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.

Emma Ramsay

Lowy Cancer Research Centre, University of New South Wales, Australia

Host Institute: Karolinska Institutet, Huddinge, Sweden

Dates of visit: 21 February – 29 March, 2013

GSAO (4-(N-(S-glutathionylacetyl)amino) phenylarsonous acid) is a novel organoarsenical compound presently completing phase I trials in cancer patients who are refractory to treatment. GSAO targets the mitochondria of proliferating endothelial cells by binding to calcium-replete adenine nucleotide translocase on the inner mitochondrial membrane. Consequently, small solutes equilibrate across the membrane causing swelling and eventual rupture of the mitochondria.

Essential to the action of the drug is the cleavage of the γ-glutamyl group from GSAO. Without the removal of this moiety, GSAO is unable to enter cells and reach its target. The enzyme, γ-glutamyl transferase (γGT), is able to remove the γ-glutamyl group. This enzyme plays a role in maintaining intracellular glutathione levels, removing the γ-glutamyl group extracellularly, and allowing the constituent amino acids to be taken up into the cell where they can be reformed as glutathione.

γGT has been shown to be differentially expressed in a number of cancers; increased expression being observed in cancers of the ovary, liver, lung, and breast and in melanoma and leukaemia. We hypothesised that tumours expressing high levels of γGT will have a greater response to GSAO than tumours with basal activity. In vitro studies have supported this hypothesis, showing a clear relationship between the γGT status of cancer cells and the response of the primary target-proliferating endothelial cells. In vivo, tumours of a high γGT cancer cell have a greater response to GSAO than basal γGT expressing tumours.

Pancreatic ductal adenocarcinoma (PDAC) is characterised by an extensive desmoplastic reaction. This consists of an extensive stroma, comprising greater than 80% of the total tumour volume. Pancreatic stellate cells are considered responsible for this reaction. We have observed with primary pancreatic stellate cells that cancer-associated cells, compared to their normal counterparts have a significantly higher γGT activity. This extends the original hypothesis of targeting the tumour site with the prodrug GSAO to tumours with high tumour cell γGT activity, to potentially targeting PDAC with GSAO. Relying on the γGT expression of the stellate cells to activate the drug and thus inducing cell death in proliferating cells.

I travelled to Sweden to learn their multi-cellular 3D spheroids and make use of their immortalised pancreatic stellate cells. This model allows for the combination of cancer cells and stellate cells within the one sphere. This is a significantly easier 3D model to work with than animals. Studies with this model are ongoing; however initial results suggest that the interaction between the cells affects the response of the spheroids to the drug.

I would like to thank EACR for the opportunity to travel to Sweden to perform this work. It was an enormously enriching experience. Along with learning a new technique I was also able to experience a different culture. I would also like to thank those I worked with at KI for all their support and guidance.

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Radiotherapy is one of the most widely used modalities for head and neck cancer treatment. During conventional radiotherapy, the normal tissues surrounding the tumour cells also get exposed to radiation and results in normal tissue adverse reactions. Oral mucositis, skin erythema and xerostomia are some of the commonly seen adverse reactions in HNC patients and the severity of these manifestations varies widely from patient to patient. Biological strategies that can detect the normal tissue side effects at an early stage can help radiation oncologists to adapt treatment strategies which limits these adverse effects and increases the therapeutic efficacy.

Research in this direction from our laboratory has demonstrated that γ-H2AX analysis (an indirect marker for double strand break) may have predictive implications for identifying the overreactors of mucositis and skin reactions in head and neck cancer patients prior to the initiation of radiotherapy (Goutham et al., 2012). This provides us with a clue that DSB repair efficiency plays an important role in determining normal tissue radiosensitivity. Further, we were interested to characterise the normal tissue radiosensitive and radioresistant individuals by analysing DSB repair capacity based on sub-pathways of Non Homologous End joining (NHEJ). It is well documented that there are at least two sub-pathways of NHEJ: one which does not require any microhomology (direct) and one that needs short regions of homology for joining/microhomology based (Verkiak et al., 2002).

For the V(D)J assay, cells are transfected with plasmid substrate along with RAG1 and RAG2 expression plasmids, and we analyse the blunt end ligation and microhomology based recombination using Not 1 and NgoMV restriction digestion respectively. I could perform these techniques using fibroblast cells derived from patients with extreme radiosensitivity. Additionally, these experiments were performed in the presence of DNA-Pk inhibitor. Further, we are interested to adapt these methods to our laboratory. During my stay, I also attended a course on laser scanning microscopy to get an overview of different techniques used on confocal microscopes.

I am grateful to the European Association for Cancer Research for providing me with the opportunity to learn and adapt DNA repair techniques to my studies. I am also thankful to Prof. Dik C van Gent and all his lab members for their kind support and guidance.

References:


Veronica Steri

University of East Anglia, School of Biological Sciences, Norwich, UK

Host Institute: Washington University, School of Medicine, Division of Oncology, St Louis, MO, US

Dates of visit: August 2013

Exploring the role of β3-integrin integrin in bone metastasis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is essential for tumour growth and spread. The concept that tumour angiogenesis may have therapeutic implications in the control of tumour growth was introduced in the early seventies and in the last decade, this theory has been translated into the clinic.

However, while meeting with success for some tumour types, many tumours are intrinsically resistant to anti-angiogenic treatment or they acquire secondary resistance that leads to tumour recurrence. The dramatic up-regulation of αvβ3-integrin that occurs in the vasculature during tumour growth has long suggested that the endothelial expression of this molecule is an ideal target for anti-angiogenic therapy to treat cancer. Overall though, αvβ3-integrin antagonists extend patient survival by only a matter of weeks.

We have recently developed a transgenic mouse model to examine the endothelial specific contribution β3-integrin makes to primary tumour growth and angiogenesis and shown that its acute depletion transiently inhibits these processes in mice. Even though our findings suggest that endothelial β3-integrin is still to be considered a good anti-angiogenic target with some room for improvement, to our knowledge there is no data on the effect of β3-integrin antagonism in the process of metastasis formation.

Bone is the most common site of breast cancer metastasis. The formation of metastasis in the bone requires mutual interaction between tumour cells and the cellular environment (including osteoblasts, osteoclasts and endothelial cells) commonly defined as “vicious cycle”, whereby tumour cells secrete factors that promote bone degradation providing a fertile ground for tumour growth. Once tumour cells start growing in the bone microenvironment, they will be likely to be resistant to conventional chemotherapy. This underlies the compelling need to develop new therapeutic targets (among which anti-angiogenic therapy might in line of principle prove effective), but these studies are limited because of the lack of suitable animal models allowing to precisely dissect the role of tumour and stromal cells in metastatic formation and progression within the bone.

The partnership with the Weilbaecher lab proved to be crucial since they have developed a mouse breast carcinoma cell line (GFP and LUC tagged) that spontaneously metastasise to bone after mammary fat pad injection and primary tumour resection in mice. The aims of our collaboration was primarily to learn how to work with the spontaneous mouse model of bone metastasis as well as to acquire the basic expertise with bone techniques in order to investigate the role of endothelial β3-integrin in the angiogenic process happening in the bone microenvironment during tumour colonisation and metastasis formation. Ultimately, but more relevantly, we aimed to set the stage for a collaborative work to test whether targeting β3-integrin might be effective in preventing or reducing bone lesions.

“I would like to thank the EACR for awarding the travel fellowship, which has allowed our lab to establish a fruitful collaboration with the Weilbaecher lab.”

Unfortunately I spent only one out of the three months originally planned in the Weilbaecher lab but I still managed to learn how to perform mouse surgical procedures, in vivo bioluminescent imaging of metastasis and bone embedding for MicroCT acquisition and analysis. It was a fantastic and extraordinary learning experience in a very exciting and motivating working environment.

I would like to thank the EACR for awarding the travel fellowship, which has allowed our lab to establish a fruitful collaboration with the Weilbaecher lab. This award also gave me the chance to acquire new knowledge and expertise which will be very helpful for my future research. Finally, I wish to thank Prof. KN Weilbaecher for letting me work in her team, for her great enthusiasm and personal support and Dr. Xinming Su for his teaching, patience and huge help.
Marta Pereira

Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga; ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Host Institute: The Institute of Cancer Research, UK

Dates of visit: February – April 2013

I am a junior postdoctoral fellow at the Life and Health Sciences Research Institute, in Portugal. I started my collaboration with Dr. Chris Jones at the Institute of Cancer Research, UK, during my PhD project and had the opportunity to spend a period in his lab. We continued working together and thanks to the EACR fellowship I was able to go back to Dr. Jones’ lab and not only strengthen our collaboration, but also gain some bioinformatic skills that will be extremely useful for my future research.

Brain tumours are the leading cause of cancer related death in paediatric patients and are responsible for the greater part of the cancer-related years of life lost across all age groups. A deep molecular knowledge of these tumours is needed in order to better understand their genesis, progression and also uncover new therapeutic targets and more successful treatments.

Genetic instability that varies from subtle changes in DNA sequence to chromosomal abnormalities constitutes a hallmark of the tumourigenic process. Our previous studies identified a remarkably stable genomic profile in paediatric brain tumours, particularly high-grade glioma, clearly distinguishing childhood cancer from their adult counterparts. Some of these tumours with few or no detectable copy number changes also seem to have microsatellite instability (MSI), a distinct type of genomic instability, where the microsatellite regions are mutated.

The MSI in brain tumours was significantly more frequent in paediatric samples, but the mutated genes in the setting of these tumours are still unknown. In this context, our goal now is the identification of novel genes and pathways differentially deregulated in these genomically distinct paediatric tumours. We have whole exome sequenced some of these genomically stable paediatric HGG samples, together with the collected germline DNA.

During my visit, I had the opportunity to follow and learn with Dr. Alan MacKay, an expert bioinformatician. Due to my EACR fellowship, I was able to gain different bioinformatic skills such as handling raw sequencing data, getting started on the statistic software R and predict sequence variants. This knowledge will be extremely helpful to manage data back at my home institution. Together with the in silico analysis, I was also validating, by Sanger Sequencing, the bioinformatically predicted variations. This work is still ongoing and we will keep working together in order to produce important results.

Together with this work, the EACR fellowship gave me the opportunity to learn new in vitro techniques with Dr. Mara Vinci. Particularly, three-dimensional tumour spheroid-based functional assays previously designed, developed and established by Dr. Vinci. These assays will be extremely helpful in the future at my home institution for drug and target molecules testing.

I would like to take this opportunity to acknowledge EACR for the support to visit Dr. Jones’ lab, which was extremely important in the context of my actual and future research. I also thank Chris and his team for welcoming me so nicely to the lab and for the wonderful scientific discussions and valuable input.

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Embryo development and carcinogenesis are regulated by common genes and signalling pathways. It has been suggested that the reactivation of developmental processes contributes to tumour progression. Despite the knowledge of common threads between embryo development and carcinogenesis, the crosstalk between both scientific areas has been limited. An important goal of my PhD project is the identification of the transcriptional targets involved in both embryo development and carcinogenesis in order to establish parallelisms between these two research fields.

As cancer models, I am focussed on gliomas, one of the most lethal human tumours, extensively studied by our group at the Life and Health Sciences Research Institute in Portugal. A developmental model organism will be used, namely chick embryos. The current state-of-the-art technique for determining genomic targets of transcription factors is Chromatin Immunoprecipitation Sequencing (ChIP-Seq). This is an experimentally challenging technology with which Dr. Fiona Wardle’s group, at King’s College, UK, has long-standing experience.

I started my collaboration with Dr. Fiona Wardle 3 months before being awarded with the EACR fellowship. Thanks to the EACR fellowship I was able to return to Dr. Fiona Wardle’s lab, which contributed in a significant manner towards this collaborative project. Furthermore, this fellowship constituted an important experience and an excellent opportunity to increase my international scientific contacts and establish new collaborations.

“This EACR fellowship was a remarkable professional experience that allowed me to make a significant step forward in my research project.”

During my visiting period, I had the opportunity to learn ChIP-Seq and ChIP-qPCR techniques and improve my knowledge and bioinformatics skills in order to analyse sequencing data. Moreover, I was able to apply this knowledge to my own samples, both embryonic and cancer samples. This work/collaboration is still ongoing and we hope to find new important data in the context of my actual and future research. I gained experience and acquired new skills that I am actually implementing at my home institution.

This EACR fellowship was a remarkable professional experience that allowed me to make a significant step forward in my research project. I would like to thank EACR for their support and for this beautiful experience. I also wish to thank Dr. Fiona Wardle and her fantastic group for sharing their expertise, for wonderful hospitality and for the pub time.

Thank you EACR.
**Satish Gupta**

International Hereditary Cancer Centre, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland & Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland

Joint diXa/Genedata workshop on “Understanding ‘omics’ data” organised by EMBL-EBI and Genedata at Basel, Switzerland

Dates of visit: 19 – 21 November 2013

I am currently a PhD student at International Hereditary Cancer Centre, Pomeranian Medical University, Szczecin, Poland under “International PhD Studies in Molecular Genomics, Transcriptomics and Bioinformatics in Cancer.” The aim of the study is “Influence of selenium concentration and variations in selenoprotein genes on cancer risk in CHEK2 mutation carriers and other unselected cancers.” My PhD subject requires efficient statistics to find the regulatory role of genetic and environmental factors in cancer patients with CHEK2 mutation. Moreover, I would like to pursue my further research on developing new bioinformatics and statistical pipelines for better and easy understanding of mechanisms of cancer biology.

Being near completion of a PhD, one always has a financial stringency to attend conferences and workshops. I would like to thank and highly appreciate EACR for accepting my application at an emergency level and granting me the Travel Fellowship. This workshop gave me a platform to learn basic and advanced statistical skills and its application in omics data analysis. I will definitely acknowledge EACR wherever needed.

Data interpretation is very important in any field of biology. The application of Genome Wide Association Study (GWAS) and Next-Generation sequencing technologies are widely used to detect biomarkers for different cancers and to understand the mechanisms of cancer biology. The data is huge and difficult to analyse and interpret. A very good statistical knowledge and basic programming is required for the interpretation of the data. Biologists with good statistical and computational skills always have an advantage in better understanding the data.

The workshop was organised by EMBL-EBI and Genedata under diXa project was focussed on the statistical applications to understand genomics and proteomics data. The workshop took place at Genedata, Basel, Switzerland where different nationalities were represented by 15 participants.

The first day session was opened by Dr. Lee Larcombe from European Bioinformatics Institute with a brief introduction about the EMBL-EBI and diXa project. The trainers responsible for the workshop were Arnd Brandenburg, Timo Wittenburger and Hans Gmünder. The sessions were divided as such to give rigorous theoretical lessons from basics to advance level statistics, simultaneously followed by practical sessions using existing data in “Expressionist software” from Genedata.

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The first day was primarily focussed on understanding different algorithms used to analyse gene expression data; NGS count data and raw profile data from mass spectrometry. The second day session includes data overview and mining methods; basics of statistics and experimental design and statistical tests followed by hands-on-session. The third day we had training on prediction and classification methods of biological data. The discussion and interaction among the group from different research areas was also very beneficial to understand the data in a different perspective. The trainers from Genedata, Basel were very open minded and knowledgeable to discuss any problems related to the subject. The short visit to attend this workshop with EACR sponsorship has opened new doors for my future career goals.
Rajendra Bahadur Shahi

Laboratory of Medical and Molecular Oncology, Department of Medical Oncology, Oncologisch Centrum, UZ-Brussel, Vrije Universiteit Brussel, Brussels, Belgium

Host institute: Computational Cancer Genomics Lab, Department of Bioinformatics and Computational Biology, Division of Quantitative Sciences, MD Anderson Cancer Center, Houston -TX, USA

Date of visit: 20 Nov 2012 - 20 March 2013

Next Generation Sequencing and Genomics of Lung cancer in Non-smokers

It has been evaluated that around 50 genomic mutations are found in human adult cancers, with variations within and in-between cancer types.

Some of these mutations are presumed more important as drivers of the malignant phenotype than others. These mutations encode for proteins that reside in approximately 12 pathways deregulated in cancer. The Epidermal growth factor receptor (EGFR) is a typical “cancer gene” that is often mutated in NSCLC. Interestingly, those activating mutations present a striking correlation with EGFR-TKI sensitivity. Thus, the presence of activating mutations in the kinase domain of EGFR is an important predictive marker for the treatment of NSCLC by reversible (i.e. erlotinib and gefitinib) inhibitors.

More than 40 different EGFR-TK activating mutations have been reported to date, including in-frame deletions and insertions as well as mis-sense mutations in the exons 18 to 21. In virtually all lung cancers in which an EGFR mutation was found, no second driver mutation is found among all of the genes known to be involved in lung cancer pathogenesis. It is also known that not all patients with EGFR activating mutations are equally susceptible to EGFR TKI. Upon treatment it is observed that after a remission of a few months to years, patients develop resistance by the emergence of a malignant clone with a second resistance mutation in the kinase domain or activation of subverting growth factor pathways.

Thus, there is a need of research to identify other mutations that cooperate with the EGFR mutations in the same or other pathways for the effective tailoring and discovery of new therapeutic targets that could be explored for co-targeting with mutant EGFR. The analogies in disease phenotype and response to targeted therapies in patients with an EGFR driven lung cancer and mice with a transgenic mutant EGFR gene suggests that this disease could have a relatively simple genotype compared to smoking related lung cancer.

Therefore it seems feasible to explore and analyse the cancer genome in full, at least at the exome level for other candidate driver mutations.

In this backdrop, being a wet-lab biologist naive to next generation sequencing (NGS), whole genome or whole exome analysis of heterogeneous tumours was a daunting but challenging task. However this was a very good opportunity to explore the genomic landscape of cancers. Thus, I was in dire need of comprehensive training under the supervision of high track lab in cancer genomics. My enthusiasm was further amplified when Prof Dr Ken Chen from Computational Cancer Genomics Lab - MD Anderson Cancer Center, a world leader in genomic structural variation analysis, accepted me as visiting student.

Dr Chen has extensive experience in analysis of genome of lung cancer, leukaemia and breast cancer as well as the designing, devolvement, and co-development of a set of computational tools such as BreakDancer, TiGRA_SV, CREST, PolyScan, SomaticSniper, and VarScan that have been widely applied to characterise individual and population genomics in various large-scale next-generation sequencing projects.

Apart from the rigorous lab work, the opportunity to attend departmental meetings, seminars and talk programmes and having face to face interaction with leaders in cancer genomics such as, Robert Weinberg, Ling Lin, Nicholas Navin were really outstanding and inspiring moments. Furthermore, it has also become a venue to meet people from different parts of the world with different cultural, educational backgrounds and with diverse ideas about research blended together for the search of penance for cancer.

A part of my visit would not be successful without the encouragement and supervision from my promoter Prof. Jacques De Greve, co-promoter Dr. Erik Teugels and colleagues. The other part is made more successful by the acceptance, guidance and help from Prof Dr Ken Chen and his lab members Yong Mao Fan Xian, Han Chen and Tenghui Chen. Well-deserved and hearty thanks to all of them.

The honour for the last part, with no doubt, goes to EACR for granting the Travel Fellowship Award and Vrije Universiteit Brussel for granting Doctoral School Travel Grants that played a pivotal role in accomplishing the goal of my PhD research and my career towards clinical cancer genomics. I hope that similar generous and prestigious travel grants form EACR will abridge different minds, people and labs around the world in the fight against cancer.

Left: Rajendra Bahadur Shahi
David Vetvicka

Institute of Biophysics and Informatics, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

Host Institute: Gray Institute for Radiation Oncology and Biology, Oxford University, United Kingdom

Dates of visit: 1 October 2012 – 3 January 2013

Our group has developed polymeric radionuclide delivery systems with multilevel targeting, where each targeting step potentiates the previous one: 1. Targeting of the whole polymeric system by Enhanced Permeation and Retention effect; 2. In the tumour tissue or in the endosome after internalisation the intercalator (ellipticine) bearing the radionuclide is released in its active form due to pH change; 3. The intercalator then concentrates the radionuclide into DNA in the cell nuclei.

We have previously shown that our non-radioactive iodinated derivative of ellipticine retains its intercalating and also cytostatic activity, therefore it has proven to be a potential candidate for the development of polymeric radionuclide delivery system. Nevertheless, studies preceding the EACR Travel Fellowship revealed that this particular ellipticine derivative doesn’t have the release properties anticipated and needed for a successful drug delivery. We have therefore designed and synthesised a new slightly different one, along with its polymer (Glycogene and poly-HydroxyPropylMetacrylAmide (pHPMA)) bound counterparts.

The in vitro drug release profiles for both polymeric carriers showed a similar trend - relatively fast release at pH 5.0 while the conjugates are stable both at pH 6.5 and pH 7.4. This means that the conjugate should be stable during transport in blood and the active intercalator should be released only after intercalation into cell in endosomes. Internalisation of the ellipticine derivatives and conjugates into cells was assayed with H2N cells on confocal microscope using the intensive ellipticine-fluorophore caused fluorescence and DAPI to stain cell nuclei. We have shown that free ellipticine derivative intercalate into cellular DNA (colocalisation with DAPI). Additionally polymer bound drug is efficiently released and also intercalates into DNA, and that is valid for both polymers used.

To assure the DNA-intercalation ability, the solution of the new ellipticinium derivative was titrated with the solution of the calf-thymus DNA and the fluorescence of the mixture was measured. As a result, the fluorescence emission of the solution gradually rises with the addition of DNA, which confirms the intercalation. Furthermore, we
tested antiproliferative activity of our compound on H2N cells. The IC50 values were not statistically significantly different when comparing ellipticine and its derivative on tested cell line. The cytotoxicity of the conjugates were not tested because we showed by HPLC that the free drugs in its original form were released from their conjugates after incubation in media.

Therefore, the data on in vitro cytotoxicity of the conjugates may be misleading due to significantly different concentrations of the drug released into the media during incubation with the cells (pH 7.4) compared to the in vivo situation. This is because in an in vivo situation, the system is opened, i.e. the released drug is being continuously removed by internalisation into cells or diffusion out of the tumour tissue.

Visiting the Experimental Radiation Therapeutics group enabled me to benefit from having an experience in a different and truly stimulating environment.

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From left to right: Bart Cornelissen, David Vetvicka, Sarah Able and Christopher Hillyar
Necrosis has for a very long time been described as a direct cause of cell death in many cases of human diseases with the term necrosis referring to an accidental, uncontrolled event. Accumulating evidence confirmed that necrosis can also occur in a regulated manner. A novel, necrotic-like, caspase-independent programmed cell death form has been recently described and termed as necroptosis. This type of cell death process requires the kinase activity of receptor-interacting protein kinase 1 and 3 (RIPK1, RIPK3) and mixed lineage kinase domain-like protein (MLKL). In the recent years the role of necroptosis in various pathologies like ischemia-reperfusion in neuronal- and myocardial tissues and in traumatic brain injury was pointed out. Moreover necroptosis as being a possible back-up mechanism of apoptosis may represent a new perspective in anticancer therapy.

Previously we studied the nature of the switch mechanism between apoptosis and necrosis and investigated the intrinsic apoptotic pathway in staurosporine-treated (generally accepted model compound for inducing intrinsic apoptosis) U937 human monocytic cells. During my PhD our aim was to see if the staurosporine-induced necrotic signalling pathway differs from the TRAIL (TNF-related apoptosis-inducing ligand)-induced one in spite of the fact that necroptosis is generally defined as a death receptor-triggered cell death pathway. We confirmed that staurosporine triggers necroptotic cell death under caspase-compromised conditions in U937 cell line. Interestingly we found that RIPK1 inhibitor withheld only the early phase of the staurosporine-triggered necroptosis in, meanwhile MLKL inhibitor showed complete arrest during longer incubation time; however these inhibitors have a similar effect on death receptor-initiated necroptosis. Based on our last (unpublished) results we hypothesise that autophagy may play a role parallel or sequential to the receptor-independent necroptosis.

For this purpose I contacted Professor Eleonore Fröhlich at the Center for Medical Research Medical University of Graz, Austria. The Microscopic Core Facility led by Professor Fröhlich provides equipment and expertise in light and fluorescence microscopic techniques. With the help of her team I aimed to reveal the contribution of autophagy in the staurosporine-induced necroptotic cell death mechanism. Since the presence of autophagosomes/autolysosomes could be the result of either increased flux of autophagy or blockage of autophagosome maturation (lysosomal fusion and degradation), I tested the effect of rapamycin, a specific inhibitor of mTOR, autophagy inducer, of chloroquine, an inhibitor of lysosome-mediated proteolysis and of starvation on staurosporine-induced apoptotic and necroptotic cells in presence and absence of necroptosis inhibitors.

The most recent studies shed light on the downstream events of necroptosis. It seems RIPK3 interacts with the mitochondrial phosphoglyceratemutase (PGAM5) that promotes mitochondrial fission and subsequently cellular necroptosis through the dephosphorylation of (Dynamin-related protein 1) DRP1. Therefore I monitored the state of mitochondria with MitoTracker Orange comparing again the effect of RIPK1- and MLKL inhibitors under necroptotic and apoptotic conditions. Moreover I followed the localisation of PGAM5 and DRP1 proteins with fluorescent antibodies.

During my three months stay I established preparation and fixation methods for the above listed immuno and organelle specific stainings on a suspension cell line and became familiar with the confocal laser scanning microscopy which was a dream of mine for a long time. The results raise questions that can be a subject of further investigation.

I am sincerely grateful to the European Association for Cancer Research for supporting me with this fellowship. The stay enabled me to acquire a technique which is indispensable for cell death research. I am deeply grateful to Professor Fröhlich and all member of the CF-MI team for welcoming me into their group, for their help, support and hospitality, and for the friendly atmosphere. I hope this visit initiates further promising cooperation in the close future.
Stanislav Avdieiev

Institute of Molecular Biology and Genetics NAS of Ukraine, Department of Biosynthesis of Nucleic Acids, Kiev, Ukraine.

Host Institution: Institut Gustave Roussy, Chromatine, Developpement et Cancer, UMR-8126, Villejuif, France.


A search for new treatment modalities of mantle cell lymphoma and glioblastoma

Despite significant progress in radio- and chemotherapy, and the growth of knowledge in the field of tumour biology, it is a constant search for new drugs that either destroy tumour cells or facilitate patient state in traditional therapy. Glioblastoma (GB), the most common and lethal form of central nervous system malignant tumour, and mantle cell lymphoma (MCL), a rare but aggressive entity of non-Hodgkin lymphoma, are known to be highly therapy resistant. Development of new treatment modalities, especially those based on polytargeted therapy is desperately needed for these diseases.

The present study was initiated to analyse the effect of compounds with distinct chemical nature, namely bradykinin (BK) antagonists and azolidinones on proliferation of different types of malignantly transformed cells: 293 cells, stably transfected by CHI3L1 oncogene (293_CHI3L1), human glioblastoma cells U373, rat glioma cells C6, mantle cell lymphoma (MCL) cells Granta, JeKo, Mino, and UPN1.

BKM-570 revealed significant antiproliferative activity in 293_CHI3L1 and U373 cells with IC50 3.8 μM and 3.3 μM, correspondingly, and strongly decreased extracellular signal-regulated kinases 1/2 (ERK1/2) and protein kinase B phosphorylation. Prominent growth inhibition was demonstrated also by BKM-570 on C6 cells with IC50 0.6 µM.

So, BKM-570 may repress tumour cells growth and its effect is mediated by the modulation of MAPK- and PI3K- signalling cascades.

5-arylidene-2-amino-4-azolone Les-28 lead to the substantial cell growth inhibition: IC50 of Les-28 was 0.16 µM for 293_CHI3L1 cells and 15 µM for U373 cells. Pyrazoline substituted thiazolone Les-4523 revealed high activity on C6 cells with IC50 0.13 µM. 50% inhibition of Mino cells growth were observed after treatment by Les-4523 at 1.58 µM. Moreover, we showed that a combination of BK antagonist and Les-28 resulted in significant growth reduction of Mino cells in contrast to very low activity while applying compounds individually.

Thus, antiproliferative properties of two different classes of molecules were shown in several distinct in vitro models of malignant transformation. Further investigations of molecular mechanisms of BK antagonists and azolidinone derivatives action and pre-clinical studies using animal models are needed for the evaluation of these compounds as new anti-cancer drugs.
What are Travel Fellowship Awards?

Co-sponsored by the Association for International Cancer Research (AICR), EACR Travel Fellowship Awards are designed to assist student and early-career researchers to further their research through learning and collaboration.

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The page contains a link to the application form in the EACR Members Area: you will need to log in as a member to download it.

Any further questions? Please email the EACR Secretariat: EACR@nottingham.ac.uk

Anita Rozsas

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Dates of visit: 1 May - 30 June 2013

Despite surgery and the development of novel therapeutic protocols, many malignant pleural mesothelioma (MPM) cases relapse and become fatal. Although MPM is distinguished by its propensity for metastatic spread via lymphatics to regional lymph nodes (LNs) and “N status” is a major determinant for the prognosis of patients with MPM, current strategies for LN staging are not sensitive enough. Consequently, there is a need for a better understanding of lymphangiogenesis of MPM. Unfortunately, because no specific markers for lymphatic endothelium were available until recently, our knowledge of the lymphatic system of malignant tumours, and especially of MPM, lags far behind that of the vascular system.

During the 4-month period of the project we were able to identify lymphatic capillaries in anti-D2-40 labelled human MPM samples. However, because podoplanin (recognised by the D2-40 antibody) is also expressed by the mesothelioma cells, in our further experiments the distinction between blood and lymphatic vessels in human MPM samples will be made by using an antibody against human LYVE-1.

We also analysed the available gene expression data of five established mesothelioma cell lines to identify the lymphangiogenic factors that may be synthesised by the mesothelioma cells and thus contributing to lymphangiogenesis within the tumour tissue.

In order to study lymph vessel formation in vivo, we also established an orthotopic model of human MPM in immune-compromised mice. The tumours displayed a growth pattern resembling the human cases including the locally invasive nature of MPM cells. Thus this model system provides an excellent preclinical platform to study and interfere with the most devastating feature of MPM, namely local invasion and rapid local recurrence.

The next step of our experiments will be the visualisation of the lymph and blood vessel capillary networks in this animal model by confocal microscopy.

The EACR fellowship was very useful because of the important ideas and advice I received from experienced clinical oncologists and cancer researchers involved in cancer research, diagnosis and therapy at the Medical University of Vienna. I would like to thank my host supervisor, Prof. Walter Klepetko, for guiding and supporting me over the fellowship period. I would also like to thank the EACR for their funding and support.