Gene expression profiling in MCL lymphoma: a focus on t(11;14)(q13, q32) breakpoints

Barat Ana, Markozashvili Diana, Pichugin Andrei, [1]Clayette Valerie, [1]Ribrag Vincent, Lipinski Marc and Vassetzky Yegor

UMR 8126, Université Paris Sud, CNRS, Institut de cancérologie Gustave Roussy, Villejuif, France.

[1]Institut de cancérologie Gustave Roussy, Villejuif, France.

vassetzky@igr.fr

Mantle cell lymphoma (MCL) is a malignant proliferation of B cells in the mantle zone of lymphoid follicles. Cytogenetic analyses have revealed that MCL is closely associated with the t (11; 14) (q13; q32). This translocation juxtaposes Ig heavy chain gene (IGH, 14q32) sequences, leading to an overexpression of a number of genes including the cyclin D1gene (CCND1). This general transcription upregulation might be due to epigenetic processes. Chromosome 11 is located in a largely heterochromatic region of the nucleus, while chromosome 14 is found in a more euchromatic context. We propose that the t (11; 14) (q13; q32) translocation induces the transposition of the 11q13 locus from a heterochromatic to a euchromatic region of the nucleus. This movement could then cause the overexpression of the genes located on 11q13. We are currently studying the nuclear dynamics of these regions, using 3D-FISH on control and MCL derived B-lymphocytes in parallel with chromosome mapping of MCL gene expression profiles of expression data, originating from Gene Expression Omnibus (Affymetrix arrays). These transcriptome studies have revealed that several genes located in the vicinity of the breakpoint on chromosome 11 are overexpressed in MCL cells.

Genes participating in the development of brain tumors and myeloproliferative diseases and their relation to signaling pathways.

[1]*Kavsan Vadym and* [2]*Ribrag Vincent.*

[1]Department of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics, NASU, Kyiv, Ukraine.
[2]UMR 1009 Normals and Pathologics Haematopoiesis, Institut Gustave Roussy, Villejuif, France.

kavsan@imbg.org.ua

Myeloproliferative and lymphoproliferative disorders (or neoplasms) are a set of diseases characterized by the abnormal proliferation of myeloid lineage of cells or lymphocytes, respectivelly. *BMP7* is a new gene involved in secondary drug resistance of mantle cell lymphoma, which was identified by microarray analysis of clinical samples, and possesses an anti-apoptotic function. High phosphorylation levels of the MAPK and PI3K kinases pathway components were shown in primary tumor cell lines, obtained from patients with increased *BMP7* expression level.

In an effort to identify genes, which might be used as molecular markers for glial tumors, we compared gene expression in glioblastoma and normal adult human brain. Serial Analysis of Gene Expression found *Chitinase 3-like 1 (CHI3L1, HC gp-39, YKL-40*) and *Chitinase 3-like 2 (CHI3L2, YKL-39)* genes among the most abundant transcripts in glioblastoma. Significant increase in 293 cells proliferation, correlated with phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and protein kinase B (AKT1), was shown after stable transfection by *CHI3L1* oncogene. Unexpectedly, dose dependent decrease in total DNA content and [^{3}H]thymidine incorporation were observed in 293 cells treated with CHI3L2, probably confirming it's tumor suppressor function.

The presence of mutual genes overexpressed in glioblastomas and mantle cell lymphoma was shown by analysis of GEO databases. Analysis of their expression in clinical samples is needed to evaluate whether they may be used as markers for mentioned diseases. Investigation of their effect on signaling pathways in mantle cell lymphomas and mechanisms which drive their overexpression will provide a new insight into pathogenesis of this myeloproliferative disorder.

Identification of non-canonical targets of mTOR during neuronal development

Jaworski Jacek

International Institute of Molecular and Cell Biology, Warsaw, Poland

jaworski@iimcb.gov.pl

Mammalian target of rapamycin (mTOR) is a protein kinase that senses nutrient availability, trophic factors support, cellular energy level, and cellular stress and adjusts cellular metabolism accordingly. In neuronal cells, mTOR activity is additionally controlled by neurotransmitters. mTOR plays several roles in neuronal development and plasticity. It is involved in the control of neuronal survival, neurite growth, and synapse formation. It has been believed for long time that all this neuronal functions of mTOR are attributable to its involvement in translational regulation. On the other hand, several studies revealed that cellular functions of yeast mTOR homologs go far beyond this canonical role. In fact, TOR plays a role in transcription, autophagy, lipid metabolism, mitochondrial function, cytoskeleton dynamics, and membrane trafficking. Dendritic arbor development is an important step during neuronal development as it defines major patterns of connectivity in the brain. We have previously shown that indeed development of dendritic arbor is regulated by mTOR and translational control is involved in this process. However, mTOR forms two functionally different complexes in mammalian cells: mTORC1 and mTORC2, and our previous studies did not answer whether only mTORC1, which is responsible for translational control, is important for dendritic growth. Our current research shows that mTORC2 acts upstream mTORC1 during neuronal development. What is more, our shRNA library screen shows that protein translation is not the only cellular process controlled by an mTORC1 during dendritic arbor growth. In fact, equally important are mTORC1-dependent control of microtubule-actin interaction via CLIP-170 protein and mTORC1 involvement in membrane trafficking.

Endocytic proteins in control of signaling and transcription

Hupalowska Anna, Pyrzynska Beata, Torun Anna, Szymanska Ewelina, Maminska Agnieszka, Miaczynska Marta

Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland

miaczynska@iimcb.gov.pl

Accumulating evidence argues that many proteins governing membrane sorting during endocytosis participate also in nuclear signaling and transcriptional regulation. Some clathrin adaptors and endosomal proteins were shown to undergo nucleocytoplasmic shuttling. Such endocytic proteins can associate with nuclear molecules, changing their localization and/or activity and may modulate the levels and specificity of gene transcription, although in most cases it is not clear how interconnected the nuclear and cytoplasmic pools of endocytic proteins are.

Two related adaptor proteins APPL1 and APPL2 represent an example of proteins performing dual functions in endocytosis and transcriptional regulation. On the one side, APPL proteins are localized to a subpopulation of Rab5-positive early endosomes termed APPL endosomes. On the other side, APPL proteins can be released from the endosomal membrane to undergo nucleocytoplasmic shuttling and to associate with nuclear proteins. We previously showed that both APPL proteins are activators of β-catenin/TCF-mediated transcription in the canonical Wnt signaling pathway. More recently, we have uncovered a novel role of APPL1 as a positive regulator of the transcriptional activity of NF-κB under basal but not TNFα-stimulated conditions. APPL1 was found to directly interact with TRAF2, an adaptor protein known to activate the canonical NF-κB signaling. Importantly, APPL1 appeared to regulate the proper spatial distribution of p65 NF-κB subunit. We could show that APPL1 increased the stability of NF-κB-inducing kinase (NIK), the key component of the noncanonical pathway, which in turn affected the transcriptional activation of p65. This places APPL1 as a novel link between the canonical and noncanonical machineries of NF-κB activation. Furthermore, as no systematic approaches were undertaken to investigate the involvement of endocytic proteins in nuclear signaling and transcriptional control, we initiated small targeted RNAi-based screens of candidate genes to study this problem. Our initial data indicate that the participation of endocytic proteins in transcriptional control is a more wide spread phenomenon.

Molecular interactions of multifunctional adaptor proteins of intersectin family

Rynditch A. V., Dergai M. V., Novokhatska O. V., Tsyba L. O., Skrypkina I. Ya., Nikolaienko O. V., Kropyvko S. V., Dergai O. V., Morderer D. Ye., Gryaznova T. A.

Department of Functional Genomics
Institute of Molecular Biology and Genetics, Kyiv, Ukraine

rynditch@imbg.org.ua

The rich binding capability of the multidomain, adaptor and scaffolding proteins of intersectin (ITSN) family has linked them to multiple functions such as clathrin-mediated endocytosis, mitogenic signalling, actin cytoskeleton rearrangements and apoptosis. Abnormalities of ITSN1 expression were associated with the endocytic anomalies reported in Down syndrome brains and early stages of Alzheimer's disease. ITSN2 was proposed to be a predictive marker for breast cancer. Despite intensive study of ITSNs, the regulation of their function in different cell processes and the role of ITSNs in disease development remain unclear.

In order to highlight a role of ITSN genes, we studied their expression in normal and pathological tissues as well as analyzed the composition of ITSN-containing protein complexes. We identified 17 alternative splicing events affecting ITSN1 pre-mRNA and found alternative transcription initiation site in the fifth intron of ITSN1 gene. ITSN1 isoforms differ in their domain organization, interaction with protein partners, localization in different tissues and stages of development. We have found neuron-specific alternative splicing of microexon 20 that affects binding abilities of ITSN1 SH3A domain and provides a mechanism for the control of brain-specific interactions of ITSN1. Using mass spectrometry analysis and *in silico* prediction we identified 11 novel protein partners of ITSN1 and ITSN2, among them adaptor proteins Ruk/CIN85, Reps1 and SHB. ITSN1, and its shortest alternatively spliced isoform ITSN1-22a forms complex with membrane-deforming proteins SGIP1 and amphyphisin, respectively. An interaction of ITSN1 with WASP-interacting protein WIP

suggests a possible role of ITSN1 in the regulation of protein complexes during invadopodia formation in cancer cells. We also identified a new neuron-specific protein partner MAP6/STOP involved in microtubule stabilization and generation of synaptic plasticity. Our results demonstrated that ITSNs could be regulated by ubiquitylation and phosphorylation. The Nedd4-like E3 ubiquitin ligase *AIP4* is involved in posttranslational modification of ITSN1 isoforms. We have also shown that ITSN2 undergoes EGF-dependent tyrosine phosphorylation in HeLa cells. Moreover, the SH2 domains of signalling proteins Grb2, Crk, Fyn, Fgr, Abl1, PLCG1 and PI3K differentially interact with ITSN2 in mouse tissues. These findings expand the role of ITSNs as a scaffolding molecules bringing together components of endocytic and signalling complexes as well as highlight the mechanisms of regulations of ITSNs functions.

Potential implication of leucyl-tRNA synthetase in tumourigenesis

Tukalo M., Boyarshyn K.,Yaremchuk A., Kovalenko O., Gudzera O., Kondratov A.

Department of Protein Synthesis Enzymology
Institute of Molecular Biology and Genetics, Kyiv, Ukraine
mtukalo@imbg.org.ua

Recent screening the novel differentially expressed genes (DEG) in A549 lung cancer line has revealed the over-expression of leucyl-tRNA synthetase (LRS) and its up-regulation has been confirmed by other methods (Shin at al., 2008). The oncogenetic potential of LRS gene (*LARS1*) was validated by siRNA knockdown and various transformation assays. On the other hand, two resent studies (Han at al., 2012 and Bonfils at al., 2012) have shown that leucyl-tRNA synthetase is a leucine sensor for TORC1, in both yeast and mammalian cells. This finding suggests a potential implication of LRS in tumorigenesis and opens a new dimension for cancer therapy. Since mTORC1 inhibitors are used for cancer therapy, the inhibitors of LRS could be used as new therapeutic regiments for cancer.

We have investigated the level of *LARS1*expression in A549 cell line, normal pulmonary epithelial cell line, tissues of kidney cancer and tissues of normal kidney. The level of LARS1 mRNA expression of in cancer cells A549 was more than 2 times higher than in cells of normal epithelium. In the case of kidney cancer in 16 samples of tumor tissue an increased expression of *LARS1* was observed in 13, and only in two its slight decrease was noted. However, only three samples showed a more than twofold increased expression, and one – more than three times. Thus, the data obtained on the samples of kidney tumors studied show a tendency of increasing *LARS1* expression. At the same time the level of expression does not allow us to consider this gene as a biomarker of tumor growth in the kidney cancer.

Multimolecular translation elongation factor eEF1H in human carcinomas.

Negrutskii B. S., Veremieva M. V., Shalak V. F., Trosyuk T. V. and El'skaya A. V.

Laboratory of Protein Biosynthesis Department of Translation Mechanisms of Genetic Information,

Institute of Molecular Biology and Genetics, Kyiv, Ukraine

negrutskii@imbg.org.ua

Multisubunit complex eEF1H comprises eEF1A and eEF1B entities. eEF1B complex serves as a GDP/GTP exchanging device for eEF1A which interacts with GTP and aminoacyl-tRNA transporting the latter to the 80S ribosome. The eEF1B complex of unknown stoichiometry consists of the eEF1Bα, eEF1Bβ and eEF1Bγ subunits. Recently, a variety of multiprotein complexes, including translation ones, were shown to release their individual components to fulfill novel functions under unfavorable circumstances. We aimed to check if independent functioning of the eEF1B subunits in human organism is also possible. Manifestation of non-coordinated changes in the subunits expression levels would be an essential evidence for the possibility of independent from complex appearance of the eEF1B constituents in cancer tissues.

Indeed, we have found non-coordinated changes in the expression level of all eEF1B subunits in cardioesophageal cancer and lung carcinomas strongly suggesting a lack of synchronized regulation of the eEF1B constituents in cancer tissues. Those findings were confirmed by immunohistochemical studies.

Importantly, cancer-related increase in the level of the eEF1B subunits was observed in the majority of cardioesophageal and lung tumor samples.

The purpose of planned research is to investigate the organization of the eEF1B complex *in vitro*, to establish a stoichiometry of the subunits in the complex, to examine a possibility of the existence of mini-complexes including not all of the eEF1B subunits. Expressed in *E. coli* the individual eEF1B subunits will be investigated by physical methods. The perspective of the project is to approach crystallization and subsequent X-ray analysis of the individual eEF1B subunits and the complex as a whole.

DNA methylation, chromatin modifications and SSLP polymorphism in the 4q35 chromosomal region in facioscapulohumeral dystrophy myoblasts and cervical carcinoma cells

[1,2]Kisseljova Natalia P., [2]Dmitriev Petr, [1]Katargin Alexey, [2]Kim Elena, [1,2]Ezerina Daria, [2]Planche Emmeline, [4]Lemmers Richard J. L. F., van der [4]Maarel Silvère M., [3]Laoudj Dalila, [2]Lipinski Marc, and [2]Vassetzky Yegor S.

[1]NN Blokhin Russian Cancer Research Center, RAMS, Russia,

[2] UMR 8126, Université Paris Sud, CNRS, Institut de cancérologie Gustave Roussy, F-94805, Villejuif, France.

[3] INSERM EA 4202 ERI25, 371 Avenue du Doyen Gaston Giraud F-34295 Montpellier, France.

[4]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands

vassetzky@igr.fr

Facioscapulohumeral dystrophy (FSHD) is a hereditary disease with a prevalence of 1 in 20,000 linked to a partial deletion of the D4Z4 repeat array on chromosome 4q. The nuclear matrix attachment region (FR-MAR) present in the vicinity of the repeats loses its efficiency in myoblasts from FSHD patients. The FR-MAR coincides with a region of sequence variation (SSLP) and contains an enhancer-blocking element. Here, we have analyzed DNA methylation patterns and histone post-translational modifications in the FR-MAR of cervical carcinoma and primary myoblasts from FSHD patients. A specific DNA methylation pattern combined with lack of histone H3 acetylation was found to be required for binding of the MeCP2 protein, a known component of the nuclear matrix, and for association of the FR-MAR with the nuclear matrix. An 8-nucleotide insert present in the SSLP appeared to enhance the association of the FR/MAR with the nuclear matrix. Together, these results constitute the first evidence that DNA and histone modifications affect MeCP2-mediated FR/MAR attachment to the nuclear matrix in normal and pathological cells. In FSHD myoblasts, release of the FR/MAR from the nuclear matrix is favored by the acetylation of the amino-terminal tail of histone H3 and the absence of the 8-nucleotide insert.

Identification and characterization of medullary breast carcinoma-associated antigens as a potential targets for breast cancer diagnostics and therapy

[1]Kiyamova R., [1]Kostianets O., [1]Shyian M., [2]Gout I., [1]Filonenko V.

[1]Department of Cell Signaling,
Institute of Molecular Biology and Genetics, Kyiv, Ukraine
[2]University College London
filonenko@imbg.org.ua;
i.gout@ucl.ac.uk

Medullary breast carcinoma (MBC) despite anaplastic features and high grade has a good prognosis that can be related to prominent lymphocytic infiltration. We applied SEREX technology (serological recombinant expression cloning) to search MBC antigens as a potential targets for diagnostics and therapy of breast cancer. In a course of MBC cDNA screening by autologous MBC patient sera we have identified 41 potential MBC antigens. Analysis of frequency of antibody responses toward these 41 antigens in sera of allogenic MBC patients and healthy individuals using plaque-spot serological assay allowed us to select 18 autoantigens which were exclusively reactive with sera of medullary breast carcinoma patients. qPCR analysis of mRNA expression for 6 of these 18 antigens (RAD50, FAM50A, PABPC4, RBPJ, HMGN2, DEK) represented by more than one independent cDNA clone isolated from the MBC cDNA library showed their differential expression profile in MBC tissue samples. However, we didn't reveal any correlation between the MBC antigens mRNA expression profiles and the level of specific autoantibodies in patient sera.

Antigens which showed cancer-related serological profile will be further investigated in a large cohorts of patients with different types of breast cancer using ELISA assay with recombinant analogues of identified antigens to clarify whether they are candidates for breast cancer diagnostics or therapy.

Influence of hypoxic stress on anti-tumor rresponse

Chouaib Salem

INSERM U753Institut Gustave Roussy
Villejuif 98805- France

salem.chouaib@igr.fr

The identification of tumor-specific epitopes as targets for antitumor cytotoxic effector cells has made possible their use in vaccination trials. However, positive clinical results have been scarce most likely because of the weak immunogenicity of these peptides, the low frequency of tumor-specific T lymphocyte precursors and the resistance of tumor cells to cytotoxic effector cells. Large established tumors, which are associated with the acquisition of tumor resistance to specific lysis, are usually not fully controlled by the immune system. It is obvious that the evasion of immunosurveillance by tumor cells is under the control of the tumor microenvironment complexity and plasticity. Hypoxia, a key component of the tumor microenvironment, is a common characteristic of locally advanced solid tumors that has been associated with diminished therapeutic responses, malignant progression, increased probability of recurrence, locoregional spread and distant metastasis. It occurs in the majority of solid tumors and is strongly correlated with advanced disease stages and poor clinical outcome. This is, in part, due to increased genomic instability in hypoxic tumors cells and enhanced resistance to cytotoxic treatments. We have made significant contributions in the understanding of the impact of hypoxic stress on the anti-tumor immune response.The influence of tumor microenvironment in particular hypoxic stress in shaping the quality of CTL response and the susceptibility of tumor target cells to CTL-induced cell death will be discussed.

Identification and characterization of a tumor-associated antigen for the development of a novel cancer vaccine approach in lung cancer

Durgeau A., El Hage F., Vergnon I., Chouaib S. and Mami-Chouaib F.

Inserm U753, Gustave Roussy Institute
Villejuif, France
cfathia@igr.fr

We identified an antigen recognized in a human lung carcinoma by a cytotoxic T lymphocyte (CTL) clone derived from autologous tumor-infiltrating lymphocytes (TIL). The antigenic peptide is presented by HLA-A2 and is encoded by the *CALCA* gene, which codes for calcitonin and which is overexpressed in several lung tumors compared to normal tissues. This peptide ($ppCT_{16-25}$) is derived from the C-terminal region of the preprocalcitonin (ppCT) signal peptide, and is processed independently of proteasomes and the transporter associated with antigen processing (TAP). Processing occurs within the endoplasmic reticulum of all tumoral and normal cells tested by a novel mechanism involving signal peptidase (SP) and signal peptide peptidase (SPP). Lung cancer cells bearing this epitope displayed low levels of TAP, but restoration of their expression by IFNγ treatment or *TAP1* and *TAP2* gene transfer inhibited ppCT antigen presentation. In contrast, TAP up-regulation in the same tumor cells increased their recognition by proteasome/TAP-dependent peptide-specific CTL, namely anti-mutated α-actinin-4 epitope-specific CTL. Thus, $ppCT_{16-25}$ is the first human tumor epitope whose surface expression requires down-regulation of TAP. Lung tumors frequently display low levels of TAP and might thus be ignored by the immune system. Our results indicate that emerging SP-generated peptides represent alternative T cell targets, which permit CTL to destroy TAP-impaired tumors and thus overcome tumor escape from $CD8^+$ T cell immunity. This new epitope is therefore a promising candidate for cancer immunotherapy. Current studies aim is to identify antigenic peptides derived from ppCT and processed by proteasome/TAP pathway to target both antigen processing-efficient and -deficient tumors. Our objective is to set up an immunotherapy strategy more adapted for the treatment of patients suffering from lung cancer.

Transcription signature of FSHD myoblasts

Dmitriev Petr, [1]Laoudj Dalila, Lipinski Marc, and Vassetzky Yegor S

[1] UMR 8126, Université Paris Sud, CNRS, Institut de cancérologie Gustave Roussy, F-94805
Villejuif, France.
[2] INSERM EA 4202 ERI25, Montpellier, France.

vassetzky@igr.fr

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disease with a prevalence of 1 in 20,000. FSHD is characterized by progressive weakness and atrophy of the facial muscles and shoulder girdle. Recently our group has tested the structure of chromatin loops in the locus 4q35 affected in the disease. We demonstrated that the chromatin loops are reorganized in FSHD myoblasts compared to healthy myoblasts (Petrov et al., 2006; Pirozhkova et al., 2008). However, the etiology of FSHD remains unknown. To elucidate the mechanism of the disease and propose strategies of treatment, we have established the transcription signature of FSHD myoblasts and found numerous pathways deregulated in FSHD, which opens a new way in treatment of FSHD.

A search for transcription signature of FSHD myoblasts

[1]*Kavsan Vadym and* [2]*Vassetzky Yegor*

[1]Department of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics, Kyiv, Ukraine

[2] UMR 8126, Université Paris Sud, CNRS, Institut de cancérologie Gustave Roussy, F-94805 Villejuif, France.

kavsan@imbg.org.ua

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder resulting from an unusual genetic mechanism. The mutation, a deletion of 3.3 kb subtelomeric repeats, appears to disrupt the regional regulation of 4q35 gene expression. The specific gene(s) responsible for facioscapulohumeral muscular dystrophy has(ve) not been identified. However, the "vacuolar/necrotic" phenotype exhibited by facioscapulohumeral muscular dystrophy myoblasts suggests that aberrant gene expression occurs early in facioscapulohumeral muscular dystrophy muscle development. In order to test this hypothesis, global gene expression profiling and in vitro characterization of facioscapulohumeral muscular dystrophy and control myoblasts were carried out. The genes involved in several cellular processes such as oxidative stress, MAPK/PI3K signaling, immune response were found to be dysregulated.

Serial Analysis of Gene Expression (SAGE) revealed a set of genes with the most pronounced changes in expression in human glioma tumor cells. The most corresponding proteins were involved in angiogenesis, host-tumor immune interplay, multidrug resistance, extracellular matrix formation, IGF-signaling, or MAP-kinase pathway. *CHI3L1* and *CHI3L2* were identified as the most upregulated genes in glioblastomas. Next, it was shown that *CHI3L1* or *CHI3L2* expression is increased significantly under pathological conditions such as inflammation or tumors. Moreover, recent research suggests that CHI3L1 and CHI3L2 acts as proliferative or proapoptotic agents, correspondently. These activities are mediated by signaling through the MAPK and PI3K cascades.

The fact that the expression profiles of two types of human disorders such as FSHD and glial tumors, have pools of genes characterized by similar functions, may indicate that the same genes or gene signatures could participate in the development of these diseases. Especially, it can be applied to the genes which are involved in the regulation of mitogenesis and cell proliferation.

MGMT repair enzyme and its protective role in the response of tumor and normal cells to alkylating compounds

Lukash L. L., Iatsyshyna A. P.

Department of Human Genetics

Institute of Molecular Biology and Genetics, Kyiv, Ukraine

lukash@imbg.org.ua

Tumor therapy with alkylating agents is limited by the development of resistance of tumor cells and undesired systemic side effects. Understanding and overcoming these limitations might be possible on the basis of our knowledge how these anticancer drugs are acting and the cells are protecting their DNA. Regarding alkylating anticancer drugs belonging to the groups of methylating and chloroethylating agents a critical site for their attack is the O^6-position of guanine in the DNA, and as a result of this interaction the adduct O^6-alkylguanine is formed. This lesion is considered to be a major cause of mutations and malignant transformation induced by alkylating agents. It also gives rise to genotoxicity and cell death through the stimulation of apoptosis.

O^6-alkylguanine is corrected by the DNA repair enzyme O(6)-methyguanine-DNA methyltransferase (MGMT) which transfers the alkyl group to the own active center cysteine and is inactivated due to a suicide mechanism. The guanine in DNA is restored despite a cell origin, normal or tumor one. The capacity of cells to repair the O^6-alkylguanine depends on levels of expression and activity of the MGMT in cell or the rate at which a cell can synthesize this enzyme. MGMT has a strong protective effect on the proliferation capacity, survival and apoptosis after the treatment of cell populations with antineoplastic O^6-alkylating agents. Thus, MGMT is the most important determinant in the alkylating drug resistance.

The *MGMT* is ubiquitously expressed in Mammals, but levels of its expression widely vary depending on the type of cell or tissue, the cell cycle phase, the developmental stage of organism. Because its expression may also differ dramatically in individual tumors, determination of MGMT activity would be important as a predictive indicator on the one side, and inactivation of MGMT in

tumors to sensitize them to antineoplastic agents is realized in experimental works and in clinical trials on the other side. Various highly efficient MGMT inhibitory compounds have been developed. However, despite effective *in vitro* studies and lack of systemic side effects in patients, a recent phase II trials did not reveal a significant increase in the efficacy of alkylating agents in the treatment of malignant glioblastomas. That is a reason why it would be desirable to develop new more efficient inhibitors and strategies of inhibitor targeting to inactivate MGMT preferably in tumors. We are going to develop highly active and specific inhibitors of human MGMT enzyme by using an approach of molecular modeling, biological screening and combinatorial synthesis jointly with Prof. Sergey Yarmoluk.

It has been shown that various environmental factors influence MGMT expression. MGMT was found to be inducible in human, rat and mouse tissues with genotoxic drugs, including O^6-alkylating agents and X-rays, glucocorticoids and cytokines. Observed variations of the *MGMT* expression indicate a complicated regulation of this gene, but molecular basis of intra- and inter-individual variation in the *MGMT* expression levels are still not fully defined. It has been shown that epigenetic and genetic factors are involved in the regulation of the *MGMT* expression.

In a result of the *in silico* search of regulatory sequences within mouse and human *MGMT* promoters we revealed many novel potential sites for inducible and tissue-specific transcription factors, but their functional activity has to be subjected to experimental confirmation. The epigenetic regulation could explain a variation of the *MGMT* gene expression during embryogenesis. The mouse *Mgmt* gene is an accessible model for study of a development- and a tissue-specific regulation of the *MGMT* gene transcription. Therefore, we propose to study status of methylation of the mouse *Mgmt* gene promoter and the gene body at different stages of embryogenesis and the regulation of the transcription of this gene in different mouse tissues in collaboration with Prof. M. Bochtler, Laboratory of Structural Biology, IIMCB.

The link between CG methylation and depletion across the kingdoms of life

Wojciechowski Marek, Czapinska Honorata and Bochtler Matthias

International Institute of Molecular and Cell Biology,
Warsaw, Poland

mail

DNA methylation occurs in prokaryotes and eukaryotes, but in different forms and with different functions. In prokaryotes, methylation is very diverse. Mechanistically, the modification can affect the N4 or C5 of cytosine or N6 of adenine. Sequence context is variable. Functionally, methylation plays a role in restriction modification systems, in DNA repair for the distinction of parental and daughter strand, and also in the control of bacterial lifestyle. Some of this is conserved in primitive eukaryotes, but in higher eukaryotes, particularly vertebrates, methylation is predominantly reduced to C5 methylation in a single sequence context (CG, more traditionally CpG), and serves to control the epigenetic state of DNA, in crosstalk with appropriate histone modifications. For eukaryotic organisms with DNA methylation, it is known that the CG sequence is not only important, but also rare: in fact, the actual number of CGs is about fourfold lower than statistically expected. In my talk, I will discuss the mechanistic link between methylation and the depletion of target sequences and I will address the question about the generality of the link across all kingdoms of life.