

EACR Travel Fellowship Reports

Travel Fellowships co-sponsored by EACR and AICR are awarded to 'Young Cancer Researchers' to provide additional support for their specialist research work in an appropriate centre of excellence

Madalina Schmidt



Home Institute –Santa Maria Children's Hospital , Iasi , Romania

Host Institute –Giannina Gaslini Institute, Hemato-Oncology Department, Genova , Italy
1st Sept 2007-28th Feb 2008



The main objective of the project for my EACR Travel Fellowship was to acquire deep knowledge about the techniques for studying genetic polymorphisms in genes that encode detoxifying enzymes in the patients with acute leukemia and bone marrow failure and to be able to implement these techniques at my home Institute.

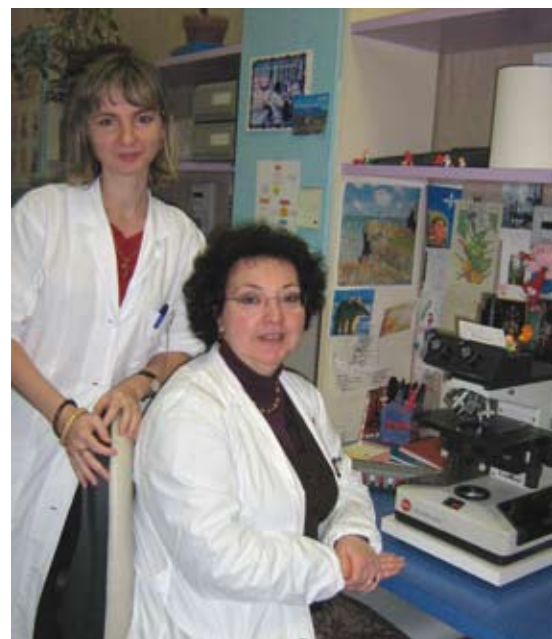
The detoxifying enzymes can influence the efficacy and toxicity of chemotherapy and also the risk for the development of leukemia. Acute lymphoblastic leukemia, the most common pediatric cancer, is a complex disease determined

by a combination of genetic and environmental factors. Important environmental carcinogens are detoxified through the glutathione S-transferases (GSTs) system which includes in humans 4 major subfamilies (α, μ, θ, π). Each of these subfamilies is composed of several members, some of which display genetic polymorphism (GSTM1, GSTT1, GSTP1). The genes coding for GSTT1 and GSTM1 exhibit a deletion polymorphism and the GSTP1 gene displays a polymorphism that causes a change in amino-acid sequence at codon 105.

During my stage in the Hematology Unit headed by Dr Carlo Dufour I attended the Leukemia/Bone Marrow Failure laboratory where has been recently performed one of the largest study on GSTT1 and GSTM1 genotypes and the largest study on GSTP1 genotypes in children with acute lymphoblastic leukemia (323 patients) about the association between the risk for childhood ALL and GSTs polymorphism. (Pigullo et al , 2007, Leukemia, 21, 1122-1124).

Due to the expert tuition of Dr. Marina Lanciotti I have learnt basic techniques of molecular biology such as DNA extraction from bone marrow and peripheral blood, PCR (polymerase chain reaction) and DNA digestion with restriction enzymes. I gained knowledge about the statistical analysis of data, I have been involved in discussions about the results of this study which do not sustain a role of GST genotypes in genetic susceptibility to childhood acute lymphoblastic leukemia.

It has been for me a great opportunity to spend 6 months in one



Madalina with Francesca Scuderi

of the leading research Institutions in Italy, in the groups of Dr Dufour and the lab space. I could see a different organisational structure and I hope I will be able to start a future collaboration between Giannina Gaslini Institute and my home Institute.

I would like to express my gratitude to the European Association for Cancer Research for this fellowship which represented a great professional experience that stimulated and encouraged my career development.

Madalina Schmidt



Francesca Fioredda, Madalina and Giovanna Valenti

Olivier De Wever



Home Institution: University of Ghent, Belgium

Host Institution: National Human Genome Research Institute, USA

I'm currently a post-doctoral researcher in the Laboratory of Experimental Cancer Research at the University of Ghent, Belgium. One of my projects focuses on the study of Rab proteins in cancer.

Rabs are members of the Ras-like small GTPase superfamily of proteins and constitute the largest branch of this family, with over 60 members in the human genome. Like other small GTPases, Rab proteins are initially synthesized as soluble proteins; for membrane attachment they require a posttranslational prenylation. Rab GTPases are localised to the cytosolic face of the limiting membrane of organelles, where they function as regulators of distinct steps in membrane traffic pathways. They cycle between a GTP-bound active and a GDP-bound inactive form, assisted by GEFs (guanine nucleotide-exchange factors) and GAPs (GTPase-activating proteins) respectively. In the GTP-bound form, the Rab GTPases recruit specific effector proteins through which they regulate vesicle formation, actin- and tubulin-dependent vesicle movement and membrane fusion and fission. It has been described that Rab GTPases play a physiological role in normal epithelial cells.

We wanted to investigate whether they play a role in cancer development and progression. Research on the biological function of up-or down regulated Rab proteins in tumour progression could open new perspectives in cancer treatment. The Rab family that we study are members of the Rab3 and Rab27 family. Our preliminary results presented at the ASCB meeting 2006 showed that transient and stable overexpression of Rab27 in MCF-7/AZ breast cancer cells stimulated invasion in vitro. Currently we work on the molecular mechanisms of Rab27-induced invasion.

The Laboratory of Dr. Gahl, NHGRI,

NIH, US, is experienced in the study of RabGTPases and vesicle trafficking. Thanks to the 'EACR Travel Fellowship', we were able to combine this know-how with the knowledge of our laboratory on functional assays to study cancer invasion and to establish a interdisciplinary international collaboration. During the 6 weeks I spent at Dr. Gahl's laboratory I was assisted by Dr. W. Westbroek, Dr. D. Maynard and Dr. A. Hendrix, three outstanding researchers who introduced me to several state of the art techniques including confocal microscopy, molecular cloning and mutagenesis.

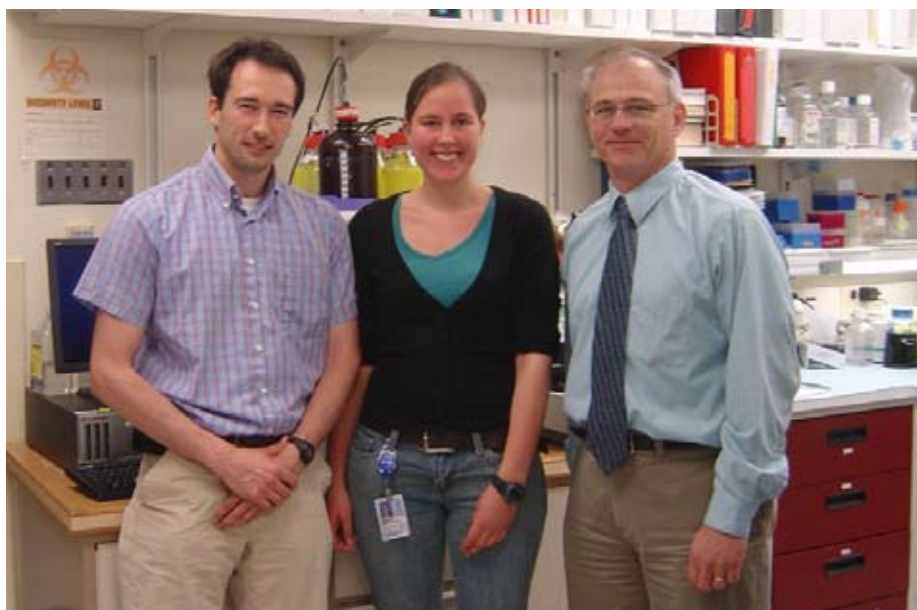
To verify whether the Rab27-induced invasion of breast cancer cell lines is dependent on its GTP/GDP cycle we developed dominant negative and constitutive active mutants of Rab27 via site directed mutagenesis. Via a collagen type I invasion assay and a Matrigel invasion assay, we show that the transient overexpression of dominant negative Rab27 is not able to induce invasion of MCF-7/AZ, while transient overexpression of constitutive active Rab27 indeed induces invasion. The GTP/GDP cycle is necessary for Rab27 induced invasion of MCF-7/AZ breast cancer cells. These results are confirmed with siRNA's against Rab27.

Next we wanted to research the molecular mechanism of Rab27 induced invasion. Since it has been described that Rab27 is involved



in actin-dependent transport and secretion of lysosomes and lysosome-related organelles, we were interested whether Rab27 overexpression influences the extracellular pH. Indeed, confluent MCF-7/AZ cells overexpressing Rab27 reach a more acidic extracellular pH (pH = 6,8) compared to MCF-7/AZ cells (pH = 7,0). Confocal microscopy showed partial co-localization of Rab27 and cathepsin B at cell-cell contacts or at cellular extensions. Preliminary results indicate that the Rab27 conditioned acidic pH is responsible for the activation of cathepsin B, which indeed might explain the Rab27 mediated effects on MCF-7/AZ. Further research is ongoing to reveal underlying mechanisms and physiological relevance.

In conclusion, I learnt a lot about how to do state of the art science and how to supervise people. Thank you EACR for this opportunity!
Olivier De Wever



Olivier De Wever with Dr An Hendrix, who will continue the Rab research at NIH with an EACR travel fellowship this year, and Dr William Gahl, head of the host laboratory

Vilde Drageset Haakensen



Home Institute: The Norwegian Radium Hospital, Oslo, Norway

Host Institute: University of Southern California, USA



The molecular basis for mammographic density
Breast cancer is a common disease, and incidence increases with age. Mammographic densities are variations of healthy breast tissue and refer to the amount of fat, connective tissue and epithelial tissue in the female breast². A high percentage of mammographic density confers a consistent four- to six fold risk of developing breast cancer^{2,3,5,6} and has been proposed as a possible surrogate endpoint for breast cancer¹.

The molecular basis for mammographic density is largely unknown. We are looking at gene expression patterns, both in peripheral blood cells as well as the breast tissue in women with high and low mammographic density in order to identify genes correlating with this phenotype. We are performing expression analyses in core biopsies and blood from 121 healthy women with varying degrees of mammographic density and 65 women with breast cancer. We are trying to identify gene expression patterns corresponding with different mammographic densities. Each woman included in the study has also answered questions about use of hormone therapy, parity and family history of breast cancer. For the expression analysis we have used the Agilent oligo 44K

microarray. I did these analyses in the Department of Genetics the Norwegian Radiumhospital headed by Prof AL Børresen-Dale, and the analysis has been completed. We have collected and scanned most of the mammograms and the questionnaires are loaded into a database. Professor Giske Ursin at USC is an epidemiologist with extensive experience on mammographic density analysis and hormones in relation to breast cancer. Through the support of the EACR Travel Fellowship scheme, I had the opportunity to stay with her for one week of intensive work on the data. During this week I first got an introduction to the computer assisted estimation of mammographic density as a percentage of the total breast. This method is established, and previously described⁴. In brief, the cranio-caudal mammograms are scanned. The operator then draws the outline of the entire breast. The computer then highlights the dense areas, and the operator verifies the densities to assure that artifacts are not included in such a way that they

that there seems to be an expected correlation between some of the hormonal parameters and degree of mammographic densities. This indicates that the methods used give representative findings.

Using what I learned during my stay at USC, we will finalize the combined analysis of the expression arrays, the mammographic densities and the questionnaire data this fall. As well as being useful for this particular study, the methodology I learned and the data retrieved will in the years to come be combined with many other analyses applied to this same dataset like large scale SNP analyses.

I am very grateful to the EACR for the Travel Fellowship, and would like to thank the committee for supporting our project! I also want to thank Prof. Ursin for all the time she spent with me and Eunjung Lee for being so patient with my programming! Now I'm looking forward to applying newly acquired skills to find out more about the biology of mammographic density! *Vilde Drageset Haakensen*



Åslaug Helland and Marit Muri Holmen with Vilde

alter the total area of dense tissues. The percentage mammographic density is then the ratio of the dense area on the total breast area. Together with Prof. Ursin and Dr Åslaug Helland, one of the project supervisors, we discussed the strategy of how to integrate results from expression arrays with data from the questionnaires and from the mammograms. I was introduced to programming of statistical software used for data analysis, and the preliminary computer analysis of the relation between hormonal data and mammographic density was started. The results of the analysis are not yet ready. What we do see from the first, preliminary analyses is

1. Boyd, N. F., Lockwood, G. A., Martin, L. J., Knight, J. A., Byng, J. W., Yaffe, M. J., and Tritchler, D. L. Mammographic densities and breast cancer risk. *Breast Dis.*, 10: 113-126, 1998.
2. Oza, A. M. and Boyd, N. F. Mammographic parenchymal patterns: a marker of breast cancer risk. *Epidemiol. Rev.*, 15: 196-208, 1993.
3. Saftlas, A. F. and Szklo, M. Mammographic parenchymal patterns and breast cancer risk. *Epidemiol. Rev.*, 9: 146-174, 1987.
4. Ursin, G., Astrahan, M. A., Salane, M., Parisky, Y. R., Pearce, J. G., Daniels, J. R., Pike, M. C., and Spicer, D. V. The detection of changes in mammographic densities. *Cancer Epidemiol. Biomarkers Prev.*, 7: 43-47, 1998.
5. Ursin, G., Ma, H., Wu, A. H., Bernstein, L., Salane, M., Parisky, Y. R., Astrahan, M., Siozon, C. C., and Pike, M. C. Mammographic density and breast cancer in three ethnic groups. *Cancer Epidemiol. Biomarkers Prev.*, 12: 332-338, 2003.
6. Warner, E., Lockwood, G., Tritchler, D., and Boyd, N. F. The risk of breast cancer associated with mammographic parenchymal patterns: a meta-analysis of the published literature to examine the effect of method of classification. *Cancer Detect. Prev.*, 16: 67-72, 1992.

Kim
De
Keersmaecker



Home institution: Human Genome Laboratory, Department of Molecular and Developmental Genetics-VIB11, K.U.Leuven, Leuven, Belgium

Host institution: Center for Molecular Medicine of the Austrian Academy of Sciences, Director's Group, Vienna Competence Center, Vienna, Austria

Dates: June-July 2007

Knowledge of NUP214-ABL1 interaction partners, and comparison of NUP214-ABL1 interaction partners with those of BCR-ABL1 might provide useful clues to elucidate the mechanism of activation of NUP214-ABL1. In addition, the identification of NUP214-ABL1 interaction partners could provide new insights in NUP214-ABL1 downstream signalling, and for designing targeted therapy for NUP214-ABL1 positive leukemia.

In the past, Dr. Superti-Furga and Dr. Hantschel have made major contributions towards a better understanding of the regulation

NUP214-ABL1 expressing T-ALL cell line. Dr. Hantschel and I performed the large scale NUP214-ABL1 immunoprecipitation experiment and prepared the samples for mass spectrometric analysis. In addition, we have pulled-down NUP214-ABL1 and its interaction partners with nilotinib and dasatinib, two second generation ABL1 kinase inhibitors. Also this immunoprecipitation sample will be analyzed by mass spectrometry. Comparison of the results of these different experiments should allow us to select the most important NUP214-ABL1 interaction partners. For these proteins, we will confirm interaction with NUP214-ABL1 in independent experiments and investigate the relevance of these interactions for NUP214-ABL1 activity and transforming capacity.

As a small side project during my stay, we have compared the *in vitro* sensitivity of BCR-ABL1 and NUP214-ABL1 to the ABL1 kinase inhibitors imatinib and dasatinib and have compared the substrate affinity and maximal velocity of the kinase reaction for these proteins. The results we obtained in this way nicely complement the results I had previously generated in my home institution in Leuven.

I would like to thank EACR for their support as this allowed me to perform a few experiments which could provide critical new elements in understanding activation and oncogenic capacity of the NUP214-ABL1 fusion protein.

Kim De Keersmaecker



“Identification of NUP214-ABL1 interaction partners by mass spectrometry.”

NUP214-ABL1 is a constitutively active ABL1 tyrosine kinase fusion protein found in 6% of T-cell acute lymphoblastic leukemia (T-ALL) patients. In the past, we have extensively compared the functional properties of NUP214-ABL1 with the more common BCR-ABL1 oncogene both in *in vitro* cellular systems as well as in *in vivo* mouse models. We showed that NUP214-ABL1 is a weaker oncogene compared to BCR-ABL1 and that its cellular localization to the nuclear envelope is important for its activation. However, we still do not completely understand how the constitutive kinase activity of NUP214-ABL1 is initiated.

of ABL1 and BCR-ABL1 kinase activity. Recently, their group in Vienna has optimized a technique by which BCR-ABL1 interaction partners were identified by large scale immunoprecipitation of BCR-ABL1 from human BCR-ABL1 expressing myeloid cells, followed by identification of all proteins in the immunoprecipitate by mass spectrometry. This technique allowed them to map the BCR-ABL1 interactome. Their technique is also applicable to NUP214-ABL1 as the immunoprecipitation is performed with an anti-ABL1 antibody that also reacts with NUP214-ABL1.

The EACR grant I received allowed me to visit this group in Vienna and to collaborate with Dr. Hantschel to apply this technique on a human



Kim with Oliver Hantschel

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Research

AICR
Cancer knows no boundaries.
Fortunately, neither do we.

Vitalina Gryshkova



Home Institution: Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine

Host Institution: University College London, London, UK

19 May to 19 June 2007



“Investigation of novel potential ovarian cancer antigen sodium-phosphate cotransporter Napillb”

I am a second year PhD student at the Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine. The main objective of my PhD project is to characterize the sodium-phosphate cotransporter NaPiIIb as a novel marker of ovarian cancer.

Ovarian cancer is the most common gynaecological cancer that is usually far advanced before it is diagnosed and is associated with poor prognosis and survival rate. So far, only a few tumour-associated markers and antigens specific for ovarian cancer have been identified. MX35 antigen is one of them. It was originally found nearly 20 years ago at Memorial Sloan-Kettering Cancer Center as an unknown antigen through the use of monoclonal antibodies termed MX35 (New-York, USA). Although the MX35 antigen had not been identified so far, the

clinical trials with humanized MX35 antibody and Fab2 fragments of mAb MX35 suggest their therapeutic potential in patients with ovarian cancer.

In the frame of a collaborative consortium on molecular cloning of MX35 antigen, our laboratory (IMBG, Kyiv, Ukraine) has recently identified the sodium-dependent phosphate transporter NaPiIIb (SLC34A2, NaPi3b, Npt2), by screening of an OVCAR3 cDNA expression library, as the potential MX35 antigen. Later on, NaPiIIb was also confirmed as the MX35 antigen by affinity purification of MX35 protein from mammalian cells, followed by mass spectrometry (New York, USA).

During my visit to the University College London I have cloned the largest extracellular domain of NaPiIIb (aa188-361) in a baculovirus transfer vector and generated recombinant baculovirus. The expression of recombinant GST/Napillb extracellular domain was tested by infection of insect cells and affinity purification on Glutathione Sepharose column. Using this strategy, I managed to purify milligram amounts of GST/NaPi2IIb-EC domain. The purified protein will be used in our future studies for the generation of specific monoclonal and polyclonal antibodies which would be important to further investigate tumour-associated functions of NaPiIIb.

I would like to thank EACR for the travel fellowship, which gave me an excellent opportunity to gain further experience in modern techniques, such as baculovirus generation, at University College London. Also I am very grateful to Prof. Ivan Gout for his time and support.

Vitalina Gryshkova



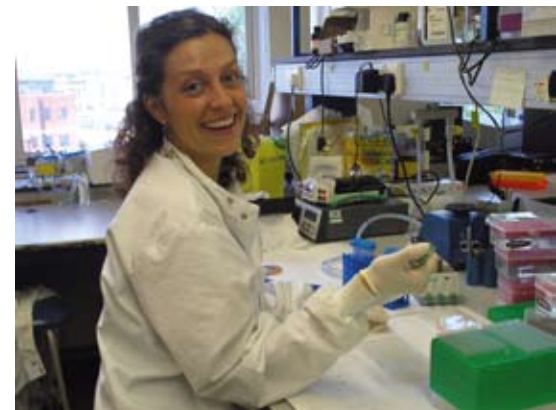
Mimosa Mortarino



Home Institution: Molecular Therapies Unit, Experimental Oncology Department, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milano, Italy

Host Institution: Bone & Joint Research Unit, William Harvey Research Institute, Barts and The London, Queen Mary's School of Medicine, London, United Kingdom

April 2007- July 2007



Prostate carcinoma (CaP) represents the second most common cause of male death due to neoplasia in industrialised countries. In the great majority of cases, at the onset, it is an organ-confined disease, which can be efficiently treated by surgery and radiotherapy. However, in one-third of the patients, the disease evolves into a more aggressive, metastatic and androgen-independent form, which fails to respond to even multi-drug treatment. To date, we still lack early diagnosis and/or prognostic tools.

Therefore, the discovery and validation of new molecules (tumour-associated antigens) and the antibodies directed against them, becomes highly important, providing the clinic with new diagnostic tests and potentially also novel therapeutic reagents.

The determination of the antigen profile for this specific type of tumour, and the identification of novel protein markers, will be performed using a post genomic approach by uniting

two technologies, namely “antibody phage display” and proteomics. During my experience in London, which is not yet completed, my work has focused on the creation of an antibody library from patients with CaP. Thanks to the EACR Travel Fellowship, I worked in the laboratory of Dr. Ahuva Nissim, Bone & Joint Research Unit, William Harvey Research Institute, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London, UK. Dr Ahuva Nissim is an expert of antibody library and phage display technology and she helped me with her expertise in the construction of the antibody library in a scFv format, from prostate cancer patients.

Selection of patients: To reach a wide variability of immune response against different antigen profiles, correlated with the stage of the disease, and in agreement with clinical staff of my Italian institute, we identified three groups of patients: 1) patients with diagnosed CaP confined to the organ and not yet treated (blood collected pre-surgery); 2) patients with no evident disease after first-line treatment (blood collected at least one month after last treatment); 3) patients with metastatic disease (blood collected pre-radiotherapy).

Library construction: Peripheral Blood Lymphocytes (PBL) have been separated by Ficol and total

RNA has been extracted. With retrotranscription and PCR, we have amplified variable domains of IgG genes using specific primers for all the human germlines, to obtain a repertoire as large as possible.

Two rounds of amplification have been performed. In the first one, we used specific primers for different V gene families and, in the second one, we used primers with restriction enzyme recognition sites.

We have made enzyme digestions of the variable VK and VH domains of the immunoglobulin and undertaken their purification.

The VK light chains have then been cloned into the phagemid vector pIT2 (kindly provided by Dr. Ahuva Nissim) and have been transfected in E.Coli cells obtaining an antibody VK library. Afterwards, VH have been cloned in the VK library, obtaining a scFv antibody library with a good diversity.

Now we are going to start with the antibody library selection on a well known tumour-associated antigen as a library validation. Moreover, I will also have the possibility to select and compare different antibody libraries, including the Nissim library (Nissim et al. EMBO J, 1994), which has already been a suitable source of specific human scFv antibody fragments.

Mimosa Mortarino

Oksana
Mayevska



Home Institute: Institute of Cell Biology, National Academy of Sciences of Ukraine

Host Institute: Cardiff School of Biosciences, Cardiff University, UK

July-August, 2007



“Study of intracellular compartmentalisation of adaptor protein Ruk/CIN85 isoforms in various types of normal and cancer cells”

Adaptor protein Ruk/CIN85 is composed of three SH3 domains, Pro- and Ser-rich sequences, and a C-terminal coiled-coil region. It was found that a Ruk/CIN85-dependent network plays important roles in receptor tyrosine kinase signalling, apoptosis and rearrangement of actin cytoskeleton and cell adhesion. The main aim of the work was to study intracellular localization of the full-length Ruk/CIN85 form in human breast adenocarcinoma MCF-7 cells that stably overexpress non-tagged and GFP-fused Ruk/CIN85, under the action of different agents which can disturb vesicular trafficking.

Realization of this project at Dr. Buchman’s laboratory at Cardiff School of Biosciences was an excellent opportunity because the host department possesses all the necessary equipment to conduct comprehensive researches, confocal



Mimosa with Dani and Stella

and electron microscopy in particular. The expression level of full-length Ruk/CIN85 form in MCF7 cells is very low. So, we used this cell line as a model to generate MCF-7 cells with a stable overexpression of non-tagged and GFP-fused full-length Ruk/CIN85. The MCF-7 2E11 clone with a high expression level of GFP-Rukl/CIN85 (this clone was named MCF7RukL/CIN85-GFP) was used for further investigations.



It was shown that the main part of GFP-fused RukL/CIN85 protein in MCF-7 cells revealed by confocal microscopy was located in the cytoplasm of MCF-7 cells. The local accumulations of this protein occurred near to cytoplasmic and nuclear membranes of single cells. We didn't observe visible changes in GFP-fused Rukl/CIN85 protein or its release from vesicular structures after the addition of the actin filament depolymerizing agent cytochalasin D to MCF7Rukl/CIN85-GFP cells. Insignificant changes of CIN85/RukL localization, observed as small dots in single cells, were detected after addition of bleberdin A (a transport

protein inhibitor in Golgi apparatus). The appearance of multiple small dots of GFP-fused RukL/CIN85 protein was revealed in the cytoplasmic and nuclear membranes of MCF7Rukl/CIN85-GFP cells incubated with the microtubule destabilizer vinblastine.

It should be noted that two types of structures containing GFP-fused RukL/CIN85 protein were observed. The above mentioned agents didn't affect structures with intensive accumulation of adaptor protein (its location was stable). We speculate that these formations can be referred to as separate structures. Other structures, characterised by small dots of protein, were very labile and they could be related to certain cell organelles or cytoskeletal elements, microtubules in particular. The detailed search for microtubule elements which can interact with CIN85/Rukl and identification of adaptor protein domains involved in this binding will be studied further using different approaches.

I would like to thank the European Association of Cancer Research for the financial support of my project, making it possible to do these studies. This EACR Fellowship gave me a perfect opportunity to advance my knowledge and to get practical skills in confocal microscopy, which will be needed for my further experimental work. I also thank Prof. Buchman VL and his collaborators for the friendly atmosphere in the laboratory and their assistance in the performance of confocal analysis.
Mayevska Oxana



Timomir Dodev, Fumitaka Ichioka, Vladimir Buchman, Oxana Mayevska and Nataliya Ninkina

Petra
Knizetova



Home Institution: Laboratory of Molecular Pathology, Institute of Pathology, Faculty of Medicine, Palacky University, Olomouc, Czech Republic

Host Institution: Research Institute in Healthcare Science, School of Applied Sciences, University of Wolverhampton, Wolverhampton, United Kingdom

22 February 2007 – 20 April 2007



Because of poor prognosis and resistance to chemo- and radiotherapy, brain tumours are amongst the most devastating sorts of human cancer. One of the most malignant sorts, glioblastoma multiforme (GBM), is associated with particularly poor prognosis and short survival. The development of targeted molecular therapy presents new opportunities, as well as new challenges. Traditionally, cancer patients are treated with radiation therapy and cytotoxic agents that aim to have a greater effect on proliferating cancer cells than they do on non-cancerous cells. For some types of cancer, this approach has translated into increased survival. The use of chemotherapy for patients with malignant brain tumours has evolved over the past decades from the use of non-specific cytotoxic

agents towards the clinical testing of drugs which may affect more specific targets on the tumour cell. Unfortunately, the effect on brain tumour patients has been modest, and median survival has remained largely unchanged over the past decade.

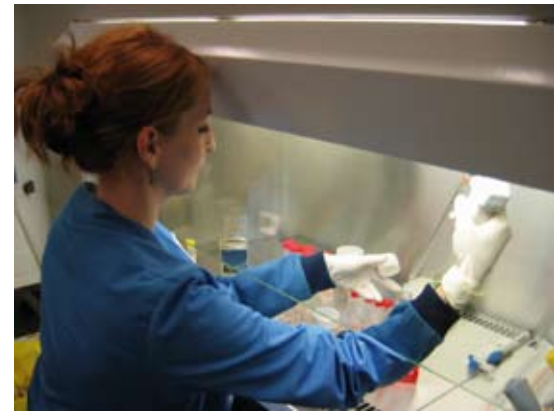


Recently, a sub-population of cancer stem-like cells (CSCs) has been identified in adult and pediatric brain tumours. They express neural stem cell markers like nestin, CD133, Sox2, and musashi-1, grow as neurospheres in serum-free conditions and produce tumours reminiscent of the tumours of origin when implanted into the brains of

immunodeficient animals.

My PhD project, dealing with molecular pathology of brain tumours, involves isolation and culture of primary glioma cell lines derived from surgical biopsies. As my future plans are to study DNA damage repair response in brain tumour derived CSCs, the EACR travel fellowship gave me a great opportunity to acquire practical experience and new technical skills under the supervision of Dr. Sarah Brown and Professor John Darling. I had met Professor John Darling, who is a group leader of the Neuro-oncology Research Group at the University of Wolverhampton, at a conference organised by my home institute in May 2006. After very impressive and fruitful discussion on brain tumours, he offered me the opportunity to come to his laboratory and improve my technical skills in growing brain tumour primary cultures and separating CD133 positive cells from these short-term cell lines. I greatly appreciated the time spent in his research group and prolonged my stay for two months.

I adapted a method of isolation and culture of CD133 positive CSCs from short-term as well as already established glioma cell lines. As the number of CD133 positive cells in these cultures is small, generally in the range from 0.2 to 2% of the total cell population, it was necessary



to develop short-term assays of apoptosis to analyze the drug sensitivity of these cells. Using assays that measured caspase 3 and 7 activity and DNA fragmentation I was able to show that CD133 positive cells were more resistant to CCNU (lomustine), a drug commonly used to treat patients with GBM, than cells separated from the same cell lines that did not express CD133. Our results demonstrated that CSCs are stably entrapped within cultures derived from surgical biopsy material from patients with GBM and such cells are retained for very long periods of culture in established cell lines from GBM. Such cells are also resistant to commonly used anticancer drugs in vitro. This might account for the relative ease with which cultures produced from GBM surgical specimens establish in vitro and that studies aimed at specifically targeting these cells might result in more effective therapies.

Petra Knizetova



Solomon, Sarah, Petra, Dona and Elton

Alessandro Salvi



Home Institution: University of Brescia,
Department of Biomedical Sciences and Biotechnology,
Division of Biology and Genetics, Italy

Host Institution:
"XI International Workshop on Molecular & Cellular Biology of Plasminogen Activation"
Stockholm, Sweden

Dates: 6/16/2007-6/20/2007



The travel fellowship awarded by EACR gave me the chance to attend the "XI International Workshop on Molecular & Cellular Biology of Plasminogen Activation" held in Stockholm, Sweden. During the workshop several aspects of Plasminogen activation were examined both in oral and poster sessions. The workshop focused on the role of plasminogen activators in development, neurobiology, vascular biology evaluating molecular aspects in processes such as cell migration, adhesion, and proliferation/apoptosis. During the presentations, the mechanisms and biological roles of other protease systems in signalling, intracellular and extracellular proteolysis were also discussed. The session dedicated to anti-cancer approaches against the dysregulation

of plasminogen activation components was very interesting because it was directly related to our own area of interest in cancer.

For many years our laboratory has been studying the plasminogen activation system and mainly we have looked at uPA/uPAR system. uPA binding to its receptor uPAR governs many biological processes such as signal transduction pathways and proliferative processes. It is responsible for plasminogen conversion into plasmin which in turn controls extracellular matrix turnover, cellular migration and growth factors activation.

Previous results obtained at the Division of Biology and Genetics, directed by Prof. Sergio Barlati, have demonstrated that correlation exists between uPA over-expression at tumoural tissue levels of patients affected by hepatocellular carcinoma (HCC) and poor prognosis (De Petro et al.; Cancer Res. 1998 May 15;58(10):2234-9). In particular it has been demonstrated that an inverse correlation exists between uPA expression and patient survival. In light of these results, I have been involved in projects on the down-regulation of uPA to understand the role of uPA in HCC and to study potential therapeutic strategies for HCC.

We have successfully down-regulated uPA by antisense oligonucleotides, antisense RNA and RNA interference technologies (RNAi) by stable expression of short hairpins RNA (shRNA), in a HCC derived cell line at high uPA expression (Salvi et al.; Mol

Cancer Ther. 2004 Jun;3(6):671-8). The results demonstrate that uPA target can reduce *in vitro* the proliferative, invasive and migrative capabilities of HCC cells and *in vivo* can limit human HCC xenografts growth (Salvi et al.; Tumour Biol. 2007;28(1):16-26). We have also evaluated that uPA RNAi leads to a down-modulation of its receptor, uPAR, and to a better organization of the EDA⁺ isoform of Fibronectin fibrils (a major extracellular matrix protein) *in vitro* and *in vivo*. Our results could help to design and test one/two target shRNA expressing plasmid constructs as therapeutic strategies *in vivo* in HCC (and other tumour cells) in experimental animal models.

We had the opportunity to submit to the workshop an abstract and a poster where our results on uPA down-modulation by RNAi both *in vitro* and *in vivo* were presented. The title was: "RNA interference for urokinase-targeting limits growth of hepatocellular carcinoma xenografts in nude mice".

Presently, we are interested in functional studies on the role of uPA in HCC and we are studying further downstream effects following uPA silencing by proteomic analysis. It was a great chance to attend the workshop in order to understand the "state of art" on plasminogen activation worldwide. The exchange of scientific opinions and experience between the young and well-known researchers in our field has widened my knowledge of the uPA/uPAR system. I would like to thank the EACR committee for this opportunity.
[Alessandro Salvi](#)



Stefania Aiello



Home Institution: Dipartimento chimico Tossicologico e Biologico, Università degli Studi di Palermo, Italy

Host Institution: Welsh School of Pharmacy, Cardiff University, Wales, UK



Andrew Westwell and Stefania Aiello

“Fluorinated 2-phenylbenzoxazoles as potent and selective antitumour agents”

This project, supported by an EACR Travel Fellowship, took place in the laboratory of Dr. Andrew Westwell (Welsh School of Pharmacy, Cardiff University, Wales, UK).

As an associate professor in Palermo, with research interests primarily in the design and discovery of new antifungal agents, the award of an EACR travel fellowship has allowed me to expand my research into the field of cancer drug discovery through application of synthetic medicinal chemistry methods.

Our project was based on the recent discovery of PMX 610, a new potent and selective small molecule antitumour agent. PMX 610 belongs to the class of fluorinated 2-phenylbenzothiazoles that have generated a great deal of interest due to their potent antitumour properties and ease of synthesis

(Westwell et al, *J. Med. Chem.* 2006, 49, 179-185). Despite in vitro activity (low nanomolar GI_{50}) in a number of cancer cell lines, further development of GW 610 has been hindered by high lipophilicity/poor water solubility (compromising potential in vivo activity), and an undefined molecular target.

This project was concerned with the synthesis and antitumour evaluation of new related chemical structures, the fluorinated 2-phenylbenzoxazoles. These new benzoxazoles were expected to have superior pharmaceutical properties (greater hydrophilicity) based on the lower logP values of benzoxazoles compared to benzothiazoles.

The benzoxazole target compounds were prepared by a two-step synthetic sequence based on a recent report (Evindar and Batey, *J. Org. Chem.* 2006, 71, 1802-1808). The first step involved reaction of 2-bromoaniline derivatives with a substituted benzoyl chloride to give an intermediate 2-bromobenzanilide. Cyclisation to the target 2-phenylbenzoxazole was then accomplished using a copper-based catalyst system. Around 20 new target compounds were synthesised in good overall yield and were fully characterised for structural identity and purity. New molecules were tested in vitro, in human cancer cell lines responsive to PMX 610; early in vitro data in human cancer cell lines sensitive to PMX 610 suggests that the new compounds are less active as potential antitumour agents.

Further biological studies are underway in collaboration with Dr. Tracey Bradshaw (University of Nottingham, UK). Our search for a new 2-phenylbenzazole compound with optimal antitumour and pharmaceutical properties continues, alongside further efforts to elucidate the mechanism of action of these agents. Publication of data from this project is expected in the near future.

We thank the EACR for the award of this Travel Fellowship, which has helped to establish a new European collaboration, and will provide the platform for further collaborative research in the future.

Stefania Aiello

Vitaly Kochin



Home Institute: University of Turku and Abo Akademi University, Finland

Host Institute: Marine Biological Laboratory, Massachusetts, USA



Vitaly Kochin with Nicolas Minc

The EACR Travel Grant to attend the Marine Biological Laboratory (MBL) summer course “Physiology: Modern Cell Biology Using Microscopic, Biochemical and Computational Approaches”.

I am currently a PhD-student in the Turku Center for Biotechnology / Abo Akademi University, Biology department. I work in the research group “Signalling in Cell Death, Survival and Homeostasis”, which is headed by Prof. John Eriksson. The research group studies signal integration and is especially interested in phosphorylation-mediated signalling. As model systems we study apoptotic, stress-mediated, and cytoskeletal signalling, and their interrelationship. My work focuses on the following projects: investigation of the mechanisms that affect formation of the death inducing signalling complex (DISC) in apoptosis by studying in vivo phosphorylation of the apoptotic regulator protein c-FLIP shown to be upregulated in different cancer types.

As a model for global phosphoproteomic analysis, I have been studying phosphorylation of



L-R: Shang Cai, Christine Field and Vitaly Kochin (with face shield).

nuclear lamins A/C. This project is performed in collaboration with Prof. Robert Goldman's group (Department of Cell and Mol. Biology, Northwestern University, Chicago, IL). I was able to identify most of the in vivo phosphorylation sites on lamin A/C and I am now trying to establish biological functions of the detected phosphosites. This will help to understand the origin and mechanisms of a wide range of diseases called laminopathies, which are caused by numerous mutations in Lamin A/C gene.

The EACR co-funded my attendance of the MBL's advanced summer course "Physiology: Modern Cell Biology Using Microscopic, Biochemical and Computational Approaches", Woods Hole, MA, USA, June, 9 – July, 29, 2007. This course was very fruitful as it greatly advanced my knowledge and practical skills in imaging techniques, biochemical assays, and computational methods used for biological data analysis and interpretation.

The beauty and high efficacy of this course is based on the way it is organized: real research on real projects is performed during the course. The course takes seven weeks along with lectures given by famous scientists. Also the course offered me invaluable opportunity to establish connections with leading scientists in the fields of cell biology and biophysics.

During the course I participated in three projects:

With Dr. Claire Waterman we studied

cell motility by measuring traction forces (computational methods) that adherent cells apply onto the ECM substrate upon treatment with drugs. We were measuring traction forces of control fish keratocytes and those treated with myosin II inhibitor – blebbistatin (biochemical methods). I became experienced with live cell imaging and computational techniques in order to visualize forces the cell applies to move. This work is part of the projects performed in Dr. Waterman's lab in NIH. The data we obtained is another step further in the research field studying the motility of cells including metastasizing cancer cells.

In the project with Dr. Tim Mitchison we were testing different drugs – small molecule inhibitors of mitosis (including anticancer drug B12536, which is in phase II clinical trials now) to characterize their effects on mitotic spindle formation. We observed

several very interesting phenotypes of spindles produced upon drug treatment. This work requires longer than a two weeks time frame, so it is planned to be continued in Dr. Mitchison's lab after the course.

During the project with Dr. Jennifer Lippinkott-Schwartz we were testing the ability of primary macrophages from Alzheimer's disease (AD) patient and age matched control, individual to phagocyte amyloid beta. It was shown before in the literature that AD macrophages are defective in phagocytosing amyloid-beta as compared to control ones. We wanted to investigate the reasons for this phenomenon in more detail. During ten days, we did a lot of live and fixed cells confocal laser scanning microscopy: we checked and quantified the ability of AD and control macrophages to phagocyte amyloid-beta, polystyrene beads, fluorescently-labelled dextran. We labelled and quantified amounts of lysosomes and actin in cells, and made a striking observation that AD macrophages almost completely lack podosomes (the primary sites of integrin stimulated actin polymerization) as compared to control cells. This results are submitted as a poster abstract for the ASCB 2007 Meeting.

I recommend this course to PhD-students and postdocs as a truly interdisciplinary approach in studying cell biology. This course has changed my life! Thank you EACR so much for your support! EACR is great!

Vitaly Kochin



"Waterman Group. Back row L-R: Claudia Kuttel, Nicolas Minc, Karen Newell-Litwa, Margot Quinlan, Benedikt Sabass, Chen Xing, Lauren Goins, Davis Pajeroski, Middle row L-R: Zeba Wunderlich, Jennine Dawicki McKenna, Clare Waterman, Katsuhiro Kita, Front row: Vitaly Kochin (sitting).

Linda Whelan



Home institution: UCD School of Biomolecular and Biomedical Sciences, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Host institution: Professor Lihua Marmorstein, University of Arizona, Department of Ophthalmology and Vision Science, 655 N. Alvernon Way, Suite 108, Tucson, AZ 85711

Reason for choosing the host institution: Prof. Marmorstein is a world renowned leader in fibulin biology.

Aim: "To examine the functional role of fibulin-4 in embryonic stem cells taken from fibulin-4 knockout/knockin mice"

Background to the project: Angiogenesis is a fundamental physiological process required for reproduction, embryogenesis and tissue repair. During angiogenesis, endothelial cells undergo a sequence of coordinated events to form functional vascular networks. The fibulins, a family of secreted extracellular glycoproteins associated with basement membranes and elastic fibres, modulate cell morphology, growth, adhesion and motility. Recently, it has been suggested that fibulins might play a role in the regulation of vascular growth and maturation during development and in lesions of injured blood vessels. The function of fibulin-4 is ill defined. Several studies indicate that fibulin-4 induces mitogenesis and exhibits oncogenic properties. Prof. Marmorstein has shown that inactivation of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice. These mice exhibited vascular defects and emphysema. We aim to examine the functional role of fibulin-4 in embryonic stem cells taken from fibulin-4 knockout/knockin mice.

Achievements:

In collaboration with Professor Marmorstein, we have successfully



Front left - Linda with colleagues in Arizona; Lihua Marmorstein is pictured centre.

isolated embryonic stem cells. Briefly, time pregnant mice (E.D, 3.5) were sacrificed by carbon dioxide overdose. The uterus was removed and the uteri horns were flushed with media to release the blastocysts. Blastocysts were transferred to culture dishes containing a feeder layer of mouse embryonic fibroblasts. After 24h, blastocysts had attached to feeder layer and inner cell mass and the outer shell were visible by light microscopy. By day 6, blastocysts had hatched from the zona pellucida. The inner cell mass was mechanically removed and dispersed into new culture dishes or genotyped. To maintain the pluripotential, leukemia growth factor was added to the media. We

aim to differentiate these cells into different lineages with the aim of understanding the functional role of fibulin-4 in cancer.

I would like to thank the European Association for Cancer Research for giving me the opportunity to visit Prof. Marmorstein laboratory. I have now acquired the skills necessary for carrying out stem cell research. These skills will be disseminated to other investigators in the Institute and will fill an integral need within our group. I would also like to thank Prof. Marmorstein for taking time out from her busy schedule to assist me in the optimisation of the technique and for the useful discussions that ensued.

Linda Whelan

