CHI3L1 and CHI3L2 – one protein family, two opposite functions

Areshkov P. O., Avdieiev S. S.
Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnogo Str., Kyiv, Ukraine 03680
chitotrio@gmail.com

Mammalian genomes code a set of homologous chitinase-like proteins. In human there are six proteins of this family, with CHI3L1 and CHI3L2, the most investigated among them. Overexpression of both genes was found in gliomas [*Shostak et al., 2003, Kavsan et. al., 2008*]. It was reported that CHI3L1, similarly to IGF1, promoted the growth of human fibroblasts and synoviocytes through MAPK/ERK1/2 and PI3K/AKT pathways activation [*Recklies et al., 2002*]. In our previous experiments we found that CHI3L2 activated those signaling pathways but had opposite to CHI3L1 effect on 293 and U373 cell growth. Suppression of cell proliferation by CHI3L2 treatment was caused by difference in ERK1/2 activation kinetics and their localization [*Areshkov et al., 2012*]. Moreover, using specific inhibitors of different steps of clathrin-mediated endocytosis we showed differences in CHI3Ls-associated MAPK and PI3K signaling initiation. CHI3L1 induces ERK1/2 and AKT phosphorylation through clathrin-coated vesicle formation, whereas stimulation of MAPK and PI3K signaling by CHI3L2 could be initiated by activated receptor clusterization and cell membrane invagination only.

Recently, *Miyatake et al.* (2013) reported that lentiviral transduction of *CHI3L2* did not decrease proliferation of mouse ATDC5 cells but significantly enhanced cell dividing. Authors explained their results by difference in cell model (mouse *vs* human cells) and manner of treatment (addition to culture medium *vs* constitutive protein production). To test this hypothesis we obtained mammalian cells that ectopically produced CHI3L1 or CHI3L2 proteins. Applying system of lentiviral gene transduction, we confirmed our results and showed opposite influence of *CHI3L1* or *CHI3L1* expression on cell growth. 293 cells stably producing CHI3L2 possess decreased proliferation rate in comparison with cells of wild type or 293 cells infected by “empty” vectors and, as expected, CHI3L1 production in 293 cells resulted in enhanced cell growth. Such function duality of cancer-associated genes has been already described [*Stepanenko et al., GENE, 2013, in press*] and our results confirmed importance of cell type and activation conditions for biological outcome.

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

Avdieiev S.[1], *Gera L.*[2], *Havrylyuk D.*[3], *Hodges R.*[2], *Lesyk R.*[3], *Ribrag V.*[4], *Vassetzky Y.*[4]

[1]Dpt Biosynthesis of Nucleic Acids, Institute Molecular Biology and Genetics, National Academy of Science of Ukraine, 150 Zabolotnogostr, Kyiv 03680, Ukraine

[2]Dpt Biochemistry and Molecular Genetics, University of Colorado, Denver, Anschutz Medical Campus, Aurora CO 80045, USA

[3]Dpt Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, 69 Pekarska str, Lviv 79010, Ukraine

4CNRS UMR8126, University Paris-Sud 11, Institute Gustave-Roussy, Villejuif 94805, France.

stasavdieiev@gmail.com

Aim. This study was initiated to analyze 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 Mino. **Methods.** MTT-based cell proliferation assay, western blot analysis. **Results.** Based on cytotoxicity test data, it was found that BK antagonists BKM-570 and BKM-1800 revealed significant growth suppressor activity on 293_*CHI3L1* and U373 cells. LC_{50} of BKM-570 was 3,8 μM and 3,3 μM for 293_*CHI3L1* and U373 cells, while LC_{50} of BKM1800 was 25,8 μM and 20 μM, correspondingly. Prominent growth inhibition was demonstrated also by BKM-570 on C6 cells with IC50 4 μM. Several azolidinones were involved in our investigation. 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 IC_{50} 0,13 μM. 50 % inhibition of Mino cells growth were observed after treatment by Les-4523 at 1,58 μM. **Conclusions**. Thus, antiproliferative properties of two different classes of molecules were shown in several distinct *in vitro* models of glioma and MCL.

This work was supported in part by State Agency for Science, Innovations and Informatization of Ukraine in frames of the project F33.4/001 "Identification of promising molecular biomarkers for monitoring of human neurodegenerative and oncological diseases".

Yeast and human SAGA acetyltransferase and deubiquitinase activities shape all the transcribed regions of the genome

[1]*Devys Didier*, [1]*Bonnet Jacques*, [1,2] *WangChen-Yi*, [2]*Kao Chen-Fu and* [1]*Tora Laszlo*

[1]IGBMC, CNRS UMR 7104, 1 rue Laurent Fries, 67404 Illkirch, France
[2]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

devys@igbmc.fr

According to the text book model, co-activators with chromatin modifying activities such as the SAGA complex are recruited at a number of well-defined loci through interactions with DNA bound activators. At these sites, the induced chromatin modifications are suggested to favor the recruitment of general transcription factors and RNA polymerase II. Therefore, it is expected that changes in gene expression upon depletion of these complexes should largely overlap their genome wide distribution. This model has been challenged by recent studies in Saccharomyces cerevisiae that revealed little overlap between SAGA locations, as assessed by chromatin immunoprecipitation (ChIP) and by gene expression defects measured by mRNA changes following downregulation of individual SAGA subunits. Thus, we asked whether ChIP of SAGA specific subunits would provide an accurate map of SAGA action. To answer this question, we assessed the genome wide distribution of the SAGA acetyltransferase and deubiquitinase activities in either yeast or human cells. Surprisingly, upon inactivation of the SAGA acetyltransferase activity, the levels of H3K9 acetylation at the promoters of almost all expressed genes were significantly reduced. Similarly, upon inhibition of the SAGA deubiquitinase activity, revealed increased histone H2Bub1 levels in the transcribed region of all expressed genes in both organisms. These results together demonstrate that contrary to the available genome wide binding data, both yeast and human SAGA is recruited to all expressed genes on which they acts both at the promoter and the transcribed regions. Therefore, besides SAGA coactivator function characterized by a stable ChIP-able binding to its genomic sites, the two enzymatic activities of SAGA have a global and genome wide action suggesting an important role in shaping the transcribed regions of the genome.

The interaction of lipophilic derivatives of siRNA with hematopoietic and tumor cells

Chernolovskaya E. L., Chernikov I. V., Meschaninova M.I., Dovydenko I. S., Venyaminova A. G., Zenkova M. A., Vlassov V.V.

Institute of Chemical Biology and Fundamental Medicine SB RAS,
Novosibirsk, Russian Federation,
elena_ch@niboch.nsc.ru

siRNAs are considered to be promising therapeutic agents for sequence-specific silencing of disease-related genes. However, the problem of siRNAs delivery into cells limits their biomedical application. Conjugation of siRNA to the molecules, which can be internalized into the cell by natural transport mechanisms, can result in the enhancement of siRNA cellular uptake. In this work we investigated the carrier-free accumulation of nuclease-resistant siRNA equipped with lipophilic residues tethered on the 5'-end of the sense strand in KB-8-5 cancer cells and normal hematopoietic cells. We showed that conjugates of siRNA (0.2–5 µM) with cholesterol effectively penetrated (up to 100 %) into the cells, whereas the uptake of the unmodified siRNA was insignificant. The efficiency of the carrier-free cellular accumulation of lipophilic siRNAs is dependent upon the type of lipophilic residues, the type of the target cells and the length of the linker connecting the lipophilic residue and siRNA strand. We found that efficacy of cellular uptake enhanced when the length of linker between siRNA and lipophilic residue increased from 3 to 12 carbon atoms. As expected, the accumulation of lipophilic siRNA in preferential blood mononuclear cells in serum free medium was higher than the accumulation in the cells in whole blood. The biological activity of cholesterol-conjugated siRNAs targeted to *MDR1* gene was tested in KB-8-5 drug resistance cells. Incubation of the cells in the presence of the conjugates resulted in the silencing of the target gene and the restoration of the sensitivity of the cells to cytostatics. The pronounced reduction of the number of living cells was observed at day 6 of cells incubation with cholesterol-containing siMDR and 300 nM vinblastine. Thus, our conjugates are able to penetrate into cells of different types without transfection reagents, to silence the expression of the target gene and to reverse the multiple drug resistance of cancer cells making their susceptible to chemotherapy.

This work was supported by RAS programs "Molecular and cellular biology" and "Fundamental sciences to medicine", by RFBR grant 11-04-01012, Ministry of Science and Education of the Russian Federation (No.14.740.11.1058) and by Interdisciplinary grant of SB RAS (No.85).

DNA import competence and mitochondrial genetics

F. Weber-Lotfi[1], D. V. Mileshina[1,2], N. Ibrahim[1,3], M. V. Koulintchenko[1,2,3], G. G. M. D'Souza[4], V. Saxena[4], Yu. M. Konstantinov[2], R. N. Lightowlers[3], A. Dietrich[1]

[1] CNRS Institute of Plant Molecular Biology, Univ. Strasbourg, Strasbourg, France, 67084

[2] Siberian Institute Plant Physiology and Biochemistry, SB-RAS, Irkutsk, Russia

[3] Institute Cell and Molecular Biosciences, Medical School, Newcastle University, UK4 School of Pharmacy, MCPHS University, 179 Longwood Ave, Boston, USA, MA 02115

andre.dietrich@ibmp-cnrs.unistra.fr

Mitochondrial genome expression is essential for organelle functional efficiency and inter-compartment cross-talk. Manipulation of mitochondrial genetics is thus of interest for a range of fundamental investigations and is appealing to treat neurodegenerative diseases caused by organelle DNA mutations. We have previously established that isolated plant and mammalian mitochondria can functionally import double-stranded DNA through an active mechanism. We **aim** to understand the mechanism(s) underlying mitochondrial competence for DNA uptake and to develop cell uptake followed by mitochondrial targeting of functional gene constructs. **Methods**: We developed DNA uptake experiments with mitochondria isolated from potato (Solanum tuberosum) or from *Saccharomyces cerevisiae* mutants defective for various nucleus-encoded mitochondrial proteins and carriers. qPCR and RT-qPCR analyses assessed the level of cell-internalized construct and putative transcription. **Results**: We used both biochemical approaches and *S. cerevisiae* genetic tools to identify the still elusive inner membrane proteins participating in mitochondrial DNA import. Strikingly, among the candidates from the inner membrane carrier family selected on the basis of biochemical data with plant organelles, only the two minor forms of the adenine nucleotide translocator turned out to be required for optimal DNA translocation into isolated yeast mitochondria. Conversely, we highlighted a putative contribution of proteins that control mitochondrial morphology in S. cerevisiae. Building on the hypothesis that the competence for DNA uptake is also a property of the organelles *in vivo*, we attempted to use nanocarriers to target DNA to mitochondria in intact cells. We explored the use of a mitochondriotropic liposomal formulation to deliver a DNA construct encoding a recoded green fluorescent protein (gfp) gene controled by a rat mitochondrial promoter into the mitochondria in live rat cells. In comparison to free DNA and vehicle controls, incubation of the cells with liposome/DNA complexes led to significant incorporation of the construct and generation of gfp mRNA. **Conclusions**: Taken together, our data imply that there are significant variations in the mitochondrial DNA import mechanism between different organisms and that even in a given organism multiple pathways might operate. Our first in vivo results suggest that mitochondriotropic liposomes can deliver DNA into mitochondria of live mammalian cells, potentially opening novel prospects for mitochondrial transfection.

The work was financially supported by the CNRS, the University of Strasbourg and a grant from the French Ministry for Research (Investissements d'Avenir / Laboratoire d'Excellence MitoCross).

Defective regulation of miRNAs target genes in myoblasts from facioscapulohumeral dystrophy patients

Petr Dmitriev[1,2], Luiza Stankevicins[1], Eugenie Ansseau[6], Andrei Petrov[5], Ana Barat[1], Philippe Dessen[3], Thomas Robert[3], Ahmed Turki[2], Vladimir Lazar[3], Emmanuel Labourer[4], Alexandra Belayew[6], Gilles Carnac[2], Dalila Laoudj-Chenivesse[2], Marc Lipinski[1] and Yegor S. Vassetzky[1]

[1] UMR 8126, Univ. Paris-Sud, CNRS, Institut de Cancérologie Gustave-Roussy, F-94805 Villejuif, France

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

[3] Functional Genomics Unit, Institut de Cancérologie Gustave-Roussy, F-94805 Villejuif, France

[4]Ambion Inc., USA

[5]IBC Generium

[6]Laboratory of Molecular Biology, University of Mons; 20 place du Parc, B700 Mons, Belgium

vassetzky@igr.fr

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant hereditary neuromuscular disorder linked to the deletion of an integral number of 3.3 kb-long macrosatellite repeats (D4Z4) within the subtelomeric region of chromosome 4q. Most genes identified in this region are overexpressed in FSHD myoblasts including the double homeobox genes DUX4 and DUX4c. We have carried out a simultaneous miRNome/transcriptome analysis of FSHD and control primary myoblasts. Of 365 miRNAs analyzed in this study, 29 were found to be differentially expressed between FSHD and normal myoblasts. Twenty-one microRNA (miR-1, miR-7, miR-15a, miR-22, miR-30e, miR-32, miR-107, miR-133a, miR-133b, miR-139, miR-152, miR-206, miR-223, miR-302b, miR-331, miR-362, miR-365, miR-382, miR-496, miR-532, miR-654, miR-660) were upregulated, eight downregulated (miR-15b, miR-20b, miR-21, miR-25, miR-100, miR-155, miR-345, miR-594). Twelve of the miRNAs upregulated in FHSD were also upregulated in the cells ectopically expressing DUX4c, suggesting that this gene could regulate miRNA gene transcription. The myogenic miRNAs miR-1, miR-133a -133b and miR-206 were highly expressed in FSHD myoblasts which nonetheless did not prematurely enter myogenic differentiation. This could be accounted for by the fact that in FSHD myoblasts, functionally important target genes including cell cycle, DNA damage and ubiquitination-related genes, escape myogenic microRNA-induced repression.

Strategies for the selection and identification of active sites inhibitors with potential therapeutic value

Claire Loussouarn, Emilie Stephan-Queffeulou, Melody Shahsavarian, Séverine Padiolleau- Lefèvre, Bérangère Avalle & Alain Friboulet

"Enzyme and Cell Engineering" Université de Technologie de Compiègne / CNRS
Rue Roger Couttolenc - CS 60319 - 60203 COMPIEGNE CEDEX – France

Alain.Friboulet@utc.fr

The research developed in our laboratory is devoted to the study of biocatalysis, mainly by creating new functions not existing in nature to explain actual life properties and to produce new tools for biotechnology and medicine. Catalytic antibodies (abzymes) were first developed as tools in chemistry, or as laboratory curiosities. Our group has shown that the structural and functional mimicry of the idiotypic network of immune system can be exploited to elicit catalytic anti-idiotypic antibodies. Monoclonal antibodies with esterase, amidase and protease activity were thus obtained. The data obtained indicate that the idiotypic network is capable, to a significant extent, to replicate sophisticated catalytic apparatus of serine proteases, and further validates the use of mimicry of enzyme active centers by the immune system for induction of catalytic antibodies.

On the other hand, different groups have clearly demonstrated the presence of antigen-specific hydrolytic antibodies in a number of inflammatory, autoimmune and neoplastic disorders, but also in the serum of healthy donors; their pathogenic effects have been demonstrated occasionally. The origin of disease-associated catalytic antibodies may have been "induced" by the antigen implicated in the disease, may result from the loss of repressive control, or may be an expression of the idiotypic network with exacerbated self-recognition in autoimmune diseases. The field of catalytic antibodies has also brought lessons to understand the molecular architecture necessary for regulating a catalytic activity, and search for innovative approaches to inhibit "detrimental" activities or to elicit "beneficial" catalytic antibodies are developed. In the present talk, results obtained in the selection of peptidic and nucleic acid (aptamers) inhibitors will be presented, using both a model monoclonal anti-idiotypic catalytic antibody, and enzymes involved in leukemia's.

RNA architectural modules and their detection in sequences

Eric Westhof

Architecture et Réactivité de l'ARN, Université de Strasbourg,
Institut de Biologie Moléculaire et Cellulaire, CNRS,
15 rue René Descartes, 67084 Strasbourg, France

e.westhof@ibmc-cnrs.unistra.fr
http://www-ibmc.u-strasbg.fr/arn/Westhof/

RNA architecture can be viewed as the hierarchical assembly of preformed double-stranded helices defined by Watson-Crick base pairs and RNA modules maintained by non-Watson-Crick base pairs. RNA modules are recurrent ensemble of ordered non-Watson-Crick base pairs. Such RNA modules constitute a signal for detecting non-coding RNAs with specific biological functions. It is, therefore, important to be able to recognize such genomic elements within genomes. Through systematic comparisons between homologous sequences and x-ray structures, followed by automatic clustering, the whole range of sequence diversity in recurrent RNA modules has been characterized. These data permitted the construction of a computational pipeline for identifying known 3D structural modules in single and multiple RNA sequences in the absence of any other information. Any module can in principle be searched, but four can be searched automatically: the G-bulged loop, the Kink-turn, the C-loop and the tandem GA loop. The present pipeline can be used for RNA 2D structure refinement, 3D model assembly, and for searching and annotating structured RNAs in genomic data.

Cruz, J.A., and Westhof, E. (2011). Sequence-based identification of 3D structural modules in RNA with RMDetect. Nature methods 8, 513-521.

Inhibitory signalling to the Arp2/3 complex steers cell migration

Roman Gorelik[1,13], Irene Dang[1,13], Carla Sousa-Blin[1,13], Emmanuel Derivery[1], Christophe Guérin[2], Joern Linkner[3], Maria Nemethova[4], Tamara Tchipysheva[5], Valeria Ermilova[5], Sophie Vacher[6], Julien G. Dumortier[7], Florence A. Giger[7], Valérie Campanacci[8], Isaline Herrada[9], Anne-Gaelle Planson[8], Susan Fetics[8], Véronique Henriot[1], Violaine David[1], Ksenia Oguievetskaia[1], Goran Lakisic[1], Fabienne Pierre[1], Adeline Boyreau[11], Nadine Peyriéras[11], Anika Steffen[10], Klemens Rottner[10,12], Sophie Zinn-Justin[9], Jacqueline Cherfils[8], Nicolas B. David7, Ivan Bièche[6], Antonina Alexandrova[5], J. Victor Small[4], Jan Faix[3], Laurent Blanchoin[2], Alexis Gautreau[1]

CNRS Gif-sur-Yvette, France

group Cytoskeleton in Cell Morphogenesis, LEBS, CNRS UPR3082 Gif-sur-Yvette 91190, France

alexis.gautreau@lebs.cnrs-gif.fr

Cell migration requires the generation of branched actin networks that power the protrusion of the plasma membrane in lamellipodia. The Arp2/3 complex is the molecular machine that nucleates these branched actin networks. This machine is activated at the leading edge of migrating cells by the WAVE complex. The WAVE complex is itself directly activated by the small GTPase Rac, which induces lamellipodia. However, how cells regulate the directionality of migration is poorly understood. Here we identify a novel protein that inhibits the Arp2/3 complex in vitro, Arpin, and show that Rac signalling recruits and activates Arpin at the lamellipodial tip, like WAVE. Consistently, upon depletion of the inhibitory Arpin, lamellipodia protrude faster and cells migrate faster. The major role of this inhibitory circuit, however, is to control directional persistence of migration. Indeed, Arpin depletion in both mammalian cells and Dictyostelium discoideum amoeba resulted in straighter trajectories, whereas Arpin microinjection in fish keratocytes, one of the most persistent systems of cell migration, induced these cells to turn. The existence of a Rac-Arpin-Arp2/3 inhibitory circuit embedded within the positive feedback loop that maintains directional migration can account for this conserved role of Arpin in steering cell migration.

Molecular and cellular effects of siRNA on RET/PTC3 junction oncogene in papillary thyroid carcinoma

Hafiz Muhammad Ali[1], *Giorgia Urbinati* [1], *Hubert Chapuis* [2], *Didier Desmaële* [2], *Patrick Couvreur* [2], *Liliane Massaad-Massade* [1]

[1]Institut Gustave Roussy UMR 8203 CNRS, Villejuif

[2]Faculté de Pharmacie UMR 8612 CNRS, Châtenay-Malabry, France

Liliane.massade@igr.fr

Papillary thyroid carcinoma (PTC) accounts of above 80-90% cases of thyroid cancer and resulted by RET fusion with 12 other genes leading to 13 different onco-fusion genes. Amongst; RET/PTC3 (RET fusion with ELE1) is the most metastatic type occurring in 20–30 % PTC cases and represents an interesting target for small interfering RNA (siRNA) strategies since it is present only in the tumour cells and not in the normal cells. Our **aim** is: i) to target the RET/PTC3 oncogene by siRNAs, ii) to assess the knockdown effects on cell growth and cell cycle regulation. **Methods**: First, we established a stable cell line named RP3 cells derived from NIH-3T3 mouse fibroblasts by transfecting a plasmid containing RET/PTC3 junction oncogene. An efficient siRNA RET/PTC3 and an effective dose were selected on finding significant RET/PTC3 gene (RT-qPCR) and protein (Western blot) inhibitions. *In vitro* siRNA RET/PTC3 effects were tested on RP3 cell viability (MTT), toxicity (LDH), invasion/migration in matrigel (IncuCyte), apoptosis (FACS, WB), RET/PTC3 gene (RT-qPCR) and protein (WB) expressions. siRNA RET/PTC3-SQ bio-conjugate was synthesized, corresponding nanoparticles were prepared and tested for their effects on in vitro gene (RT-qPCR) and protein (Western blot) silencing. In vivo tumour growth inhibition and gene and protein silencing efficiency of these nanoparticles was later assessed as well, on administration in nude mice via intravenous route (cumulative dose = 2.5 mg/kg). **Results**: *In vitro* siRNA RET/PTC3 was found to significantly inhibit cell viability, LDH activity, invasion/migration, RET/PTC3 gene and protein expressions compared to siRNA control and siRNA RET/PTC1 at 24h, 48h and 72h post-transfection incubations. It was found to increase cell death in RP3 cells by apoptosis and necrosis (Annexin/PI, WB). By Western blot, an increased caspase-3 expression was found from 24h to 48h while PARP1 expression was increased from 24h to 72h post-transfection. In vivo, the RP3 cells were found to be tumorigenic in nude mice compared to mother NIH/3T3 cells. *In vivo* NPs siRNA RET/PTC3-SQ were found efficient in significant ($p<0.001$) tumour growth, gene and protein inhibitions. **Conclusions**: We established a siRNA efficient and specific to RET/PTC3 oncogene that we are able to deliver by "squalenoylation". These results open new prospects in the treatment by siRNAs of PTC with RET/PTC3 junction and offers a new non cationic plate-form for the siRNA delivery.

This work was supported by ANR "P2N Nanosqualonc", ANR-11-NANO-0003, http://www.anr.nanosqualonc.com

Interplay of the base excision repair and mismatch repair pathways in active DNA demethylation

Inga Grin and Alexander A. Ishchenko

Université Paris Sud, Laboratoire « Stabilité Génétique et Oncogenèse » CNRS, UMR 8200, Institut de Cancérologie Gustave Roussy, Villejuif, France.
Alexander.ISHCHENKO@gustaveroussy.fr

Active DNA demethylation in mammals occurs via hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by the Ten-eleven translocation family of proteins (TETs). 5hmC residues in DNA can be further oxidized by TETs to 5-carboxylcytosines and/or deaminated by the AID/APOBEC family proteins to 5-hydromethyluracil (5hmU). Excision and replacement by regular C of these 5mC-derivatives is initiated by several DNA glycosylases including: thymine-DNA glycosylase (TDG), methyl-binding domain protein 4 (MBD4) and single-strand specific monofunctional uracil-DNA glycosylase (SMUG1) in the base excision repair (BER) pathway. Recently, it has been shown that non-canonical DNA mismatch repair system (MMR) can repair both alkylation and oxidative damage to DNA. Here, we used a phagemid based DNA substrate, containing defined oxidative base lesions and methylation marks to identify and characterize different DNA repair pathways: BER, nucleotide incision repair and MMR, which may be involved in active DNA demethylation. Our data obtained from cell-free extracts repair assays support the role of MMR in the active DNA demethylation in mammalian cells.

Deciphering the molecular mechanisms underlying the three-dimensional folding of the *Drosophila* genome into physical domains

Caroline Jacquier,[1] *Tom Sexton,*[1] *Eitan Yaffe,*[2] *Ephraim Kenigsberg,*[2]
Frédéric Bantignies,[1] *Benjamin Leblanc,*[1] *Michael Hoichman,*[2]
Hugues Parrinello,[3] *Amos Tanay,*[2] *and Giacomo Cavalli.*[1]

1Institut Génétique Humaine, UPR 1142, CNRS, 141 rue de la Cardonille, 34396 Montpellier, France

2Department of Computer Science and Applied Mathematics and Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel

3Montpellier GenomiX IBiSA, 141 rue de la Cardonille, 34396 Montpellier Cedex 5, France

caroline.jacquier@igh.cnrs.fr

The spatial organisation of the genome has been correlated with transcriptional regulation for years: co-expressed genes share specialised sites within the nucleus for their expression or repression, and regulatory chromatin loops bring distal elements such as enhancers in contact with their target genes. Very recently, technical advances (the “Hi-C” method) have allowed such chromatin interactions to be assessed systematically. A major finding to come from the genome-wide maps of such interactions is the conserved organisation of metazoan genomes into well-demarcated physical domains, whereby interactions are strong within domains and are sharply reduced when crossing domain boundaries. Such domains contain co-expressed genes and overlap extensively with active and repressive epigenetic marks, suggesting that the spatial and functional organisations of the genome are closely linked. However, the mechanisms establishing and maintaining these potentially important functional modules are not known.

In drosophila, a systematic screen of chromatin profiles revealed a striking enrichment for insulator proteins, such as CP190, Beaf-32 and CTCF, and for the mitotic spindle protein Chromator at domain boundaries. These observations strongly suggest that insulator binding sites actively define domain demarcations. To test this hypothesis, we are establishing genome-wide maps of chromatin interactions using the Hi-C method we developed in the lab, as well as chromatin profiles of epigenetic marks in various genetic backgrounds lacking these boundary-binding factors. These data will enable us to assess if chromatin folding into physical domains is impaired in absence of these factors and if epigenetic domains are consequently remodelled.

Progressive parkinsonism by acute dysfunction of excitatory amino acid transporters in the rat substantia nigra

Maxime Assous, Laurence Had-Aissouni, Paolo Gubellini, Christophe Melon, Imane Nafia, Pascal Salin, Philippe Kachidian, Lydia Kerkerian-Le Goff

CNRS/Aix-Marseille Université, IBDML UMR7288, 13009, Marseille, France

lydia.kerkerian@ibdml.univmed.fr

Parkinson's disease (PD) is characterized by the progressive degeneration of substantia nigra (SN) dopamine neurons, involving a multifactorial cascade of pathogenic events. Central players in PD pathogenesis, such as mitochondrial dysfunction and oxidative stress, can affect the function of excitatory amino acid transporters (EAATs), which play a major role in preventing excitotoxicity and maintaining physiological levels of GSH. Here we explore the hypothesis that dysfunction of excitatory amino acid transporters (EAATs) might contribute to the vicious cycle sustaining degeneration of SN DA neurons. Acutely-induced dysfunction of EAATs in the rat SN, by single unilateral injection of their substrate inhibitor L-trans-pyrrolidine-2,4-dicarboxylate (PDC), triggers a neurodegenerative process mimicking several PD features. Dopamine neurons are selectively affected, consistent with their sustained excitation by PDC measured by slice electrophysiology. The anti-oxidant N-acetylcysteine and the NMDA receptor antagonists ifenprodil and memantine provide neuroprotection. Besides oxidative stress and NMDA receptor-mediated excitotoxicity, glutathione depletion, autophagy and neuroinflammation characterize the primary insult. Most interestingly, the degeneration progresses overtime with unilateral to bilateral and caudo-rostral evolution. Transient compensatory changes in dopamine function markers in SN and the target striatum accompany cell loss and axonal dystrophy, respectively. Motor deficits appear when neuron loss exceeds 50 % in the most affected SN and striatal dopamine tone is dramatically reduced. As a possible cellular insight for neuron death progression, subthalamic nucleus metabolic activity shows an asynchronous bilateral increase. These findings outline a functional link between EAAT dysfunction and several PD pathogenic mechanisms/pathological hallmarks, and provide the first acutely-triggered model of progressive parkinsonism.

Telomere length regulation in *Hansenula polymorpha*

Alexander N. Malyavko[1], *Elena M. Smekalova*[1], *Maria I. Zvereva*[1], *Andrey V. Mardanov*[2], *Nikolai V. Ravin*[2], *Konstantin G. Skryabin*[2], *Eric Westhof*[3], *Olga A. Dontsova*[1]

[1]Faculty of Chemistry, Lomonosov Moscow State University,
119999 Moscow, Russia and
Belozersky Institute, Moscow State University,
Leninskie Gory 1, Bldg. 40, 119991 Moscow, Russia.
[2]Centre "Bioengineering" of RAS,
Prosp. 60-let Oktyabrya, bld. 7-1, Moscow 117312, Russia
[3]Architecture et Réactivité de l'ARN, Université de Strasbourg,
Institut de Biologie Moléculaire et Cellulaire du CNRS,
15 rue René Descartes F– 67084 Strasbourg, France
malyavkoan@gmail.com

Aim. To characterize telomerase from *Hansenula polymorpha*. **Methods**. Telomerase assay, telomeric PCR, pyrosequencing of PCR products. **Results**. Telomerase sustains telomere homeostasis and provides unlimited proliferative potential for unicellular eukaryotes, as well as stem, germ and cancer cells. The two core components of telomerase complex are telomerase RNA (TER) and telomerase reverse transcriptase (TERT). After each round of replication chromosomes lose telomeric repeats. TERT maintains telomere length by reverse transcription of the short template region of TER. We found that telomerase from yeast *Hansenula polymorpha* elongated its substrate by one additional nucleotide in vitro (compared to the expected sequence from the predicted template region). We showed that this nucleotide (dT) was reverse transcribed from adenine, which was positioned beyond the predicted template boundary. The product of such elongation could not be recognized by telomerase as a substrate that should compromise effective telomerase action at telomeres. Sequencing of PCR products of telomeres provided evidence for the incorporation of this noncognate dT nucleotide into telomeres in vivo. The involvement of this event in telomere length regulation was confirmed by mutational analysis of the template region of *H. polymorpha* TER. **Conclusions**. *H. polymorpha* telomerase utilizes reverse transcription of the noncognate nucleotide to control telomere elongation.

Materials of this work are part of the article, which is currently in press (Smekalova et al. "Specific features of telomerase RNA from Hansenula polymorpha". RNA).

The $tRNA_3^{Lys}$ packaging complex involves association of human mitochondrial LysRS with the polyprotein GagPol from HIV-1: a new pharmacologic target?

José Dias, Lydia Kobbi, Martine Comisso, Aurélien Nail, Anne Szklarz, and
Marc Mirande

Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France

Marc.Mirande@lebs.cnrs-gif.fr

Cytosolic and mitochondrial LysRS are encoded by alternative splicing of a single gene and can only be distinguished according to their very N-terminal sequences. Beyond its role in translation, mitochondrial LysRS (mLysRS) is also hijacked from the host cell following HIV-1 infection to carry the primer tRNA3Lys into the virions1. Using monospecific antibodies, we previously showed that only mLysRS is taken up in viral particles along with tRNA3Lys, the primer for reverse transcription of the HIV-1 genome. We screened all the viral proteins to identify the partners of LysRS responsible for the formation of the tRNALys packaging complex. We showed that mLysRS associates with the Pol domain of GagPol. This interaction is highly specific, as assessed by the KD value of about 5-10 nM between mLysRS and Pol. More specifically, the transframe (TF) and integrase (IN) domain proteins of Pol interact with the catalytic domain of LysRS2. A model of the assembly of the mLysRS:$tRNA_3^{Lys}$:GagPol packaging complex is proposed, which is also consistent with the release of its different components after maturation of GagPol into the virions. Maturation of the precursor of mLysRS upon its mitochondrial targeting is a prerequisite to form a complex with tRNA3. These data open new perspectives for the search of a new class of inhibitors of the HIV-1 development cycle that would block the packaging of tRNA3Lys into viral particles.

1Kaminska et al. J. Virol. (2007) 81: 68-73.
2Kobbi et al. J. Mol. Biol. (2011) 410, 875-886.
3Dias et al. Biochemistry (2012) 51, 909-916.

Structure of mammalian proto-oncogene eEF1A2 and its functional implications

B. Negrutskii, *V. Shalak, A. Yaremchuk, T. Crepin, O. Novosyl'na, D. Vlasenko, M. Tukalo, A. El'skaya*

State Key Laboratory of Molecular and Cellular Biology,
Institute of Molecular Biology and Genetics NAS of Ukraine.
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680

negrutskii@imbg.org.ua

eEF1A2 is the isoform of mammalian translation elongation factor which is also involved in the carcinogenesis. Comparison of spatial structures of the eEF1A isoforms reveals different organization and oligomeric properties of eEF1A2 and eEF1A1. Crystal structure of preferentially monomeric, naturally folded and post-translationally modified eEF1A2 in the complex with GDP uncovers major structural and functional difference in nucleotide binding and exchange procedures in the mammalian and prokaryotic analogues. Novel mechanism of the guanine exchange factor eEF1B action in mammalian cells is described.

Stress granules and the proliferative rate of epithelial cells

Loic Hamon, Ouissame Bounedjah, Bénédicte Desforges, Patrick A. Curmi, David Pastre

Institut National de la Santé et de la Recherche Médicale (INSERM),
UMR829; Université Evry-Val d'Essonne;
Laboratoire Structure-Activité des Biomolécules Normales et Pathologiques,
Evry 91025, France.

david.pastre@univ-evry.fr

Following severe oxidative, heat or osmotic stresses, and also after some viral infections, micrometric cytoplasmic bodies composed of mRNAs and many RNA-binding proteins appear in the cytoplasm of eukaryotic cells. For more than one decade, these non-membranous granules called “stress granules” were the subject of intense research. The most popular model is that stress granules are formed after the massive release of mRNAs from polysomes with the help of self-attracting RNA-binding proteins like TIA-1 and G3BP, this process being microtubule-dependent, at least for oxidative stress after arsenite exposure. These granules then could allow the reprogrammation of the translational response via the sequestration of mRNAs encoding housekeeping proteins in stress granules and the exclusion of mRNAs encoding important proteins like heat shock proteins in order to allow their translation. Here we will show that elevated polyamine contents and highly dynamical microtubules are important factors that allow the formation of stress granules in proliferating cells, in contrast with their quiescent counterpart. Many interesting aspects remain to be tackled especially regarding the potential advantages/disadvantages resulting from the formation of stress granules in proliferating cells.

The prion protein: an unexpected link between base excision repair and neurodegeneration

Anne Bravard[1]*, Frédéric Auvré*[1]*, Jacqueline Bernardino-Sgherri*[1]*,*
Damiano Fantini[1]*, Zhou Xu*[2]*, Ludmilla Sissoëff*[2]*, Olivier Etienne*[1]*,*
Mathieu Daynac[1]*, GianLuca Tell*[3]*, François Boussin*[1]*, Jean-Philippe Deslys*[2]*, and*
J. Pablo Radicella[1]

[1]Institute of Cellular and Molecular Radiobiology, CEA, Fontenay-aux-roses, France ;
[2]Institut des Maladies Emergentes et des Thérapies Innovantes, CEA,
Fontenay-aux-roses, France ;
[3]Department of Biomedical Sciences and Technologies, University of Udine, Italy.

pablo.radicella@cea.fr

A conformational change in the highly conserved and ubiquitously expressed prion protein (PrPc) to yield an abnormal form (PrPSc) is associated with the generation of prions, the infectious agent of transmissible spongiform encephalopathies. Whether the resulting pathology is due to the accumulation of PrPSc aggregates or to a loss of function of the normal PrPc is still an open question since, despite decades of investigation, the physiological role of the normal PrPc remains elusive. A protective effect against oxidative stress has been shown, but the underlying mechanisms have not been determined. Here, by using animal and cellular models, we unveil a key role of PrP in the DNA damage response. We found that exposure of neurons to a genotoxic stress activates PRNP transcription leading to an increased amount of PrP in the nucleus where it interacts with APE1, the major mammalian endonuclease essential for base excision repair (BER), and stimulates its activity. Preventing the induction of PRNP results in accumulation of abasic (AP) sites in DNA and impaired cell survival after genotoxic treatment. The reduced activity of APE1 in brains from Prnp-/- mice is associated with a defect in the repair of induced DNA damage in vivo. Brains from mice at early stages of prion infection also display a reduced APE1 activity, suggesting that loss of the PrP protective function on DNA plays a role in neuronal death and could be implicated in many neurodegenerative processes.

Increased mobility of broken MLL gene ends in human lymphoid cells treated with DNA topoisomerase II poison etoposide

Sergey I. Glukhov[1], *Mikhail A. Rubtsov*[1], *Daniil A. Alexeyevsky*[4], *Andrei V. Alexeevski*[3,4,5], *Olga V. Iarovaia*[2], *Sergey V. Razin*[1,2,6]

[1]Dpt Molecular Biology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

[2]Institute of Gene Biology RAS, Moscow, Russia

[3]A.N. Belozersky Institute for Physical and Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

[4]Dpt of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia

[5]Scientific Research Institute for System Studies (NIISI RAN), Moscow, Russia

[6]LIA 1066 French-Russian Joint Cancer Research Laboratory, Villejuif, France-Moscow, Russia

sergey.v.razin@usa.net

The mixed lineage leukaemia (MLL) gene is frequently rearranged in secondary leukaemias, in which it could fuse to a variety of different partners. Breakage in the MLL gene preferentially occurs within a ~8 kb region that possesses a strong DNA topoisomerase II cleavage site. It has been proposed that DNA topoisomerase II-mediated DNA cleavage within this and other regions triggers translocations that occur due to incorrect joining of broken DNA ends. To further clarify a possible mechanism for MLL rearrangements, we analysed the frequency of MLL cleavage in cells exposed to etoposide, a DNA topoisomerase II poison commonly used as an anticancer drug, and positioning of the broken 3'-end of the MLL gene in respect to inherent chromosomal territories. It was demonstrated that exposure of human Jurkat cells to etoposide resulted in frequent cleavage of MLL genes. Using MLL-specific break-apart probes we visualised cleaved MLL genes in ~17 % of nuclei. Using confocal microscopy and 3D modelling, we demonstrated that in cells treated with etoposide and cultivated for 1 h under normal conditions, ~9 % of the broken MLL alleles were present outside the chromosome 11 territory, whereas in both control cells and cells inspected immediately after etoposide treatment, virtually all MLL alleles were present within the chromosomal territory. The data are discussed in the framework of the "breakage first" model of juxtaposing translocation partners. We propose that in the course of repairing DNA topoisomerase II-mediated DNA lesions (removal of stalled DNA topoisomerase II complexes and non-homologous end joining), DNA ends acquire additional mobility, which allows the meeting and incorrect joining of translocation partners.

DNA-protein and protein-protein interactions in nucleotide excision repair and their regulation

N. I. Rechkunova[1]*, Y. S. Krasikova, E. A. Maltseva, M. V. Sukhanova, O.I. Lavrik*[1]
Institute of Chemical Biology and Fundamental Medicine, 630090 Novosibirsk, Russia
[1]Department of Natural Sciences, Novosibirsk State University, 630090, Novosibirsk, Russia
nadyarec@niboch.nsc.ru

Aim. Nucleotide excision repair (NER) is one of the major mechanisms to prevent genomic DNA instability. This process removes a wide range of lesions distorting the double helix and bulky chemical adducts resulting from environmental factors or chemotherapeutic agents. The coordination of the assembly of the NER complexes is achieved through multiple DNA-protein and protein-protein interactions. The interactions of key NER proteins, XPC-RAD23B, XPA, and RPA with DNA structures mimicking NER intermediates and their cooperation within DNA-protein complexes have been analyzed. **Methods.** The topography of the NER protein complexes with damaged DNA was determined by photoaffinity labeling technique using DNA structures containing photoreactive 5I-dUMP residues in the certain positions either in damaged or in undamaged strands and fluorescein group linked to uridine residue as a lesion. Electrophoretic mobility shift assay (EMSA) and fluorescent depolarization measurements were used for the analysis of DNA-protein binding. **Results.** Positioning of human and yeast DNA damage recognition complexes, XPC-RAD23B and Rad4-Rad23, respectively, on damaged DNA was determined. Under conditions of equimolar binding to DNA both proteins exhibited the highest level of crosslinks to 5I-dUMP located exactly opposite the damaged nucleotide. Both proteins bind to the damaged 15 nt bubble-DNA structure mimicking in size the "transcription bubble" DNA intermediate with the highest affinity. RPA and XPA are very abundant proteins that are absolutely required for NER functioning. Both RPA and XPA proteins stimulate XPC binding to the damaged DNA and enhance the level of XPC-DNA crosslinks. An influence of the substitution of lysine in DNA binding cleft of XPA by glutamate (XPA K141E, K179E, and K141/179E; recombinant plasmids were kindly provided by Dr. Hanspeter Naegeli, University of Zürich) on the interaction with various DNA structures was analyzed. Both K179E and K141E mutations result in moderate decrease in DNA binding affinity and do not influence on the protein positioning on partially open DNA duplex. Tandem mutation K141/179E dramatically reduced XPA affinity to DNA. The results allow suggesting the key role of XPA orientation for positioning the NER preincision complex. Poly(ADP-ribose)polymerase-1 (PARP-1) is one of the candidate to participate in NER regulation. Using EMSA, the influence of PARP1 on binding of XPC-RAD23B and XPA to model DNA-structures modulated by PAR synthesis was revealed. **Conclusions.** Obtained data fill the gap between biochemical results for XPC-RAD23B and X-ray structure for yeast ortholog Rad4-Rad23 and exhibit a significance of protein-protein interactions for the correct assembling of the NER machinery. PARP1 can be regarded as the universal regulator in DNA repair processes.

This work was supported by RFBR (grants 12-04-31323, 12-04-33162, 13-04-00538, 13-04-93107, 13-04-40197-comfi); the Russian Ministry of Education and Science (contract 14.B37.21.0188); the Program of the RAS Presidium 'Molecular and Cellular Biology'

Search for molecules with combined antioxidant and DNA-binding activities

E. Rostoka[1,2], S. Isajevs[2], E.Buraka[1,2], L. Baumane[1], N. Sjakste[1,2]

[1]Latvian Institute of Organic Synthesis, Aizkraukles Street 21, Riga LV1006, Latvia;

[2]Faculty of Medicine, University of Latvia, Raina bulv. 19, Riga LV1050, Latvia

Search for substances protecting DNA against oxidative damage is a topical problem. In our work we have tried to assess possible antioxidant and DNA-protecting activities in a vast group of compouds: natural compounds - baicalein, quercetin, kaempferol, indole-3-carbinole, luteolin, lycopene, myricetin, separately and combinations and artificial 1,4-dihydropyridines (1,4-DHP) synthesized in Latvian Institute of Organic Synthesis. Several natural compounds (baicalein, luteolin, quercetin, indole-3-carbinol) decreased NO production in different tissues and organs of intact rats. Intraperitoneal injection of lipopolysascharide (LPS) to the animals caused a drastic increase of NO production levels in all tissues studied. The highest production of nitric oxide was detected in liver. Baicalein and luteolin decreased NO outburst in several organ. In contrast, administration of the indole-3-carbinol enhanced the LPS-induced increase of NO production, quercetin produced a similar effect. No influence of indole-3-carbinol and quercetine on iNOS expression in liver was observed in the control group. Luteolin up- regulated the gene expression. Baicalein decreased level of the gene expression. Level of transcription was still decreased when baicalein was given in combination with luteolin. The LPS induced iNOS gene expression on high level. Quercetin and kaempferol significantly decreased the LPS-triggered iNOS mRNA expression. Enhancement of the iNOS mRNA expression by indole-3-carbinol was observed. The same trend was observed when indole-3-carbinol was supplemented by quercetin. Baicalein decreased the gene expression triggered by LPS, the effect was better pronounced if it was combined with luteolin also in sepses model. Interestingly, in brain cortex indole-3-carbinol inhibited iNOS mRNA expression in control group. LPS triggered a marked increase in iNOS gene mRNA copies compared to LPS-untreated animals. Indole-3-carbinol and lycopene enhanced this effect. In the 1,4-DHP study Metkarbaton appeared to be the only effective hydroxyl radical scavenger in Fenton reaction among twenty tested 1,4-DHP. AV-153-Na, AV-154-Na, AV-153-Li, PP-150-Na, PP-54-NH_4 manifested ability to protect plasmid DNA against damages caused by peroxynitrite *in vitro*. Compound AV-153 evidently interacted with DNA, as addition of the sonicated rat liver DNA to AV-153 solutions caused pronounced hyperchromic and bathochromic effects on the spectra. Reciprocally addition of the compound to DNA solutions caused hypochromic effect in DNA spectra. Similar effects were observed when intact pTZ57R plasmid was used for titration. Oxydative modifications of DNA bases by peroxynitrite did not change much binding affinity of the compound. However the effect was much stronger when sonicated plasmid was taken for titration. The binding mode did not change when ionic strength of the solution was changed from 5mM to or 150mM of NaCl, it became stronger at 200 mM. Interaction of the compound with AT-rich DNA of *Staphilococcus aureus* ot GC-rich of *Micrococcus luteus* were much weaker compared to interaction with DNA of similar AT and GC content. Data of in vivo studies will be also reported.

The work was supported in part from the European Regional Development Foundation project No 2010/0315/2DP/2.1.1.1.0/10/APIA/VIAA/026

New repair pathways for free radicals induced complex DNA damage

Ibtissam Talhaoui[1], Doria Alilia, Armelle Vigouroux[2], Bulat Aikeshev[1], Nicolas E. Geacintov[3], Solange Morera[2], Alexander A. Ishchenko[1], Murat Saparbaev[1]

[1]Groupe «Réparation de l'ADN», Université Paris Sud, Laboratoire « Stabilité Génétique et Oncogenèse » CNRS, UMR 8200, Institut Gustave Roussy, Villejuif, France
[2]Laboratoire d'Enzymologie et de Biochimie Structurales (LEBS), CNRS, Gif-sur-Yvette Cedex, F-91198, France
[3]Chemistry Department, New York University, New York, NY 10003-5180

Murat.SAPARBAEV@gustaveroussy.fr

Oxidative DNA lesions are believed to be a major type of endogenous damage leading to human degenerative disorders including cancer, cardiovascular disease and neurological syndromes. The clinical features of inherited human DNA repair deficient disorders such as Cockayne syndrome and Fanconi anemia point to complex nature of endogenous oxidative DNA damage which may include bulky adducts (cyclopurines, exocyclic bases), interstrand and intrastrand DNA crosslinks. Conversely, severe biological effects of ionizing radiation and drugs such as bleomycin, mitomycin and neocarzinostatin are correlated with clustering of lesions within a single helical turn of the DNA molecule. At present, the precise repair mechanisms for the complex DNA damage (CDD) are poorly understood. Oxidized DNA bases are substrates for two overlapping pathways: base excision repair (BER) and nucleotide incision repair (NIR). In the BER pathway a DNA glycosylase cleaves the N-glycosylic bond between the abnormal base and deoxyribose, leaving either an abasic site or single-stranded break in DNA. Alternatively, in the NIR pathway, an apurinic/apyrimidinic (AP) endonuclease incises oxidatively damaged DNA in a DNA glycosylase-independent manner, providing the correct ends for DNA synthesis coupled to the repair of the remaining 5′-dangling damaged nucleotide. In the present work we studied alternative repair pathways for bulky G-T intrastrand crosslinks and spiroiminodihydantoin (Sp) adduct when present in DNA. Here we demonstrate that bacterial, yeast and human AP endonucleases can directly repair G-T crosslinks and Sp residues in the NIR pathway. Furthermore, we describe genetic dissection of human AP endonuclease 1 functions in BER and NIR pathways and construction of NIR-deficient mammalian cell lines. The potential biological roles of the BER and NIR pathways in counteracting complex DNA damage are discussed.

Poly(ADP-ribose) glycohydrolase in DNA repair and replication: towards potential applications in anticancer strategies ?

Giuditta Illuzzi[1], Elise Fouquerel[1,2], Aurélia Noll[1], Jean-Christophe Amé[1], Marie-José Nzambi-Khonde[1], Carole Beck[1], Barbara Camuzeaux[1], Françoise Dantzer[1], Valérie Schreiber[1]

[1]IREBS-Biotechnology and Cell Signalling, UMR7242-CNRS, Université de Strasbourg, Labex Medalis, 300 Bd Sébastien Brant, BP 10413, 67412 Illkirch, France.

[2]Present address: University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, Pennsylvania 15232, United States

valerie.schreiber@unistra.fr

Poly(ADP-ribosyl)ation is a post-translational modification of proteins involved in a wide number of biological processes including DNA repair, transcription, cell differentiation or cell death. The regulation of poly(ADP-ribose) produced in response to DNA damage by the poly(ADP-ribose) polymerases (PARPs), and degraded by poly(ADP-ribose) polymerase (PARG) is critical for the damaged cell fate. The role of the founding member of the PARP family PARP-1 is highly documented in the DNA damage response. Moreover, PARP inhibitors are involved in clinical trials to potentiate the action of anticancer clastogenic drugs as well as for their cytotoxic effect on tumours harbouring mutations in genes involved in double strand breaks repair by homologous recombination, such as BRCA1/2. Far less is known about PARG and the role of its different isoforms in the cell response to DNA damage. A major goal of our laboratory is to determine whether PARG could also be considered as a promising target for anticancer strategies. We have shown previously that the absence of PARG increases radiosensitivity and affects the repair of radioinduced single (SSB) and double (DSB) strand breaks. We have also demonstrated a functional link between PARG and the repair/replication factor PCNA: binding to PCNA contributes to PARG recruitment to laser induced DNA damage sites and to replication foci. This latter observation prompted us to investigate the contribution of PARG in DNA replication. Whereas PARG appeared dispensable for normal replication, PARG-deficient cells showed increased sensitivity to the replication inhibitor hydroxyurea (HU). These cells showed defect in S-phase restart after prolonged but not short HU-treatment, conditions known to trigger replication fork collapse and formation of double strand breaks that are repaired by homologous recombination. Our current studies are aimed to determine the underlying mechanisms involving PARG and poly(ADP-ribose) in the cell recovery from replication stress.

Project funded by CNRS, Université de Strasbourg, Labex Medalis, Ligue contre le Cancer (Equipe Labellisée), EDF.

Association of the *PSMA3* gene polymorphisms with multiple sclerosis

Tatjana Sjakste[1], *Jolanta Kalnina*[1,2], *Natalija Paramonova*[1], *Olga Sugoka*[1]

[1]Genomics and Bioinformatics, Institute of Biology of the University of Latvia;
[2]Faculty of Medicine, University of Latvia;
tanja@email.lubi.edu.lv

Multiple sclerosis (MS) is the most common autoimmune disorder of the central nervous system. The cause of MS is still poorly understood and different metabolic pathways seem to play a role in disease development. Failure of ubiquitine proteasome system (UPS) efficiency had been recently implicated to MS pathogenesis. The **aim** of the present study was to analyse the association between MS in Latvian population and allelic variants of the *PSMA3* gene encoding one of T1A peptidase, which is a 20S core alpha subunit of a constitutive and immune-proteasomes. **Methods**: The rs2348071 SNP of the *PSMA3* gene (c.543 + 138G > A) has been genotyped in 281 MS patients (201 women) being diagnosed on relapsing – remitting (188 subjects) or secondary progressive (93 subjects) course of the disease, versus 191 control subjects (117 women) without inflammatory and any autoimmune disorders. **Results**: In controls the rs2348071 locus showed allele and genotype presentation similar to other Europeans with MAF about 30 % and genotype GG being most frequent (53 %). In both female and male cohorts of MS patients the minor allele A was observed slightly more frequent than in controls ($P < 0.05$), frequency of both GG and AA homozygotes was decreased (about 30 % and 5 % respectively) in favour of heterozygote GA genotypes (from 61 % to 71 % in patients of relapsing – remitting and secondary progressive course of disease respectively) that was significantly higher than in controls ($P < 0.0001$; OR = 3.539 [95 % CI 2.409 – 5.198] according to co-dominant model). The *PSMA3* c.543 + 138 G > A nucleotide substitution potentially could affect binding of several transcription factors, sequence similarity to splicing signals and other modulators of gene expression and finally influence 20S proteasome functionality. **Conclusion**: The rs2348071 heterozygous genotype appears to be the MS risk factor in Latvian population.

The work was supported in part from the European Regional Development Foundation project No 2010/0315/2DP/2.1.1.1.0/10/APIA/VIAA/026

Both integration of plasmid DNA into genome and constitutive overexpression of CHI3L1 promote chromosome instability and phenotype changes in 293 cells

Stepanenko A.A.[1], Andreeva S.V.[1], Mikitenko D.A.[1], Huleyuk N.[1], Vassetzky Y.S.[2], Kavsan V.M.[1]*

[1]State Key Laboratory of Molecular and Cellular Biology,
Institute of Molecular Biology and Genetics,
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680
[2]CNRS, Université Paris-Sud 11, Institut de Cancérologie Gustave Roussy,
94805 Villejuif, France
a.a.stepanenko@gmail.com

To study the patterns of CCAs/NCCAs after genotoxic stress (integration of foreign DNA into genome and overexpression of oncogene *CHI3L1*) and to establish the link between changes of karyotype and malignant phenotype, we used two distinct 293 cell lines (variant 1 and variant 2) with different morphology, proliferation level, aggressiveness, and degree of CIN. Empty vector stably transfected 293_pcDNA3.1 cells variant 1, 293_pcDNA3.1 cells variant 2, and 293_*CHI3L1* cells (nonclonal) were derived from 293 cells variant 2. 293_*CHI3L1* cells clone 1 and clone 2 were obtained from 293 cells variant 1.

293 cells variant 1 and variant 2 differed in many marker chromosomes and degree of CIN (CCA/NCCAs) and resembled different phases of evolution (step-wise phase and punctuated/discontinuous phase) according to Heng et al. [Heng et al., 2010-2013]. Analysizing 293 cell derivatives we conclude that genotoxic stress (as integration of foreign DNA into genome and overexpression of oncogene *CHI3L1*) potentiates CIN during somatic cell evolution and tumorigenesis. Karyotypes of cell lines after genotoxic stress evolved stochastically and were individual with different patterns of CCAs/NCCAs. The pattern of CCAs/NCCAs of cells depended on the nature of genotoxic stress, phase of cells evolution, the nature of applied stress, and time lapse (number of passages), which cells were in culture after genotoxic stress before karyotyping. Artificial manipulations of specific molecular mechanisms (e.g., CHI3L1 oncogene overexpression) into a dominant form strongly promoted cancer evolution, drastically reducing genome heterogeneity. The pattern of CCAs/NCCAs reflected tumorigenic properties of cells. The interplay between CCAs and NCCAs is a driving force for cellular genetic heterogeneity, phenotypic plasticity, and clonal population diversity that is essential to cancer evolution.

The study of Hsp60 role in thyrod cancer progression

[1]Sidorik L.L., [1]Kapustian L., [1]Chornyy S., [2]Gurtovyy V., [3]Pogrebnoy P., [2]Khozhaenko Yu., [4]Gagarkin N., [2]Usenko V. S.

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
[2]Pathomorphological laboratory BIONTEK, Dnipropetrovs'k, Ukraine
[3]Kavetsky' Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine
[4]Mechnikoff' Clinical Hospital, Dnipropetrovs'k, Ukraine
sidorik@imbg.org.ua

Background. A wide range of tumor cells or tissues have been shown to express atypical level or localization of Hsp60. Hsp60 expression in breast or gastric cancer is associated with poor prognosis and resistance to chemotherapy or radiation therapy. Hsp60 expression in thyroid cancer has not been studied extensively. The aim of our study was to evaluate the possible changes in Hsp60 cellular content and localization in thyroid gland' cells in normal state and upon pathology by quantitative immunohistochemical analysis and to exam the correlation between anti-Hsp60 autoantibodies level and the degree of thyroid gland lesions in this group of patients. **Methods**. Sera from 49 patients (46 females and 3 males, 20–57 years old, 12 – nodular hyperplasia of thyroid gland, 12 – Hashimoto's thyroiditis, 18 – follicular adenoma, 6 –papillary and 1 follicular carcinoma) and 12 healthy donors (as a control) were used for determination of anti-Hsp60 antibodies level by ELISA. To evaluate the content and localization of Hsp60 in thyroid tissue of such patients and upon pathology we used immunohystochemical analysis. As a control, autopsy material of 12 thyroid tissue without morphological signs of thyroid pathology was used. Hsp60 expression was evaluated according to the percentage of positively stained cells. **Results.** The increased anti-Hsp60 autoantibodies level has been detected in sera more than 50 % of patients with non-malignant thyroid gland pathology (Hashimoto's thyroiditis, nodular hyperplasia, follicular adenoma) and in 86% of TC patients sera by ELISA method. This finding correlates with the degree of thyroid gland lesions in this group of patients obtained by the immunohistochemical study. **Conclusion**. Significant increase of anti-Hsp60 antibodies level was determined in sera of patients with thyroid pathology. The highest titers of anti-Hsp60 antibodies in sera of patients with thyroid cancer has been revealed. Changes of Hsp60 expression and cellular localization in thyroid cancer tissue have been detected in comparison with normal ones. The increase of Hsp60 expression and elevated level of anti-Hsp60 autoantibodies was associated with morphological signs of pathology - lymphoid infiltration and sclerotic changes of tissue. The working model of Hsp60 involvement in thyroid cancer progression is proposed.

Specificity of DNA import into isolated mitochondria from plants and mammals

M. V. Koulintchenko[1,2], N. Ibrahim[2], E. S. Klimenko[1], Yu. M. Konstantinov[1], F. Weber-Lotfi[2], A. Dietrich[2]

1 Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences, 132 Lermontova Str., Irkutsk, Russian Federation, 664033

2 CNRS Institute of Plant Molecular Biology, University of Strasbourg, 12 General Zimmer Str., Strasbourg, France, 67084

mk100171@yahoo.com

The **aim** of the project is to study different features of DNA import into plant mitochondria and into human mitochondria. **Methods.** We developed DNA uptake experiments with isolated plant mitochondria, using as substrates various sequences associated or not with the specific TIRs present at each end of the 11.6 kb linear plasmid from rapeseed (*Brassica napus* L.). Further substrates for mitochondrial import were the S1 and S2 linear plasmids from maize (*Zea mays*). **Results. T**he efficiency of the import of large DNA molecules into plant mitochondria depends on the sequence and (ii) the specificity of DNA import can be mediated by the presence of certain elements in their sequence, especially TIRs at the ends of the molecules. Conversely, the efficiency of DNA import into mammalian mitochondria seemed to depend neither on the DNA sequence, nor on its size. We addressed the possible universal role of the TIRs of mitochondrial linear plasmids from *Z. mays* and *Brassica* in the mechanism of DNA import into plant mitochondria. Using a vector containing the TIRs (327 bp) from the 11.6 kb *B. napus* plasmid, we obtained several DNA constructs and tested the importance of the size and DNA structure on the import efficiency into plant mitochondria. The DNA sequences of *Z. mays* linear plasmids, S1 and S2 were also cloned and used as substrates for import assays. Using radioactively labeled DNA substrates and the *in organello* potato (*Solanum tuberosum*) mitochondrial import system, it was established that the import efficiency has a non-linear dependence on DNA size: DNA fragments of 6–7 kb in size can be imported into *S. tuberosum* mitochondria more effectively than molecules with a 4 kb size. It was also shown that import into plant mitochondria of DNA molecules of “medium" sizes, *i.e.* between 4 and 7 kb, barely has any sequence specificity: neither TIRs from the 11.6 kb *Brassica* plasmid, nor TIRs from the *Z. mays* S-plasmids influenced DNA import into *S. tuberosum* mitochondria. Conversely, the role of the TIRs from the 11.6 kb linear plasmid in the import of large DNA molecules was established earlier for *Brassica rapa* mitochondria. **Conclusions.** The data obtained support a species-specific import mechanism of the mitochondrial linear plasmids, and more generally of large DNA molecules, into plant mitochondria, which needs further investigation.

The work was financially supported by a grant from the Russian Fund for Basic Research (12-04-01400) and the French Ministry for Research (Investissements d'Avenir / Laboratoire d'Excellence MitoCross).

Role of DDC1 as a link between check point and DNA repair in yeast *Saccharomyces cerevisia*

M.V. Sukhanova, C. D'Herin[1], P.A. van der Kemp[2], S. Boiteux[1], O.I. Lavrik
Institute of Chemical Biology and Fundamental Medicine, 630090 Novosibirsk, Russia
[1]CBM, UPR 4301, CNRS, 1A rue de la Férollerie, Orléans, France
[2]CEA, iRCM, UMR217, CNRS, 18 route du Panorama, 92265, Fontenay aux Roses, France
lavrik@niboch.nsc.ru

Aim. The aim of this study was to identify key nick sensor proteins operating in yeast *Saccharomyces cerevisiae* by using original approach that was developed under study of nick sensor proteins in mammalian cells. **Methods.** Photoreactive DNA structures mimicking intermediates of base excision repair (BER) were used for affinity modification of interacting proteins in the crude cell extracts. The protein identification was performed by MALDI–TOF-MS peptide mapping. The cell extracts were produced from wild–type yeast *Saccharomyces cerevisiae* and mutants deleted in specific genes. **Results.** To characterize proteins that interact with BER/single strand break repair DNA intermediates in cell extracts, we used photoreactive DNA duplex containing nick carrying photoreactive group. This photoreactive DNA was incubated with the yeast cell extract and after UV irradiation a number of proteins were labeled. Two of the crosslinked proteins were identified by MALDI–MS as catalytic subunit of DNA polymerase epsilon and Ddc1 checkpoint protein. Labeling of DNA polymerase epsilon catalytic subunit with the nick-containing DNA indicates that this DNA polymerase is involved in DNA repair synthesis in yeast. Ddc1 crosslinks to DNA nick independently of the other components of checkpoint clamp, Mec3 and Rad17, suggesting Ddc1 alone is able to recognize DNA single-strand break. In addition, the absence of Ddc1 protein greatly influences the overall pattern of proteins crosslinked to DNA nick and stimulates proteolysis of topoisomerase 1 crosslinked to DNA. We suggested that this effect is due to Ddc1 capacity to prevent proteolytic degradation of the DNA–protein adducts. DNA duplexes containing 3′-recessed (5′-ss/dsDNA) or 5′-recessed end (3′-ss/dsDNA) carrying photoreactive group were used for photoaffinity labeling of proteins in cell extracts. It was found that Ddc1 was crosslinked by the 5′-ss/dsDNA independently of the other components of checkpoint clamp. The detectable crosslinking of subunits of Ddc1-Mec3-Rad17 clamp with 3′-ss/dsDNA was not observed. The p70 subunit of RPA (RPA p70) was the predominant crosslinking product with 3′-ss/dsDNA. Cell extracts deleted for Ddc1 (ddc1) did not display labeling of full-length RPA p70 with neither 5′-ss/dsDNA nor 3′-ss/dsDNA. It is interesting that RPA p70 undergoes a proteolytic cleavage in ddc1 extract. Addition of purified Ddc1 or C-terminal fragments of Ddc1 to ddc1 extract slightly inhibits of RPA p70 cleavage. **Conclusions.** The interaction of Ddc1 with DNA nicks may provide a link between the DNA damage checkpoint and BER pathways in yeast. Genetic and biochemical studies in *S. cerevisiae* determined that Ddc1-Mec3-Rad17 checkpoint clamp and RPA act at the early steps of the checkpoint response to recognize DNA repair/replication intermediates. Our results reveal a novel and potentially important property of Ddc1 in preventing of proteolytic degradation of RPA and other key DNA binding proteins.

The work was supported by RFBR (grants 11-04-00559, 12-04-92601, 12-04-33162, 13-04-93107, 13-04-40197-comfi); Program of RAS "Molecular and Cell Biology".

Biological effects of therapeutic siRNA against TMPRSS2-ERG fusion oncogene for the cancer prostate treatment

Giorgia Urbinati, Quentin Rousseau, Hafiz M. Ali, Liliane Massaad-Massade
Institut Gustave Roussy UMR 8203 CNRS France
Liliane.massade@igr.fr

Prostate cancer is one of the most common tumors in men worldwide and the second leading cause of death among all cancer types in western countries. Combined with surgery, hormone therapy is the first line treatment administrated for hormone-dependent prostate cancers, however when the pathology turns into a castration independent phase, the treatments become less specific. New specific molecular targets would then be highly useful to develop innovative personalized medicines. Among these targets Tomlins et al. decrypted in 2005 a chromosomal rearrangement resulting in the fusion of TMPRSS2 gene with members of the E26 Transformation-Specific (ETS) family and in particular E26 related gene (ERG). The TMPRSS2-ERG genomic fusion is found in 50 % of localized and 30 % of metastatic prostate cancers and this particular chromosomal rearrangement lead to more aggressive cancer phenotypes and worst cancer-specific survival rate. Our **aim** is: i) to target the TMPRSS2-ERG oncogene by siRNAs, ii) to assess the knockdown effects on cell growth and cell cycle regulation. **Material and Methods**: the human VCaP cell line that harbours the TMPRSS2-ERG rearrangement was used. siRNAs specifically directed against TMPRSS2-ERG oncogene were designed to knockdown ERG in the VCaP cells. They were transfected in VCaP then, total RNA was extracted and First-strand cDNA was generated with M-MLV (Reverse Transcription), real time PCR Q-RT-PCR was used to assess the knockdown efficiency. Western blot was performed using ERG antibody and β-actin was used as loading control. Genes affected by the ERG knockdown were identified by microarray (MA) analysis followed by Q-RT-PCR validation. Then, siRNA TMPRSS2-ERG effects on cell cycle growth (MTT tests), cell cycle and apoptosis (flow cytometry) were studied. **Results**: ERG mRNA and protein levels were dramatically down-regulated after siRNA transfection in VCaP cell line. Microarray analysis showed regulation of apoptosis related genes by TMPRSS2-ERG siRNA which was confirmed by Western blot and fluorescent kit assays and regulation of other genes involved in intracellular protein trafficking. In addition, cell viability was compromised and angiogenesis appeared to be affected. **Conclusion**: We success to design efficient and specific siRNAs TMPRSS2-ERG against ERG oncogene. Now, we are bioconjugated them with lipids to obtain nanoparticles in order to evaluate their potential antitumor effects *in vivo*.

This work was supported by ANR "P2N Nanosqualonc", ANR-11-NANO-0003, http://www.anr.nanosqualonc.com

miRNA boost in the tumor and miRNA drop in the blood serum caused by treatment with RNase A promote an attenuation of tumor malignancy

Zenkova M. A., Mironova N. L., Patutina O. A., Brenner E. V., Kurilshikov A. M., Vlassov V. V.

Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

marzen@niboch.nsc.ru

In recent years, ribonucleases are regarded as perspective anticancer drugs. Novel data, suggesting an important role of miRNAs in mediating tumor growth and invasion, have provided researchers with a new area to search for possible molecular ribonuclease targets. Previously, antitumor and antimetastatic properties of pancreatic ribonuclease RNase A were demonstrated and therapeutic efficacy was shown to correlate with increase in ribonuclease activity of the blood plasma of experimental animals (Patutina O.A. et al., Biochimie 2011). We hypothesized that RNase A affects some regulatory pathways of tumorgenesis through the cleavage of circulating oncogenic miRNAs. Here by high-throughput SOLiD sequencing technology we performed analysis of genome-wide profiles of miRNAs in tumor and serum of mice bearing Lewis lung carcinoma after the treatment with RNase A. Sequencing data revealed that treatment with RNase A resulted in an apparent alteration in the levels of 123 tumor-derived miRNAs and 139 serum miRNAs. We observed drop of most miRNAs in blood serum and very unusual miRNA boost in tumor tissue. Data of qPCR of selected miRNAs mir-29b, mir-21, mir-10b, mir-451a, mir-17, mir-18a, mir145, mir-31 and let-7g confirmed the observed effect of RNase A. The miRNA boost in tumor tissue was accompanied by the overexpression of miRNA processing genes drosha/RNASEN, xpo-5, dicer1 and eif2c2 (Ago-2). It was assumed, that while penetrating into cells, RNase A or its proteolytic fragments, may act as a transcription factor like Angiogenin promoting miRNA overexpression. In bloodstream RNase A may cleave circulating non-coding RNAs such as tRNAs, snoRNAs, rRNAs and snRNAs with generation of short RNAs that can compete with miRNAs for binging with Ago-2 and displace them thus facilitating further miRNA degradation by RNase A. Thus tumoricidal activity of RNase A is explained by the change of miRNA profiles in tumor tissue and bloodstream and switch off the miRNA signature from malignant to more normal promoting the attenuation of tumor malignancy.

This work was supported by RAS programs “Molecular and cellular biology” and “Fundamental sciences to medicine 2012-24”, Scientific schools №2972.2012.4, Fellowship of the President of Russian Federation for young scientists 2307.2012.4 and by RFBR grant 11-04-01012.

Ku80 interaction with apurinic/apyrimidinic sites depends on the structure of DNA ends

A.A. Kosova[1,2], S.N. Khodyreva[1,2], O.I. Lavrik[1,2]

[1]Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, 630090, Russia

[2]Novosibirsk State University, Novosibirsk, 630090, Russia

kosova.anastasiya@gmail.com

Apurinic/apyrimidinic (AP) sites are among the most frequent DNA damages. Of particular interest is repair of AP sites in clustered DNA damages presented by combinations of AP sites, oxidized bases and single strand breaks within 1-2 turns of DNA helix. Such lesions arise in DNA under the action of ionizing radiation or radiomimetic drugs. The aim of this study was to identify the human cell extract protein specifically interacting with (AP) site in partial DNA duplex containing 5′- and 3′-dangling ends and mimicking clustered DNA damage. Methods. The Schiff-base dependent cross-linking of proteins to AP DNA (borohydride trapping) in combination with gel electrophoresis and MALDI-TOF-MS was used to identify the protein. Chromatography was used to enrich the cell extract in target protein and to purify the protein. Results. For identification of the protein we used the fraction of HEK 293 cell extract proteins eluted from heparin-sepharose. The human cell extract protein which forms a major covalent adduct with an apparent molecular mass of 100 kDa with AP DNA duplex with dangling ends was identified as Ku80 subunit of Ku antigen by peptide mass mapping based on MALDI-TOF-MS data. However, if AP DNA duplex has blunt ends, the predominant product of cross-linking has an apparent molecular mass of 90 kDa. The protein forming this adduct was previously identified in our laboratory as Ku80. Appearance of Ku80 adduct with lowered electrophoretic mobility characteristic for AP DNA with dangling ends can reflect existence of two different modes of Ku80 binding with DNA or highly efficient cross-linking of DNA with another Ku80 isoform. To discriminate these alternatives, we purified Ku antigen from HeLa cells near to homogeneity. Purified Ku antigen was shown to form the covalent adducts with the same mobility as observed in cell extracts. Conclusions. Ku80 subunit of Ku antigen can specifically interact with AP DNA forming the Schiff base mediated adducts, which electrophoretic mobility depends on the structure of DNA ends. The difference in electrophoretic mobility can be caused by cross-linking of AP DNA to distinct target amino acids that appears to reflect unequal positioning of AP DNAs in the complex with Ku antigen.

This work was supported by RFBR, project 13-04-01426, GDRI program, the RAS program "Molecular and Cellular Biology", and the Ministry of Education and Science of Russia, project 14.B37.21.0188.

Structure and function of oncogene-transformed immortal cells

[1]*Kavsan V.*, [2]*Kulagova T.*, [3]*Kuznetsova T.*, [2]*Semenkova G.*, [1]*Stepanenko A.*, [4]*Vassetzky Y.*

[1]State Key Laboratory of Molecular and Cellular Biology,
Institute of Molecular Biology and Genetics,
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680
[2]Belorussian State University,
Minsk, Belorussia
[3]N.I. Pirogov Russian National Research Medical University,
Moscow, Russia
[4]CNRS UMR8126, Institut de Cancérologie Gustave Roussy,
Villejuif, France

Vadym06@yahoo.com

Previously we have characterized the new oncogene CHI3L1, overexpressed in glioblastoma, and obtained malignant 293_CHI3L1 cells, stably producing CHI3L1 angiogenic oncoprotein. 293_CHI3L1 cells proliferated faster and acquired higher ability to anchorage independent growth. Here we report the atomic force microscopy and functional characteristics of these cells. The constitutive CHI3L1 expression leads to the increased resistance to the damages by oxidative substances and promotes chromosome instability in 293 cells. According to the data and last clinical investigations, anti-cancer therapy should be aimed not to the individual genes, but to the physiological effect they caused. We propose complex treatment of gliomas including multi-target inhibitors, which can be delivered to the brain tumor by specific nanoparticle vector.

Ca/calmodulin-dependent phosphorylation of endocytic scaffold ITSN1

D. Ye. Morderer, O. V. Nikolaienko, I. Ya. Skrypkina, O. V. Rymarenko, S. V. Kropyvko, L. O. Tsyba, A. V. Rynditch.

State Key Laboratory of Molecular and Cellular Biology,
Institute of Molecular Biology and Genetics NAS of Ukraine.
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680

dmytromorderer@gmail.com

ITSN1 is an endocytic scaffold protein with a prominent function in synaptic transmission. It is known that Ca signaling is crucial for regulation of functioning of synaptic proteins. The **aim** of this work was to test the possibility of Ca/calmodulin-dependent phosphorylation of ITSN1. **Methods**. Affinity chromatography, *in vitro* kinase reaction, Western blotting, gel staining with fluorescent stains. **Results**. We show that the fraction of calmodulin-binding proteins is able to phosphorylate recombinant fragments encoding the coiled-coil region and SH3 domain-containing region of ITSN1 in the presence of Ca ions and calmodulin. **Conclusions**. The coiled-coil region and SH3 domain-containing region of ITSN1 undergo Ca/calmodulin-dependent phosphorylation *in vitro*, suggesting possible regulation of ITSN1 by Ca signaling.

This work was partially supported by
Programme of Joint European Scientific Associations of NAS of Ukraine (Project “Human pathology, from the molecular to the cellular level”, F1-2013) and
State Fund for Fundamental Researches (Project F33.4/001).

Coculture of spheroids of human fibroblasts and HeLa cells to study tumor-stroma interaction

Shkarina K. A., Cherednyk O. V., Tykhonkova I. O., Khoruzhenko A. I.

State Key Laboratory of Molecular and Cellular Biology
Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680

i.o.tykhonkova@imbg.org.ua

Background. *In vivo* the cancer develops in the complex microenvironment major cellular component of which are stoma fibroblasts. The question about relationship between cancer and stroma cells requires additional comprehensive analysis. Earlier it was shown that fibroblasts are capable to inhibit the growth and proliferation of tumor cells in the early stages of oncogenesis, whereas in the later stages activated tumor -associated fibroblasts, demonstrate the ability to stimulate proliferation, invasion and angiogenesis. Three-dimensional culture is promising way of modeling the different stages of carcinogenesis in vitro. This approach allows to reproduce some morphological and molecular characteristics of initial tissue more precisely than traditional monolayer culture. **Aim**. To develop a model to study the interaction between tumor and stromal cells in three-dimensional culture.**Methods**. Cultivation of HeLa cell lines and human dermal fibroblasts in monolayer and three-dimensional culture, immunofluorescent and immunohistochemical analysis. **Results**. In this work we present an approach based on a direct interaction between the cells of multicellular tumor spheroids and spheroids of fibroblasts. Subsequent immunofluorescence analysis allows to determine an origin of cells in the area of their contact.
Conclusions. This model will be useful to study the basic mechanisms of carcinogenesis, and to find targets for anticancer therapy.