grant writing, scientific ethics, etc. The first week was devoted to bringing students up to speed in different techniques and approaches. We had 2 day theoretical and practical sessions in microscopy, biochemistry, and programming (primarily Matlab). During the biochemistry session we purified kinesin from the optical lobes of squid and then used it in a microtubule assay – it was really fun!

After the first week we started a 6 week period, in which every two weeks I worked with a different faculty member and a teaching assistant (advanced graduate student or postdoctoral fellow from that laboratory). During that time, I worked in teams of Biologists, Mathematicians or Physicists on an original research question. The projects were always discussed with the faculty and often we were given the opportunity to address our own questions which I found to be really exciting! Often, the TAs had prepared many of the critical reagents (e.g. clones, protein preparations, cell lines) so we only had to do the fun part: the experiments! Usually after the morning seminar we would sit down with our faculty and students to get oriented for the day’s research activities. During the remainder of the day, we worked with faculty and TAs on experiments. After dinner, it was back to the laboratory! The lab work was always really intense and sometimes we would have to work until really late hours. During the rotations we also always had the opportunity to spend some time with the faculty members in a more relaxed environment, such as in barbecues, softball practice, in 4th of July preparations, etc. At the end of the two weeks we had a big symposium in which every group would present the work developed during the rotation.

This course greatly advanced my skills on imaging, development of biochemical assays and computational biology. Those skills are crucial to further my research project as I need to carefully quantify processes and to be able to model them. Moreover, these techniques were developed in the context of really exciting research projects, such as the study of cell symmetry in daughter cells after cell division, the study of the microtubule GTP cap and microtubule dynamics, and finally the characterisation of the recently described actin like filament AlfA in bacteria. In this research I was supervised first by Wallace Marshall (UCSF), then by Joe Howard (Max Planck Institute), and by Dyche Mullins (UCSF), each one in a two week module. These are all outstanding researchers and in consequence I learnt a lot about how to do science. Additionally, since the course brings together students and faculty from different disciplines and many leaders in the field of cell biology, I had the opportunity to do truly interdisciplinary research and to direct me towards future labs for my Post-Doc. This course has already had an impact on my research since I have applied both programming skills and microtubule in vitro techniques on experiments that are ongoing in the lab. Not less important, this course helped me in being more confident as a scientist, showing me that I can easily start projects in new areas and work with different people. I am truly thankful to EACR for sponsoring me and for having given me the opportunity to take this course.
Selective activation of glucocorticoid receptor transrepression as a new approach to targeted therapies for haematological malignancies

Haematological malignancies are the most common form of childhood cancer, accounting for almost 50% of all cancer diagnoses. Every year more than one hundred children, the patients of the Department of Children Oncology and Haematology of the Blokhin Cancer Research Centre RAMS, are treated by chemotherapy usually combined with glucocorticoids (GCs), causing undesirable side effects, in particular metabolic imbalance, osteoporosis, diabetes and Cushing syndrome. Standard GCs manifest their biological effects through the glucocorticoid receptor (GR), a well known transcription factor, which can regulate gene expression positively (transactivation) or negatively (transrepression). Therapeutic effect of GCs is mainly mediated via GR transrepression: negative interaction between GR and other transcription factors. Undesirable side effects occur mainly via GR transactivation mechanism in DNA-dependent manner.

As a PhD student of Blokhin Cancer Research Center, I have developed a keen interest in studying new glucocorticoid analogues with reduced side effects. Selective GR activators (SEGRA) that shift GR functions towards transrepression and have improved therapeutic index, hold a great potential for the blood cancer chemotherapy. Anticancer effect of GR ligand of this type, 2-(4-acetoxyphenyl)-2-chloro-N-methylammonium chloride, also called CpdA, was recently characterised in the Laboratory of Dr. Irina Budunova on prostate cancer cells. It was shown that CpdA prevents GR transactivation but activates selectively transrepression mechanism.

“This opportunity strengthened my PhD thesis and gave me a strong basis for my future career.”

The goal of this study was to analyse CpdA effects on blood cancer cells in comparison with GCs, and to find out whether it possesses the ability to activate selectively GR transactivation, but does not affect transactivation. As a model system of haematological malignancies we chose two leukaemia (K562 and CEM) and three lymphoma (NCEB, Granta, Jeko) cell lines. In this work we successfully used the whole panel of modern molecular biological techniques, and the main approach was the infection of the cells with lentiviral vectors. This method I was able to master through the collaboration with Dr. Budunova, who used it in her previous work and had all the necessary lentiviral vectors, equipment and area certified for the manipulations with viruses.

Our preliminary results revealed that the most pronounced response to GCs and CpdA treatment was shown for CEM (leukaemia) and NCEB (lymphoma) cells, and these effects correlated with the higher GR expression level. We obtained transgenic cell lines with stable luciferase expression under GRE-containing promoter and found that CpdA, unlike GCs, did not induce transactivation in these cells.

Effects of these compounds on transrepression potential were determined in cells infected with lentiviral luciferase vectors under the promoters of a number of GR-regulated TFs. GCs and CpdA in luciferase assay exerted a noteworthy similarity in the overall negative effect on the activity of NF-κB and AP-1 and their related genes.

We demonstrated GR-dependence of the effects observed on CEM and NCEB cells with down-regulation of GR by lentiviral infection with plasmid expressing shRNA to GR. GCs and CpdA revealed much less cytotoxic, proapoptotic and anti-proliferative effects on the cells with down-regulation of GR compared with the cells infected by empty vector. Also inhibition of GR expression led to significant reduction of both transrepression and transactivation.

As GCs are usually used for the treatment of blood cancers in combined chemotherapy with different anti-cancer drugs including proteasomal inhibitors, we investigated combined effects of CpdA with proteosomal inhibitor Bortezomib. We revealed that Bortezomib induced caspase-dependent apoptosis in both CEM and NCEB cells, and CpdA as well as GCs exerted potentiating effect on Bortezomib cytotoxic and proapoptotic action.

I would like to thank EACR for the unique opportunity to perform this project in the Laboratory of Dr. Irina Budunova. Besides having mastered new laboratory technique, I thoroughly enjoyed and learned from many inspiring discussions with everyone in the Budunova Laboratory. These discussions about science in general, GR role in cancer and my project strengthened my PhD thesis and gave me a strong basis for my future career. I would really like to thank Dr. Budunova and all the members of her team for welcoming me into their group, for their help, guidance and hospitality.
Serological markers of human papillomavirus (HPV) infection in Human Immunodeficiency Virus (HIV) positive and HIV negative Australian homosexual men

Different types of Human Papillomaviruses (HPV) cause the majority of anogenital warts, and anal cancer. However, little is known about HPV infections in homosexual men, a population at high risk of developing anal cancer.

Therefore, I started collaborating with Prof. Andrew Grulich from the University of New South Wales (UNSW) in Sydney, who initiated two large cohort studies among Human Immunodeficiency Virus (HIV) positive and HIV negative Australian homosexual men. Prior to my trip to Sydney, he sent me some 5,000 serum samples of these men to the German Cancer Research Center (DKFZ) in Heidelberg, where I generated HPV serological data for these cohorts. During my stay at UNSW, we jointly analysed the data.

The collaboration was very fruitful, combining my virological and serological knowledge with Andrew’s epidemiological and statistical expertise. We just submitted four abstracts to the upcoming International HPV Meeting, describing prevalence, incidence and risk factors for HPV seropositivity in HIV positive and HIV negative homosexual men and its relation to anogenital warts. I personally profited from expanding my knowledge about epidemiological methods and data analysis, giving me also the chance to write one or more first author publications in this research discipline. This will also help me in bridging these two scientific disciplines and to sharpen my profile as a molecular epidemiologist.

“I have had the chance to write first author publications in this research discipline.”
PTPN2, also known as TC-PTP, is an intracellular, non-transmembrane phosphatase which is most abundantly expressed in haematopoietic cells. In line with the expression pattern, PTPN2 knockout mice (PTPN2-/-) suffer from severe defects in haematopoiesis affecting lymphoid, myeloid and erythroid lineages and succumb within three to five weeks due to immunological malfunctions. Hence, PTPN2-/- mice display systemic inflammation along with increased levels of inflammatory cytokines IFNγ, TNFα, IL-12 and elevated nitric oxide production. The abnormal cytokine environment in PTPN2-/- mice is especially intriguing in light of the association of inflammatory cytokines and the development of autoimmune diseases.

Today, PTPN2 is established as an important modulator of growth factor-and cytokine-induced protein tyrosine kinase-mediated signalling pathways and mitogenic signalling. Several direct substrates of PTPN2 have been identified including different members of the JAK/STAT signalling pathway and several receptor protein tyrosine kinases (4). Most of the known PTPN2 substrates have been identified under normal physiological conditions using small-scale substrate-trapping techniques (5). However, both in vivo and in vitro substrate trapping approaches face certain limitations. In addition, those experiments have been exclusively carried out in cell lines that can be easily transfected such as COS7 or HEK293T cells, which are derived from kidney cells and thus not represent the T-cell context that we were interested to study upon our finding that PTPN2 plays a role in the pathogenesis of T-ALL.

Thanks to an EACR Travel Fellowship I was a visiting doctoral researcher in the Department of Biochemistry and Molecular Biology of the University of Southern Denmark, in the lab of Prof. Blagoy Blagoev. The overall goal of the collaborative project was to identify novel substrates of PTPN2 in leukemic cells through generation of large-scale profiles of the phosphotyrosine proteome using SILAC-based mass spectrometry. Prof. Blagoev is an expert in the field of quantitative phospho-proteomics and the department possesses state-of-the-art equipment and a solid bioinformatics platform, which made it the ideal place for the initiation of our intended study.

The main purpose of my visit to Odense was to acquire all necessary skills needed to set up the entire procedure at the K.U. Leuven and to perform experiments independently to the point of LS-MS/MS analysis. Within those weeks not only have I been well trained on all techniques by many helpful people, but we have also been successful in generating and analysing the first large scale data sets.

Next to the academic benefit, the opportunity to spend a period of time in a different research environment and interact with diverse people of various levels was an extremely valuable personal experience. I am deeply grateful to Prof. Blagoev and all members of his group and the Department of Biochemistry and Molecular Biology who welcomed me in the lab and made my stay memorable. I am glad to have had the opportunity to initiate a promising and valuable collaboration between the host and guest lab.
The study of natural compounds for potential drug development is a very rapidly growing field worldwide and more than one third of all pharmaceutical agents in use today are of natural origin\(^1\). Protolichesterinic acid (PA), a natural compound isolated from an Icelandic lichen, is a potent inhibitor of 5- and 12 lipoxygenase and has shown an anti-proliferative and pro-apoptotic effects on several types of cancer cells\(^2,3,4\).

The aim of this part of my PhD study was to investigate if protolichesterinic acid shows anti-proliferative effects on other types of cancer cells than already tested, and also if the effects could be mediated through the PI3K signalling pathway, which mediates the functional responses involved in tumour progression. There is evidence that PI3K pathway can also be activated by 12(S)-HETE, a product of 12-lipoxygenase\(^5\).

In the laboratory of Prof. Suzanne Eccles I used the Sulforhodamine B clorimetric assay for cytotoxicity screening of PA on several cancer cell lines of different origins. Results indicate that PA has anti-proliferative effects on all tested cell lines at similar concentrations as seen before. It was of particular interest to take advantage of the high throughput microplate 3D functional assay developed at the Sutton laboratory and test PA in the 3D spheroid model. Results indicated a decrease in average diameter of the spheroids and a loss of viability, which would be very interesting to follow up and investigate further, especially the longtime effects of PA on the spheroids.

**“I will continue with this work and hopefully get to collaborate more in the future.”**

Using MSD biomarker detection assays it is possible to measure the total and phosphorylated levels of protein targets within a single small-volume sample. The method is based on MSD plates that have been pre-coated with captured antibodies, the sample and a solution containing the labeled detection antibody is added and a MSD SECTOR® Imager used for analysis. Inside the SECTOR® Imager, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. Three different cell lines previously tested by cytotoxicity screening in the laboratory were chosen for the MSD assay. The cells were treated with PA and incubated for different timepoints. The cells were lysed and added to different antibody coated MSD plates. Results indicated that the effects PA are more likely to be mediated through the ERK pathway rather than the Akt pathway after 24 hours of treatment, no effects were seen at earlier timepoints. I will continue with this work and hopefully get to collaborate more with Prof. Suzanne Eccles and her team in the future.

I would like to thank the European Association of Cancer Research for the fellowship and for giving me the opportunity to gain experience both in the scientific field and meeting new interesting people.

References


Mieke van Hemelrijck

Department of Cancer Epidemiology, King’s College London, UK

Host Institute: the Institute of Social and Preventive Medicine, Zurich

When I applied for a Travel Fellowship from the EACR, I wanted to improve my epidemiological knowledge by studying how nutritional components and genetic changes might influence prostate cancer biology. I had never conducted a study within the discipline of nutritional epidemiology and was very keen on learning about the extremely complex set of variables that define a person’s diet. I also wanted to learn about genetic epidemiology as this is a relatively new discipline that seeks to elucidate the role of genetic factors and their interaction with environmental factors in the occurrence of disease.

Therefore, I was very excited when I finally arrived in Switzerland at the end of February. For the next three months I was going to work at the Institute of Social and Preventive Medicine. Under the supervision of Professor Sabine Rohrmann I focused on gene-environment interactions, more specifically on the association between heterocyclic amines and the risk of prostate cancer.

A view of Zurich, where Mieke completed her epidemiological research

**Background**

A western diet has long been considered as a potential risk factor for prostate cancer. In the context of meat consumption, evidence is weak for an association between both red and processed meat intake and PCa risk. However, the intake of grilled meat is thought to be related to PCa risk since high-temperature cooking of meat leads to formation of mutagenic heterocyclic aromatic amines (HCA), which have been shown to induce tumours in experimental animal models. Cooking at higher temperatures and for longer periods of time both result in the formation of more HCA. To date, several studies have evaluated the association of intake of meat cooked at high temperature and PCa risk, but results are inconsistent.

The association between PCa risk and intake of the three most mass-abundant HCAs detected in cooked meat, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), and 2-amino-3,4,8-dimethylimidazo [4,5-f]quinoline (DiMeIQx), was also studied in our European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. Even though meat consumption, cooking methods, and degree of browning of the respective food items were assessed with a detailed questionnaire, the results did not indicate that HCA intake, as consumed in a regular diet, was associated with PCa risk. One of the reasons for these null-findings might be that the association between HCA intake and PCa risk is modified by different genotypes/polymorphisms in genes encoding for HCA-metabolising enzymes. Therefore, I aimed to assess interactions with other polymorphisms for the association between HCA and PCa risk in a case-control study nested within the EPIC-Heidelberg cohort, a study in which my mentor Professor Rohrmann is extensively involved.

**Host Institute**

Professor Sabine Rohrmann, head of the Division of Cancer Epidemiology and Prevention at the Institute of Social and Preventive Medicine in Zurich, has extensive expertise in this relatively new discipline of nutritional epidemiology as well as genetic epidemiology. The training at the Institute of Social and Preventive Medicine in Zurich was an excellent opportunity to learn new epidemiological methods and I am most grateful to the EACR for the contribution they made towards the cost of my research visit.

Division of Cancer Epidemiology and Prevention at the University of Zurich, has extensive expertise in studying how nutritional components and genetic changes might influence PCa biology. I was very keen on learning about the technical principles underlying genetic and nutritional epidemiology as in a next step it will then be possible to apply these methods and assess how different genotypes are modifying associations that I have been studying previously.

**Achievements**

The manuscript describing the findings from the work that I conducted is currently under peer review and will be published soon. Briefly, our study indicated that the association between HCA intake and PCa risk is modified by polymorphisms in genes such as GSTT1, GSTM1, CYP1A1, and MnSOD; however these modifications are not necessarily concordant with the underlying biological hypotheses. Considering genetic variation is thus an important step in elucidating the mechanism of action between meat intake and risk of PCa. Other large studies are needed to investigate the complex interplay of polymorphisms in gene encoding HCA-metabolising enzymes and intake of different HCA.

This training at the Institute of Social and Preventive Medicine in Zurich was an excellent opportunity to learn new epidemiological methods and I am most grateful to the EACR for the contribution they made towards the cost of my research visit.
Bárbara Rivera Pinto  

*Universidad Autónoma de Madrid, Spain*

*Host Institute: Human Cancer Genetics Programme, McGill, USA*

Colorrectal cancer is a complex and heterogeneous disease. It is one of the most common cancers in the western world; while the majority of CRC cases are sporadic a significant minority is due to inherited genetic mutations. Among the hereditary colorrectal predisposition syndromes, the Familier Adenomatous Polyposis (FAP) comprises a complex disease in which neoplasia is not limited to colorrectum. A large relation of extracholonic manifestations such as osteomas, upper gastrointestinal polyps, desmoid tumours, hepatoblastoma, papillary thyroid carcinoma, gastric and duodenal some brain tumours are associated to FAP.

FAP it is probably the most depicted one in terms of causative gene mutations. APC gene mutations are responsible for 50-80% of the cases while MUTYH is mainly responsible for the attenuated phenotype cases in which APC mutations were not found (up to 40%). Approximately 20% of the classic FAP patients are negative for APC/MUTYH mutation. These cases represent a problem in order to assess genetic counseling, surveillance and management of the patients and their relatives. Novel detections techniques and broad screening approaches in APC, MUTYH and other genes involved in carcinogenesis such as Spindle Assembly Checkpoints are needed to further discard their implication in the syndrome and the molecular bases underlying FAP syndrome. Our research project is focused in on the molecular characterisation of classical FAP families with APC/MUTYH negative mutation test. We search for germline mutations and study different mechanisms of inactivation of the genes involved. Moreover we are interested in the molecular protein expression patterns of the tumours. Our ultimate goal is the identification of the genes responsible for these negative FAP cases.

Our previous studies in FAP patients suggest that other genes apart from APC/MUTYH can be involved in this syndrome. On the other hand other mechanisms of inactivation of those APC and MUTYH genes apart from point mutations or great rearrangements can also be underlying the syndrome in apparently negative families. My stay in Dr. Foulkes’ lab has been a great opportunity to test the possible role for Spindle Assembly Checkpoint genes in these families with materials and techniques not available in our lab.

“The EACR fellowship allowed me to get involved in a new deep sequencing project in order to find out new genes involved in colorectal cancer hereditary syndromes in the Inuit population.”

Over the past 5 years Dr Foulkes’ group has worked on various aspects of inherited susceptibility to cancer. The main focus is on the clinico-pathological features of and outcome following hereditary breast cancer and on detailed mutation analysis in hereditary colorectal cancer.

Dr Foulkes’ lab described for the first time the involvement of BUB1B in gastrointestinal neoplasia with a comprehensive approach that fully characterised the role of this gene in a multiple polyposis patient: ‘Homozygous BUB1B mutation and susceptibility to gastrointestinal neoplasia’ Río Frio et al, NEJM 2010.

Regarding colorectal cancer etiology and particularly gastrointestinal poliposis their main objective is to establish if alterations in BUB1B or other members of the SAC or mitotic checkpoint complex (MCC) play a larger role in colorectal neoplasia. Their strategy represents a comprehensive approach to genotype, study the allelic expression and functionally characterise the importance of nine MCC genes: BUB1,
BUB1B, BUB3, ZW10, ZWINT, ZWILCH, TTK (MPS1), MAPRE1 (EB1) and KNTC1.

During my stay in their lab I had the opportunity to work on the project by selecting the target SNP’s of each gene, genotyping colorectal cancer patients and matching healthy controls in order to collect 100 heterozygous cases and controls, and contributing to the design of the allelic imbalance study and methodology. Moreover the EACR travel fellowship gave me the opportunity of including in this interesting project our APC/MUTYH negative FAP families. The cDNA study constitutes the second part of the project and is being developed in Dr Foulkes’ lab with upcoming results.

Furthermore I was able to test the protein expression of BUBR1 by immunohistochemistry in more than 200 polyps samples included in 3 Tissue microarrays. These polyps’ samples correspond to adenomatous polyps of our APC/ MUTYH negative families and polyp samples from APC positive patients, MUTYH positive patients, sporadic polyps, hyperplasic polyps and normal colorrectum epithelium and will allow us to compare the different profiles of expression among those groups.

Apart from those studies, the EACR fellowship allowed me to get involved in a new deep sequencing project in order to find out new genes involved in colorectal cancer hereditary syndromes in the Inuit population. It was a great opportunity to work with such a special population. I would like to thank everybody at Cancer Genetics Program at McGill for all the help and the hospitality and to the EACR for supporting this research and giving me this opportunity.

Ferenc Renyi-Vamos

National Institute of Oncology, Budapest, Hungary

Host Institution: Department of Thoracic Surgery, Medical University of Vienna, Austria

Dates of stay: Oct-Nov 2010 and Jan-Feb 2011

Bone marrow-derived progenitor cells in lung cancer induced lymphangiogenesis

I am a junior postdoctoral researcher at the National Institute of Oncology, Budapest. As a thoracic surgeon and clinical oncologist, I am working on the role of lymphangiogenesis in thoracic malignancies, at present mainly focusing on endothelial progenitor cells. Thanks to this EACR Travel Fellowship I was able to visit the Translational Thoracic Oncology Lab at the Department of Thoracic Surgery, Medical University of Vienna from Oct till Nov 2010 and from Jan till Feb 2011.

Although the spread of malignant cells to lymph nodes (LNs) is a common event and LN metastasis is a key prognostic factor in various malignancies, including lung carcinomas, contrary to haemangiogenesis, the de novo formation of lymphatic capillaries (i.e. lymphangiogenesis) and their role in the metastatic process have only recently become key questions of cancer research. Nevertheless, it is well established now that lymphangiogenesis (i.e. in situ lymph vessel sprouting), facilitated by vascular endothelial growth factor receptor-3 (VEGFR-3) signalling, contributes to tumour growth and metastasis. However, more recent evidence also suggests that tumour lymphatics do not necessarily derive from endothelial sprouting; instead, similar to the mechanism of vasculogenesis, tumour lymphatics can also arise through “lymphvasculogenesis”, a process by which bone marrow-derived lymphatic/vascular endothelial progenitor cells (LVEPCs) are recruited and differentiate in situ into mature endothelial cells to form new lymphatic capillaries.

Non-small cell lung cancer (NSCLC), a serious public health problem in the western world, has a predilection to metastasise to lymph nodes (LNs). Since our group found elevated lymphatic/vascular endothelial progenitor cells (LVEPCs) levels in patients with small cell lung cancer, and both we and others have recently demonstrated the clinical significance of the analogous cell population (haemangiogenic endothelial progenitor cells) in the haemangiogenic process of NSCLC, our current research investigates whether LVEPCs are also involved in tumour-associated lymphangiogenesis of human NSCLC. During my 8 week placement in Vienna we initiated a bilateral research program on LVEPC in NSCLC by starting the collection of PB and fresh frozen tumour samples from surgically treatable NSCLC patients and, moreover, by verifying the methodologies (measuring the numbers of circulating LVEPCs by FACS analysis and the circulating levels of vascular endothelial growth factor-C and -D by ELISA). Having returned from my placement I am able to exploit all the techniques I learnt, as well as carry on the project in my home institute.

I would like to thank the EACR committee for the support of my travel fellowship in Vienna. The support and the kind hospitality of the colleagues at the Department of Thoracic Surgery, Medical University of Vienna is also gratefully acknowledged.

Ferenc in front of the Anna Spiegel Research Building of the Medical University of Vienna, which houses the Translational Thoracic Oncology Lab of the Department of Thoracic Surgery.
Identification of genomic prognostic predictors and oncogenic drivers in hepatocellular carcinoma.

I am a PhD student at HCC Translational Research Laboratory in Barcelona. Thanks to the EACR Travel Fellowship I have been working for six months in the Liver Cancer Program at the Division of Liver Diseases in Mount Sinai School of Medicine in New York. The main objective of this longer-stay was to identify genomic prognostic predictors and oncogenic drivers in 250 samples of early hepatocellular carcinoma (HCC) patients treated with surgical resection. Once these drivers are identified, we wish to generate a gene signature that recognises aggressive HCC that could be useful for the decision-making process.

Hepatocellular carcinoma is the sixth neoplasia in terms of global incidence and the third cause of cancer-related mortality worldwide. Its incidence is dramatically increasing in United States and Europe, mainly due to the increase of hepatitis C virus infection. In addition, less than 30-40% of liver cancer patients in Western countries are eligible for potential curative therapies (i.e. percutaneous ablation, surgical resection or liver transplantation). Survival of these treated patients is jeopardised by tumour recurrence, with a relapse rate close to 70% within 5 years after surgery. The positive results of the phase III trial with sorafenib demonstrate the importance of the molecular targeted therapy in HCC and the oncogene addiction discovery. Since most of HCC patients are diagnosed at advance stages with a median survival ratios of around 1 year even treated with sorafenib, we think that a more accurate understanding of the molecular profile of this neoplasia could be useful to improve its prognosis assessment and to release novel targets for molecular therapies.

"I would like to thank all the people in the lab for their unconditional help and advice"

To achieve the aim for my stay, I have been working and learning different skills such as managing the clinical data and the statistic package SPSS, performing the extraction, quantification and qualification of the DNA from two different kind of tissues (i.e. Formalin-Fixed Paraffin Embedded (FFPE) and fresh frozen (FF)) and handling the final expression results with “in silico” programs like Gene Pattern, Ingenuity Pathway and GSEA. This last step was done using colleagues’ data because the arrays of the samples related to my project are still in process. Then, I was able to get the necessary skills to manage our future data at my home institution.

I am very grateful to the EACR for the Travel Fellowship that gave me the opportunity to get new skills in the lab and to advance my knowledge in the bioinformatics world. Moreover, I would like to thank all the people in the lab for their unconditional help and advice. It was a great experience, into the lab, and outside of it, New York City is awesome.
immunomagnetic enrichment and cell extraction protocols.

I acquired many transferable skills: Specificity of the enrichment and extraction protocols. Both whole blood and MNC fractions of healthy donors were tested regarding the specificity of cell extraction and enrichment protocols with immunomagnetic beads coated with BerEP4 and KS1/4. No CK20 signal were observed in all examined blood samples of healthy donors, demonstrating the specificity of the used assays. Sensitivity of the enrichment and extraction protocols: in the tumour cell spiking experiments with whole blood samples the sensitivity of the CK20 RT-PCR assay were higher using immunomagnetic beads coated with mAb BerEP4 Dynabeads, whereas 102 HT29 cells spiked in 5 ml blood (20 cells/ml) were detected using the mAb KS1/4 coated beads. Blood spiking experiments were repeated several times to confirm the above mentioned results; The observed higher sensitivity of tumour cell detection after isolation of the MNC fraction prior to immunomagnetic CTC enrichment were used Ficoll gradient centrifugation before further immunomagnetic enrichment and detection of CTC in the blood of CRC patients, detection of tumour cells in blood samples of CRC patients, RNA extraction and nested RT-PCR, CK20 transcripts were detected after immunomagnetic enrichment of tumour cells either derived from blood samples spike with HT29 cells or from blood samples draw from CRC patients.

I am very pleased that with the support of EACR I became deeply familiar in the area of different EpCAM-specifc antibodies in the Heterogeneous detection of circulating tumour cells in patients with colorectal cancer. I am very thankful to my Host Institute especially to my mentor Professor J. Schmidt. All staff were very friendly and attentive and helped me to acquire practical information and new technologies.

My visit to Heidelberg was very useful for my professional advancement. The knowledge and experience I have got in Prof. Schmidt’s lab will be very useful for my department and my home institute. The friendships and relationships I developed will help us to collaborate in future. The knowledge gained will help me to plan independently my practical work and scientific study. During my fellowship I participated as a visiting scientist in workshops and seminars held in host institute and DKFZ. Also I visited Stockholm and had a poster presentation “Latest developments in Diagnosis and Therapy of Cancer” in the 2011 European Multidisciplinary Cancer Congress organised by ECCO.

This scientific visit was very important and very useful. It was organised very well and the program was very interesting and full of new information. I had the opportunity to meet colleagues, to network and to openly discuss treatment options with international cancer specialists.

Now, coming back to Georgia I will use the knowledge and skills, methods and techniques learned in the University of Heidelberg for a similar project that the Laboratory of National Cancer Centre is planning to implement. The similar project that I plan to conduct within my department in Georgia will significantly stimulate the beginning of implementation of modern investigation and staff will be trained for this inevitable project that will be equally important for our department’s staff qualification.

I would like to thank EACR for the great chance to visit the romantic city of Heidelberg in the heart of Europe and its world famous university. Thank you very much for the award and for the fine memories.
Diana Car
Rudjer Boskovic Institute, Zagreb, Croatia


The EACR Travel Fellowship enabled me to travel to the 1st HEALING Summer School on Hh-Gli Signalling, entitled Hh-Gli Signalling in Development, Regeneration and Cancer. Since I am a PhD student working on a project on Hh-Gli signalling in cancer, this summer school was a great opportunity to deepen my knowledge.

The Hedgehog-Gli (Hh-Gli) signalling pathway is highly conserved through evolution and plays a crucial role in embryonic development of many organisms, from Drosophila to humans. In adult tissues this pathway is mainly inactive, but in the last decades, its aberrant activation has been implicated in various tumours, benign and malignant ones. The Hh-Gli signalling pathway is activated by the binding of its ligand Hedgehog to the receptor Patched. After ligand binding, Patched relieves its repression of Smoothered, allowing activation of downstream signalling, through the Suppressor of Fused, which, in turn, leads to the activation and nuclear translocation of the zinc finger transcription factor Gli. Recently the primary cilium, a sensory organelle, has been identified as the key location for protein interactions and signal transduction, but many details regarding the mechanisms of signal transduction are still unknown. In the absence of Hh signal, SuFu acts as a negative regulator of the signalling pathway because it inhibits Gli mediated transcription. Gli is being phosphorylated by three kinases, PKA, GSK3 and CK1 to promote proteasome dependent degradation.

Our laboratory is investigating the role of Hh-Gli signalling in cancer. We showed that this pathway is aberrantly activated in various cancer types by genetic as well as epigenetic changes. The regulation of its activity in cancer cell lines, by adding exogenous inhibitor or activator, can modulate pathway gene expression and cell features, such as the cell’s ability to migrate. We are also interested in the mechanistics of the Hh-Gli pathway to improve our understanding of the interactions between the components, particularly between Gli and its regulators SuFu and GSK3. Modulation of pathway activity has influence on the localization of these proteins depending on the activation.

The summer school consisted of lectures and discussions in small groups. The lectures were given by experts in the field of Hh-Gli signalling and they gave us an overview of the pathway itself, from its discovery to the latest breakthroughs. Cytonemes were described as a new means of transporting the Hh ligand from producing to receiving cells. Also, advances in understanding the function of Hh in cancer stem cells were emphasised, identifying it as a strong inducer of stemness which stimulates self renewal. Hh can trigger cancer in a wide variety of tissues and maintain cancer stem cells. The discussions were organised in small groups consisting of 7 students and one of the Summer School teachers. Since the weather was nice and warm these discussions were held outside in a relaxed surrounding, inbetween swimming breaks and lectures. My group had the opportunity to talk to Suzanne Eaton (Dresden, Germany) working on lipid metabolism and Hh signalling, and Thomas Kornberg (San Francisco, USA) specialized in morphogens, cytonemes and pattern formation. We also had the chance to talk to Alberto Gulino (Rome, Italy), working in the field of Hedgehog signalling in development, cancer and cancer stem cells, as well as to Valerie Wallace (Ottawa, Canada) working on Hedgehog-Gli signalling in the retina and stem cells in eye therapies. These discussions were very helpful, since we were able to discuss specific issues and problems regarding our research with experienced scientists and other students.

I would like to thank the EACR for awarding me this Travel Fellowship and giving me the opportunity to take part in this Summer School, meet young scientists from all over the world and the opportunity to visit such a nice island as Crete. The knowledge and experience gained there will be of great value to my future research.
Stimulation of autophagy may be upregulated; it can induce autophagy. Beclin-1 is a haploinsufficient and when tumour suppressor protein beclin-1 is knocked down, autophagy promoting induction can be used as a therapeutic modality. The first autophagic cell death (programmed cell death type II) is a highly conserved process that allows cells to capture cytoplasmic contents through the formation of double-lipid membrane vesicles (autophagosomes) and target them for degradation through the fusion of autophagosomes with lysosomes, creating single-membrane autolysosomes. Subsequently, autophagosomes merge with lysosomes and digest the organelles, leading to cell death. In contrast to apoptosis, autophagic cell death is caspase-independent and does not involve classic DNA laddering. Cancer cells in general tend to undergo less autophagy than their normal counterparts, supporting the contention that defective or suppression of autophagic cell death plays a role in the process of carcinogenesis. In fact, studies of carcinogen-induced cancer models showed that cancer cells are more resistant to undergo autophagy than premalignant or normal cells. However, the mechanisms underlying the inhibition of autophagy in cancer cells have not been well characterised. Two lines of evidence support autophagy induction can be used as a therapeutic modality. The first, autophagic cell death can be induced by antineoplastic agents. Second, autophagy promoting tumour suppressor protein beclin-1 is a haploinsufficient and when upregulated it can induce autophagy. Stimulation of autophagy may be destructive to cancer cells and that therapies that prevent inhibition of autophagy or directly promote autophagy may lead to enhanced tumour killing and response.

We and others have observed that the expression of TG2 is elevated in many drug-resistant and metastatic tumours and tumour cell lines. Because TG2 expression confers protection from apoptosis, it can be expected that increased expression of TG2 contribute to the development of aggressive phenotypes in cancer cells. Indeed, failure of cells to undergo cell death can render cells not only resistant to chemotherapeutic drugs but also promote their survival in stressful environments of the foreign tissues (metastasis). In support of this contention, our recent results demonstrated that TG2 expression in intrinsically resistant and metastatic pancreatic cancer cells constitutively suppresses the autophagic death in these cells. Downregulation of endogenous TG2 by siRNA or rottlerin resulted in massive vacuolization and formation of autophagosomes associated with induction of LC3-II, eventually resulting in autophagic death of pancreatic cells. These results clearly suggest that expression of TG2 protects pancreatic cancer cells from self-destruction. Moreover, TG2 mRNA and protein expression in pancreatic cancer cells is regulated by PKCδ, suggesting that PKCδ is the master regulator of autophagy in pancreatic cancer cells. Treatment of cells with rottlerin reduced activated/phosphorylated mTOR whereas treatment with Rapamycin, a specific inhibitor of mTOR, did not induce autophagy in pancreatic cells suggesting that mTOR may not be involved in TG2-mediated protection of cells from autophagy. Our studies also revealed that autophagy that is induced in response to PKCδ inhibition or TG2 downregulation is mediated by Beclin-1. Therefore, targeting TG2 may represent an effective and novel approach for treating pancreatic cancer. Regulation of autophagy due to aberrant expression of TG2 needs to be studied in other solid tumours such as breast and ovarian cancers. Dysregulation of autophagy due to over expression of TG2 may have important implications in terms of tumour progression. Thus, TG2 expression in cancer cells may contribute to the development of drug resistance and metastatic phenotypes by conferring protection from stress-induced autophagy. These findings indicate that autophagy might have different effects in tumours and in normal cells. It is not clear whether autophagy acts as a tumour suppressor or protects cancer cells from anticancer-therapy-induced stress. If autophagy induction can cause tumours to regress, then activating autophagy could be a useful therapeutic approach. Therefore, to develop improved cancer therapies, we need to identify the molecules, which positively or negatively regulate autophagy in cancer cells.

I would like to take this opportunity to thank the European Association for Cancer Research for the Travel Fellowship Award which has contributed to my future research. Your support allowed me for improving my knowledge and experience which is very important to my career of cancer research.

Kivanç Görgülü
Celal Bayar University Faculty of Medicine, Turkey
Host Institute: M.D. Anderson Cancer Center, U.S.
Dates of visit: 25th July 2011 - 14th January 2012
Targeting Autophagy as a Novel Therapy for Pancreatic Cancer

Autophagy and apoptosis are highly regulated forms of programmed cell death and play crucial roles in physiological processes such as the development, homeostasis and elimination of unwanted cells. Autophagic cell death (programmed cell death type II) is a highly conserved process that allows cells to capture cytoplasmic contents through the formation of double-lipid membrane vesicles (autophagosomes) and target them for degradation through the fusion of autophagosomes with lysosomes, creating single-membrane autolysosomes. Subsequently, autophagosomes merge with lysosomes and digest the organelles, leading to cell death. In contrast to apoptosis, autophagic cell death is caspase-independent and does not involve classic DNA laddering. Cancer cells in general tend to undergo less autophagy than their normal counterparts, supporting the contention that defective or suppression of autophagic cell death plays a role in the process of carcinogenesis. In fact, studies of carcinogen-induced cancer models showed that cancer cells are more resistant to undergo autophagy than premalignant or normal cells. However, the mechanisms underlying the inhibition of autophagy in cancer cells have not been well characterised. Two lines of evidence support autophagy induction can be used as a therapeutic modality. The first, autophagic cell death (programmed cell death type II) is a highly conserved process that allows cells to capture cytoplasmic contents through the formation of double-lipid membrane vesicles (autophagosomes) and target them for degradation through the fusion of autophagosomes with lysosomes, creating single-membrane autolysosomes. Subsequently, autophagosomes merge with lysosomes and digest the organelles, leading to cell death. In contrast to apoptosis, autophagic cell death is caspase-independent and does not involve classic DNA laddering. Cancer cells in general tend to undergo less autophagy than their normal counterparts, supporting the contention that defective or suppression of autophagic cell death plays a role in the process of carcinogenesis. In fact, studies of carcinogen-induced cancer models showed that cancer cells are more resistant to undergo autophagy than premalignant or normal cells. However, the mechanisms underlying the inhibition of autophagy in cancer cells have not been well characterised. Two lines of evidence support autophagy induction can be used as a therapeutic modality.
The molecular heterogeneity of breast cancer (BC) has been well studied in the past decade. Priority is to identify robust and clinically applicable markers of disease strata. TP53, a tumour suppressor mediates key tumour related processes such as cell cycle, apoptosis and DNA repair. Many available chemotherapeutic options are also known to depend on TP53 mediated apoptosis. While about 30% of BC express mutant TP53 with higher likelihood of metastasis, chemo-resistance and rapid progression, TP53 as a marker can provide ample possibilities as a predictive as well as prognostic marker.

Studying and inferring the coordinated patterns of gene expressions and networks that vary according to the TP53 status in BC by applying innovative methods, can provide clues about pathways and processes perturbed due to coordinated regulation and combinatorial influence of key potential markers in association of TP53 status in BC.

With this goal, I have been working on a project for reverse engineering the gene expression networks and inference of differential perturbation of pathways according to the TP53 status.

I am a doctoral researcher with a background in medicine and computational biology, working with Akershus University Hospital, University of Oslo. My PhD project focuses on systems biology and network based diagnostics in BC using innovative bioinformatics approaches.

Thanks to the EACR travel fellowship I got an opportunity to work at the BioMaPS Institute for Quantitative Biology of Rutgers University, USA. I had met Prof Gyan Bhanot, who is a group leader at BioMaPS group working on applying the bioinformatics methods in translational medicine. After being introduced with my project, I was offered an opportunity to visit BioMaPS. I would like to thank Prof Bhanot and his team for the time and support.

We have a large collection of BC gene expression data from centres located in Scandinavia. Generalisability of findings from several studies might have limitations mainly because of the size of datasets, platform-related limitations, and inter-laboratory variations. For the pursuit of my project, I have compiled a cross-platform dataset of about 438 cases with known TP53 mutations. TP53 mutation data, which is based on prescreening exon 2-11 using temporal temperature gradient gel electrophoresis (TTGE), is available from the respective publications.

I have been currently working on validation of the novel differential pathway perturbation and network inference method on independent dataset. A publication has been submitted to British journal of cancer, which is under revision.

Himanshu Joshi

Akershus University Hospital, University of Oslo, Norway

Host Institute: BioMaPS Institute for Quantitative Biology, Rutgers University, USA

Dates of visit: May-July and September-November 2011

Inference of gene expression networks of breast cancer in context of p53 mutation status

The scientific meeting was held in the picturesque downtown of Amsterdam. It was easy to find De Rode Hoed, the place of the meeting, which was rebuilt from an old church. The course was well organised, Kathryn Wass and Rachel Warden from EACR helped the participants, and answered every question. All the speakers were renowned experts, and most of them tried to give an advanced and extensive knowledge on their topics.

The first day was a little bit like a teaser trailer: all the speakers talked about the general importance and specific challenges the challenge of molecular pathology through reporting concrete examples. The cell signalling based molecular approach was in the focus of the lecture of Professor Richard Marais, who is a very impressive presenter. The lecture went from the communication of two cells through receptor tyrosine kinases to the complexity of the subway network of Tokyo. For me one of the most important events was his second day presentation on the molecular pathology of melanoma – the issue

István Kenessey

Semmelweis University, 2nd Department of Pathology, Hungary

Attended 1st EACR-OECl Joint Training Course “Molecular Pathology approach to cancer”, Amsterdam, the Netherlands, 7th - 9th March 2011

My PhD project was the investigation of the molecular background of metastasis in malignant melanoma. Currently I am in the Pathology Resident Program, however I am continuing my research as well. Furthermore I would like to pursue my research carrier along with my clinical duties following board certification. In the near future cancer treatment will be based on the molecular background, therefore as a young pathologist it is a must to be familiar with modern molecular pathology methods. For this very reason I applied for the fellowship to attend the EACR-OECl Training Course “Molecular Pathology approach to cancer”.

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closely related to my former PhD project.

I’ve never worked with the tumours of the central nervous system, but I was always intrigued by this area, therefore the presentation of Peter Collins on the molecular pathology of gliomas was very exciting for me. The organ specific scientific program included presentations on the molecular pathology of soft tissue tumours (by Jonathan Fletcher), bone tumours (by Pancras Hongendorn), prostate cancer (by Carlos Cordon-Cardo).

In my home Institute a number of researchers focus on the biological role of claudin molecules in different tumours, therefore another interesting lecture was Jorge Reis-Filho’s talk about a specific subtype of triple negative breast tumours, namely the claudin low group. Professor Mark Ladanyi spoke about the molecular pathology of colorectal cancers and lung cancers, where epidermal growth factor receptor has a critical role, and about specific treatment agents, tyrosine kinase inhibitors and antibodies are applied (e.g. gefitinib and cetuximab) in new protocols. At the end of the day in one hour we heard about the molecular background of haematopathology in two excellent presentation (by Carel van Noesel and Steven T. Pals). The day’s last session was the presentation of Matt van de Rijn about a very hot topic, the role of tumour associated macrophages in the progression of tumours.

The Wednesday program focused the practical aspects of molecular pathology. For example Marc van de Vijver (the other member of the organiser board) talked about the usually applied molecular test in breast cancer – which topic has special significance in routine pathology.

In summary, the thorough knowledge about the molecular background of pathological processes I’ve gathered in the course will definitely be important for my professional development both in my scientific and clinical career. Therefore I would like to thank the European Association of Cancer Research for this opportunity.

Oleksandr Kondratov

Institute of Molecular Biology and Genetics, Kyiv, NAS Ukraine

Host institute: Karolinska Institutet, MTC, Stockholm, Sweden

Dates of visit: 11th Jun 2011 - 6th Apr 2011

The main goal of my research in the Karolinska Institute was the creation of KRC/Y K712 stable cell line and performing of gene-inactivation test in SCID mice. The members of WNT-family are proteins that participate in cell signalling through canonical (β-catenin dependent) and non-canonical (β-catenin independent) pathways. The role of WNT-proteins in cancer development is dual because for one members (WNT2, WNT7A, WNT10B) it shows oncogenic features, for other members (WNT5A, WNT7A) - suppressor features. Data about participation of WNT7A gene in human cancer have an ambiguous nature: in lung cancer it was characterised as tumour suppressor, on the contrary, in ovarian cancer it showed increasing of expression of WNT7A gene. Recent data show that the product of WNT7A gene plays a role in epithelial-mesenchimal transition. Earlier we have shown that this gene is inactivated frequently in the clear cell renal cell carcinomas (ccRCC). The WNT7A was inactivated by DNA methylation of promoter region in 60% (17/28) of cases and LOH in 75% (15/20) cases of ccRCC. We supposed that WNT7A gene may plays role of tumour suppressor gene in ccRCC.

When I started work in Karolinska Institutet MTC it was decided to divide research into branches: 1) Creation of KRC/Y stable cell line; 2) Determination of WNT7A suppressor functions into in vivo tests prior to gene-inactivation test. During this work the system of Bsd/Dox selection was adjusted to WNT7A gene to prevent leakage of promoter and expression of WNT7A gene during expression. Stable cell line KRC/Y K712 pETE-WNT7A was established.
In frame of this work colony-formation and cell proliferation have been done. *In vitro* tests have shown that restoration of WNT7A expression significantly decreases the number of colonies and leads to a reducing of cell growth. Data obtained from *in vitro* tests said about potential tumour suppressor function of WNT7A gene and allow to start *in vivo* experiments to verify suppressor function of WNT7A gene in clear cell renal cell carcinomas.

The EACR travel fellowship allowed me to visit one of the leading cancer research centres – Karolinska Institutet: MTC, where I have mastered cell culture work in particular stable cell line establishment, Tet-regulated selection and a range of *in vitro* tests like colony formation and cell proliferation tests. And I hope the final data obtained up to now will characterise WNT7A gene at level that allows me to write article to journal about mechanism and (briefly) role of WNT7A gene inactivation in clear cell renal cell carcinomas. Unfortunately the period of staying in KI MTC did not allow me to start *in vivo* testing of suppression function of WNT7A gene in SCID mice. Particularly, it can be explained by difficulties in KRC/Y K712 cell line establishment.

I came back to Ukraine with the prospect to continue *in vivo* experiments. I am going to include acknowledgments to EACR in an article about inactivation and the role of WNT7A gene in ccRCC. I know I did not fulfill the plan according to SCID-mice experiments. I am disappointed. But I am working obstinately to finish an article about inactivation and function of WNT7A in ccRCC.

"Hopefully this short stay can be considered a seed for future collaborations."

This comprised three arms of 30 volunteers each, to whom a certain diet was attributed: (i) one diet was rich in flavonoids, but not supplemented; (ii) one was a normal isocaloric diet with adequate amounts of fruit and vegetables; and (iii) one was based on supplementation of the normal diet with additional flavonoids in the form of green tea and soy products. A urine sample was obtained from each participant at baseline, after each of the four weeks that the trial lasted, and one year after the end of it.

My main objectives were to compare metabolic profiles between the three arms of the trial, and to examine the differences in the metabolic profiles over time within each arm. These were achieved by applying a t test, per ‘metabolite’ (spectral peak), between pairs of arms at particular time points, and also between time points for each arm of the trial. In collaboration with Dr. Marc Chadeau-Hyam (Department of Epidemiology and Biostatistics), I compared my top-ranked results (lowest p-values) with those obtained by applying different statistical methods (e.g., t test vs. orthogonal partial least-squares) on the same data, and found some differences that need further analysis.

In addition, I learned about the analytical method of $^1$H NMR and
had the chance to discuss with Dr. Hector Keun (Department of Biomolecular Medicine) and Dr. Toby Athersuch (MRC-HPA Centre for Environment & Health) on the strengths and limitations of such method in comparison to gas or liquid chromatography-mass spectrometry. This was important since recently in Madrid we started a pilot study on metabolomics, using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, and bladder cancer.

During my visit and although this was not the main aim, I also had the opportunity to collaborate with Dr. Shu-Chun Chuang (Department of Epidemiology and Biostatistics) and contribute to the analysis of the association between certain biomarkers of inflammation/infection and lung cancer.

Hopefully this short stay can be considered a seed for future collaborations and studies on metabolomics and cancer etiology and prognosis. Finally, I would especially like to thank Prof. Paolo Vineis, and his group, for hosting me, and EACR for giving me one of the 2011 Travel Fellowship Awards.

AICR, the Association for International Cancer Research, is working towards saving lives that are being lost to cancer. In future, those lives will be saved by improvements in prevention, diagnosis and treatment, but those advances can only be made tomorrow by investing in research today. That’s why AICR funds the very best cancer research proposals we receive, wherever in the world the projects will take place.

Over the last 30 years AICR has invested over £134 million into cancer research; supporting over 1750 projects in 32 different countries. Each year we award approximately £8.5 million to new research projects around the world.

Research Projects
- 2 grant rounds per year – April & October
- Support for a project grant is typically 3 years, but 2 year, and 1 year pilot studies are also considered
- Proposals must be on basic or translational cancer research
- Specific funds available for prostate and bowel cancer projects

Please visit our website for further information

www.aicr.org.uk/research.stm