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Research networks: the good, the bad and the GDRI MCEHP

European commission and its member states has been favoring research networks over research carried out by individual research laboratories for many years. Some of the networks were indeed successful and led to enhanced cooperation between member labs, but the vast majorities of the networks were created with the sole aim to obtain specific funding. These formal research networks did not foster collaboration between participating labs. The principle that governed the creation of the International Research Network (Groupement de Recherche International) "From Molecular to Cellular Events in Human Pathologies" was very different. From the beginning, in 2007, our network was composed of laboratories with the proven record of collaboration. The network grew and accommodated new partners and new collaborations were born between the participants. The results are quite impressive: more than XX joint publications in international peer-reviewed, many national, international and European joint grants, creation of several international laboratories and research groups. We hope that that IX meeting "From Molecular to Cellular Events in Human Pathologies" that will be held in Lviv on September 19-22, 2016 will open new perspectives in collaborative research involving scientists from France, Ukraine, Russia, Latvia, Georgia, Poland and Austria.

Yegor Vassetzky

SEA you later alli-GATOR – a dynamic regulator of the TORC1 stress response pathway

S. Dokudovskaya
UMR8126, Institut Gustave Roussy, Villejuif, France

The highly conserved mechanistic Target of Rapamycin Complex 1 (mTORC1) controls the eukaryotic cell growth and response to a variety of signals, including nutrients, hormones and stresses. The pathways that convey upstream signals to mTORC1 are frequently deregulated by mutations in cancer and other diseases. Moreover a number of proteins that function upstream of TORC1 in response to different stresses are tumor suppressors. We have recently identified a novel upstream regulator of mTORC1 – the multiprotein SEA/GATOR complex. Several components of GATOR are mutated in different cancers and involved in the resistance to the anti-cancer drugs. I will provide an overview of the upstream regulation of the mTORC1 pathway and its role in cancer, with a special attention to the function of SEA/GATOR in this process.

Novel therapeutic agents in the development of effective drug combinations to treat glioma

S. Avdieiev[1], A. Stepanenko[1], L. Gera[2], R. Hodges[2], V. Ribrag[3], Y. Vassetzky[3]

[1] Laboratory of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics NAS of Ukraine, 150 Zabolotnogo str, Kyiv 03680, Ukraine;

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

[3] Gustave Roussy Institute, 39 Camille-Desmoulins str., Villejuif 94805, France

Background. Most physiological and pathological processes are multi-factorial in nature. They are affected or controlled by multiple effectors in sequential or parallel form. In order to effectively control a pathological process, it is necessary to modulate multiple related effectors concomitantly. Glial tumors are driven by multiple molecular aberrations that cannot be controlled by a single targeted agent. So, it is possible to expect that the combined multitarget anti-cancer therapy aimed simultaneously at different elements of the tumor formation mechanisms will be more effective and will promote the extension of patients' life. **Results.** To find out which drug combinations will enable the development of therapeutic regimens with improved effectiveness and decreased toxicity, the cytotoxic effects of several bradykinin antagonists (BA) were analyzed for different glioblastoma (GB) cell lines. Among all the BA under investigation, BKM-570 appeared to be the most effective, with IC50 values of 4 μM and 3.3 μM in the rat glioma C6 and human glioblastoma U251 cell lines, respectively. BKM-570 suppressed ERK1/2 and AKT1 phosphorylation in U251 cells. Temozolomide (TMZ), the first-line anti-gliomic drug used in clinics, has only a temporary positive effect and severe side effects in GB patients. We showed that the combination of BKM570 and TMZ led to significant potentiation of TMZ cytotoxicity at sub-therapeutic concentrations. Recombinant proteins with cytotoxic properties are promising agents for complex therapeutic applications. We revealed that the glioma-associated protein CHI3L2 inhibited the viability of U251 cells more effectively than TMZ. Furthermore, the combination of CHI3L2 and BKM-570 resulted in an additive cytotoxic effect. A CHI3L2-mediated decrease of cell viability was associated with a G1/S transition arrest. CHI3L2 provoked the dramatic reduction of pRB phosphorylation and significant decrease of cyclin D1 expression, as well as a substantial increase in the p53 level. In addition to the accumulation of p53, we observed the upregulation of CDK inhibitor p21. Therefore, a G1/S arrest in CHI3L2-treated cells could be realized via activation of pRB, downregulation of cyclin D, and activation of p53. **Conclusions.** BKM-570 significantly potentiates the activity of TMZ. The CHI3L2 protein inhibits glioma cells viability. The reduced cell viability after the CHI3L2 treatment could be associated with the activation of pRB, downregulation of cyclin D, and activation of p53. **Acknowledgments.** This work was supported in part by a JOINT UKRAINIANROMANIAN R&D PROJECT "Identification of proper combinations of targeted agents capable of simultaneous blocking of proliferative and invasive pathways in glioblastoma".

Catalytic antibodies: chemically laboratory-induced catalysts, randomly selected entities, environmentally-induced or constitutive physiopathological biocatalysts?

M. Shahsavarian, H. Isber, S. Padiolleau-Lefèvre, B. Avalle & A. Friboulet

Génie Enzymatique et Cellulaire, Sorbonne Université – Université de Technologie de Compiègne – CNRS, FRE3580, CS 60319, 60 203 Compiègne cedex , France

Catalytic antibodies are the antibodies that are able to chemically alter the antigen for which they are specific. The initial reports described catalytic antibodies that were obtained by immunization of animals using transition state analogs of several chemical reactions. Alternatively to the ligand-based approaches for generating catalytic antibodies, using structural and electrostatic complementarity or chemical reactivity of the immunogen, another strategy for the catalytic antibodies selection was based on the intrinsic property of the immune system to mimic antigens, via the idiotypic network. On the other hand, the antibodies with enzymatic properties also develop spontaneously in vivo. For example, IgG able to hydrolyze the vasoactive intestinal peptide, DNA, thyroglobulin, pro-coagulant factor VIII, or myelin basic protein have been described in patients with asthma, systemic lupus erythematosus, Hashimoto's thyroiditis, hemophilia A, or multiple sclerosis, respectively. Because catalytic antibodies in the human had been reported under pathological conditions, it was long thought that they are endowed with a pathogenic role, or that, at least, they are a hallmark of immune dysregulation and uncontrolled inflammation. However, the catalytic antibodies of the IgM, IgG and IgA isotypes have also been reported in the normal blood, milk of healthy mothers and saliva. Combinatorial methods allow achieving breakthrough in modern enzymology and drug design. The main advantages of the phage display technology are that (i) it is less time-consuming (around 4–5 selection cycles), (ii) the selection process is less laborious in application than a screening approach, and (iii) it is possible to use the libraries displaying recombinant antibody fragments suitable for human applications. A great number of combinatorial antibody libraries have been constructed and are available that allow a comparison of catalytic antibody genes with each other and an observation of the remarkable features that could help us to evaluate the origin and the physiological significance of the presence of catalytic entities in the antibody population. The recent experimental and statistical results obtained with combinatorial antibody libraries will be discussed.

Implication of mRNA binding proteins in translation regulation studied by AFM imaging

L. Hamon

Laboratoire Structure-Activité des Biomolécules Normales et Pathologiques,
INSERM U1204 and Université d'Evry, Université Paris-Saclay, France
loic.hamon@univ-evry.fr

Translation regulation plays a key role in the control of protein level, which encompasses the regulation of mRNA translation initiation and ribosome processing by mRNA binding proteins. Translation regulation is of particular interest when cells are exposed to stress conditions: the expression of house-keeping proteins should be repressed while that of mandatory for the cell survival, should be enhanced. Such sorting functions have been suspected for stress granules (SGs) which are micrometric aggregates appearing in the cytoplasm of stressed cells and containing mRNA and numerous mRNA-binding proteins. The absence of membrane around these granules leaves mRNA and protein free to shuttle in and out of granules but also leads to their intrinsic instability. Due to this, they cannot be isolated for further characterization. We proposed recently to reconstitute artificially these granules *in vitro* using the specific proteins found enriched in SGs like TIA-1 and to check, using AFM approach, if some RNA-binding proteins are able to release mRNA from these granules *(Bounedjah et al. Nucleic Acids Research, 2014)*. We focused our study on YB-1, an mRNA-binding protein present in SGs, which forms the isolated complexes with mRNA easily identifiable on the AFM images. When YB-1 is added to the mRNA/TIA-1 granules we observed the dissociation of the granules and the appearance of the isolated mRNA/YB-1 complexes. These results were further confirmed in a cellular context after the overexpression of YB-1. YB-1 is also known to interact with specific mRNA sequences/structures and could potentially release specific mRNA from SGs. Moreover, when mRNAs are saturated at elevated YB-1 concentration, (about 30 nucleotides per YB-1), the translation is repressed. This suggests a link between SGs and translation regulation via YB-1. However, how YB-1 could exert its putative function in translation repression while a large amount of YB-1 is required to stop translation remains elusive. One possibility is that YB-1 does not bind to mRNAs homogeneously but rather accumulates on specific mRNA targets via cooperative binding. Using AFM in combination with gel mobility shift assays, we determined the characteristics of the binding of YB-1 to mRNA *(Kretov et al. Nucleic Acids Research, 2015)* and these results offer a novel view on the translation repression orchestrated by YB-1 or other mRNA binding proteins.

Poly(ADP-ribose) polymerases covalently modify strand break termini in DNA fragments *in vitro*

Ibtissam Talhaoui[1,2], Natalia A. Lebedeva[3], Gabriella Zarkovic[1,2], Christine Saint-Pierre[4], Mikhail M. Kutuzov[3], Maria V. Sukhanova[3], Bakhyt T. Matkarimov[5], Didier Gasparutto[4], Murat K. Saparbaev[1,2], Olga I. Lavrik[3,6,*], and Alexander A. Ishchenko[1,2,*]

[1] Laboratoire «Stabilité Génétique et Oncogenèse» CNRS, UMR 8200, Univ. Paris-Sud, Université Paris-Saclay, F-94805 Villejuif, France

[2] Gustave Roussy, Université Paris-Saclay, F-94805 Villejuif, France

[3] SB RAS Institute of Chemical Biology and Fundamental Medicine, Lavrentiev Av. 8, Novosibirsk 630090, Russia

[4] Université Grenoble Alpes, CEA, INAC/SCIB-UMR E3/LAN, F-38000 Grenoble, France

[5] National Laboratory Astana, Nazarbayev University, Astana 010000, Kazakhstan

[6] Department of Natural Sciences, Novosibirsk State University, 2 Pirogova St., Novosibirsk 630090, Russia

* *Alexander.ISHCHENKO@gustaveroussy.fr, lavrik@niboch.nsc.ru*

Poly(ADP-ribose) polymerases (PARPs) use nicotinamide adenine dinucleotide (NAD+) to catalyze the synthesis of a long branched poly(ADP-ribose) polymer (PAR) attached to the acceptor amino acid residues of nuclear proteins. PARP1 is activated up to 500-fold when bound to DNA strand breaks. *In vivo*, when DNA is in the chromatin form, PARPs work on single- and double-strand DNA breaks (SSB and DSB) by recruiting and assembling DNA repair factors. The phenomenon of NAD+-dependent PARylation was discovered more than 50 years ago, but it is still unclear how this post-translational modification governs a multitude of cellular processes including DNA repair, transcription, chromatin dynamics and cell death. Here, we investigated interactions of PARP enzymes with the intermediates of DNA excision repair. Our results revealed that both mammalian PARP1 and PARP2 can covalently modify DNA oligonucleotide duplexes by addition of multiple poly(ADP-ribose) units to DNA strand break extremities. PARP1 and PARP2 preferentially react with the recessed and nicked DNA duplexes, respectively. Based on the biochemical and mass spectrometric data, we propose that PARPs can utilise DNA termini, as an alternative of 2'-hydroxyl of ADP-ribose, to catalyse PAR chain elongation either *via* 2',1''-*O*-glycosidic ribose-ribose bond, or *via* phosphodiester bond formation between C1' of ADP-ribose and phosphate of a terminal nucleotide in DNA duplex. Therefore, DNA can be regarded as a substrate for PARP1 and PARP2, which catalyze a post-replicational modification of DNA. The covalent DNA PARylation is a reversible process since PARG removes the PAR polymer from DNA with high efficiency and restores native DNA structure. Finally, this newly discovered type of post-replicational modification of DNA mediated by PARPs provides an heuristic insight into molecular mechanisms involved in DNA repair, transcription and chromatin dynamics in eukaryotic cells.

Changes in the level of blood cell-free circulating mitochondrial DNA during experimental adrenaline-induced myocardial injury in rats

N. P. Sudakov[1,2,3], O. A. Goldberg[1], A. I. Katyshev[4], S. B. Nikiforov[1], B. G. Pushkarev[1], I. V. Klimenkov[2,5], S. A. Lepekhova[1,3], K. A. Apartsin[1,3], Yu. M. Konstantinov[2,4,*]

[1] Irkutsk Surgery and Traumatology Research Center; [2] Irkutsk State University; [3] Irkutsk Research Center, Siberian Branch of the Russian Academy of Sciences; [4] Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences; [5] Limnological Institute, Siberian Branch of the Russian Academy of Sciences.

** yukon@sifibr.irk.ru*

Background. Myocardial infraction is a major cause of mortality in industrialized countries. The set of cardiomarkers currently used in clinical practice is not always sufficient for selecting therapeutic strategy to treat myocardial infraction, and this justifies a high priority for the search for novel biomarkers of cardiomyocyte injury. Currently, the level of blood mitochondrial DNA (mtDNA) is measured for predicting development of complications and mortality from malignant tumors, septic processes, as well as for estimating probability of lethal outcome in intensive care unit patients. The aim of our study was to examine the changes in a level of blood cell-free circulating mtDNA and compare them with the dynamics of cardiospecific cytolytic markers during adrenaline induced myocardial injury in rats. **Results.** The concentration of mitochondrial DNA in serum was measured after centrifuging the blood at 16,000g for 20 min to remove platelets and membrane particles carrying mtDNA. The mtDNA was quantitatively assayed using real time PCR with reaction mixture containing SYBR Green (Maxima SYBR Green/ROX qPCR Master Mix; Thermo Fisher Scientific Inc., USA). The 230bp long fragment from the *16S rRNA* gene (forward primer: 5′TGCAGAAGCTATTAATGGTTCG3′, reverse primer: 5′TTGGCTCTGCCACCCTAATA3′) was amplified. A set of samples with predetermined concentration of the 490bp long fragment from the *16S rRNA* gene (forward primer: 5′TAGGGTAACAGCGCGACCTA3′, reverse primer: 5′GTTGGGGCCTTTGCGTAA3′) containing the analyzed 230bp long sequence was used as quantitative standards. For performing real time PCR, a DT lite amplifier (DNA technology) was used. It was shown that 24 h after injection of adrenaline solution, the activity of creatine phosphokinases in the blood serum as well as lactate dehydrogenase and aspartate significantly increased, which served as an objective sign of developing hypoxic injury of cardiomyocytes at this time point. The level of cell-free circulating mtDNA in the blood increased 1.5 fold compared to the control group ($p_u = 0.2$). The activity of creatine kinases (total and MB fraction) and lactate dehydrogenase tended to decline 48 h after injecting adrenaline solution, however staying at a higher level than in the control group. Notably, at 48 h the level of circulating mtDNA in the blood of the experimental animals increased 2 fold and reached the maximum during the whole period of observation ($p_u = 0.07$). It was found that 72 h after injecting adrenaline solution, the activity of all cytolytic biomarkers declined to the normal range observed in the control group. The data of pathomorphologic examination of myocardial samples at this time point showed the formation of multiple foci of lysis of muscular fibers infiltrated by macrophages and polymorphonuclear phagocytes. Foci of inflammation were located both in the central region of the myocardium and in the immediate vicinity of endocardium and pericardium. In some cases, macrophages diffusely infiltrated the cardiac tissues. The morphological changes described above were accompanied by 1.5 fold elevated level of cell-free circulating mtDNA in the blood serum (72 h) compared to the control group ($p_u = 0.03$). As a whole, based on the data obtained in studies of the changes in cell-free mtDNA under different pathological states (myocardial infarction, cancer, trauma, diabetes, sepsis a. o.), the level of circulating mtDNA in blood can be considered as an informative systemic parameter reflecting the structural and functional state of mitochondria in pathologically altered organs and tissues in the body. If this suggestion is correct, the measurement of cell-free circulating mtDNA might be used along with other methodological approaches to assess the efficacy of cell-based therapy by applying mitochondria-targeted antioxidants. **Conclusions.** Overall, the data obtained demonstrate the opportunity for using the amount of cell-free circulating mtDNA measured in the blood serum as a novel biomarker of acute ischemic myocardial conditions. *Funding: The work was supported program GDRI of CNRS and Russian Foundation for Basic Research (project no. 15-54-16010) and Russian Foundation for Basic Research (project no. 15-04-05046).*

ITSN1, TKS4 and verprolin family members WIP and CR16-containing macromolecular complexes in invadopodia

Sergii Kropyvko, Tetyana Gryaznova, Olga Gubar, Valentyna Kryklyva,
Mariia Burdyniuk, Liudmyla Tsyba, Alla Rynditch

Institute of Molecular Biology and Genetics of NASU,
150 Zabolotnogo Street, Kyiv 03680, Ukraine,
s.v.kropyvko@imbg.org.ua

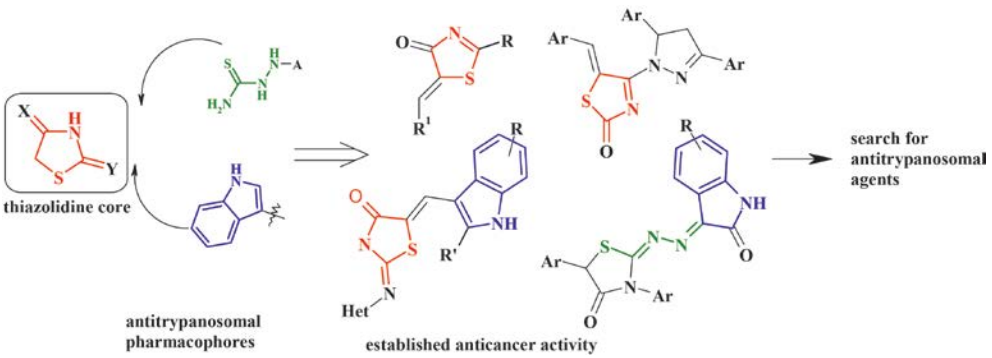
Aim. We still do not understand the mechanisms of controlling cell invasion and metastasis associated with protrusive actin enriched subcellular structures – invadopodia. Meantime, identification of factors leading to metastasis is necessary for creation of effective and novel anti-cancer therapeutics. Molecular components of invadopodia suggested a tight integration between signaling, membrane trafficking and cytoskeletal rearrangements. **Methods.** We used GST pull-down assay, Western blot analysis, immunofluorescence and confocal microscopy. **Results.** Investigation revealed that intersectin scaffolding proteins localized to invadopodia and formed macromolecular complexes with other adaptor proteins TKS4, TKS5 and GRB2, kinase SRC, as well as with the representatives of verprolin family WIP, WIRE and CR16 which control the actin cytoskeleton dynamics by direct interaction with actin and nucleation promoting factors N-WASP and cortactin and activate the ARP2/3 complex required for invadopodia formation. Novel interactions have been found for the key components of invadopodia from Tks family: 14 new partners for TKS4 and 6 partners for TKS5 which significantly enlarged molecular understanding of their functional interactions. The ata on TKS4 localization to RAB4 – associated vesicles suggested the TKS4 possible role in their transport and sorting. Interactions of TKS4 with other partners may indicate their participation in membrane trafficking and remodeling, signal transduction and actin cytoskeleton rearrangements. The formation of ITSN1-based macromolecular complexes with small adaptor of verprolin family CR16 via SH3 domains of ITSN1 in a complex with actin has been demonstrated. We also showed that the extent of ITSN1 localization to actin cytoskeleton was dependent on the presence of CR16. The findings highlight the dynamic content of macromolecular complexes important for the invadopodia formation and functions.

Dual anticancer and antitrypanosomal activity as a tool in new drug-like molecules design

A. Kryshchyshyn[1], P. Grellier[2], R. Lesyk[1]

[1] Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Ukraine, Pekarska 69, Lviv 79010, Ukraine

[2] National Museum of Natural History, UMR 7245 CNRS-MNHN, Team BAMEE, CP 52, 57 Rue Cuvier, 75005 Paris, France



Human African Trypanosomiasis (HAT, sleeping sickness) belongs to the so called world's neglected tropical diseases caused by the two subspecies of Trypanosoma brucei (Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense). Despite the reducing in the number of HAT cases in recent years, about 65 million people remain at risk nowadays. The problems associated with the treatment of sleeping sickness consist in toxicity, availability of parenteral route of administration only and often not sufficient effectiveness of the existing drugs, such as suramin, pentamidine, melarsoprol and eflornithine. Chagas disease is caused by the Trypanosoma cruzi and is one of the most widespread parasitic diseases in Latin America. Only two drugs nifurtimox and benznidazole are used for the treatment of acute stage of Chagas disease, being of limited efficacy during chronic phase. Taking into account that no new antitrypanosomal drugs were approved since 90th, the development of novel effective non-toxic antitrypanosomal drugs is one of the important tasks of modern drug design process. Thiazole and thiazolidinone derivatives are associated with the various types of biological activity, such as hypoglycemic, antibacterial, antiviral, anticancer, etc. Moreover, recent studies had shown their antiparasitic potential. There are a number of different approaches to the development of new antitrypanosomal agents. One of such consists in the investigating of known drug-like molecules and drugs with the established activity for other possible pharmacological effects. Encouraged by the results of the study of antitrypanosomal properties of anticancer agents – danusertib, lapatinib, canertinib and bortezomib we aimed to investigate the trypanocidal activity of thiazolidones with sufficient anticancer profile. A number of thiazole based compounds with an indole fragment in the molecules, as well as thiosemicarbazone derivatives with high levels of tumor cell growth inhibition were selected and tested in *in vitro* assays on Trypanosoma bucei brucei (Tbb) and Trypanosoma brucei gambiense (Tbg). Based on the literature data and the results of our previous findings, some pharmacophores responsible for the tripanocidal activity were established. The presence of such structural fragments in the molecules was one of the selection criteria. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells. The Tbb and Tbg blood stream forms were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors. After a 72-h incubation, the resazurin solution was added in each well and fluorescence was measured. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that in the absence of drug. IC_{50} levels of the studied compounds were within micromolar ranges indicating that thiazole derivatives may be a promising class for antitrypanosomal hit-compounds search. Considering that danusertib, lapatinib and canertinib are the kinases inhibitors, the protein kinases of the parasite should be investigated as possible targets of the thiazole derivatives trypanocidal action.

Poly(ADP-ribose) polymerase 1 and regulation of DNA repair.

O. I. Lavrik[1],[2]

[1] Institute of Chemical Biology and Fundamental Medicine SB RAS, Russia

[2] Novosibirsk State University, Novosibirsk, 630090, Russia
lavrik@niboch.nsc.ru

Motivation and aim: The phenomenon of nicotinamide adenine dinucleotide (NAD+)-dependent poly(ADP-ribosyl)ation catalyzed with PARP1 was discovered long time ago, but it is still unclear how this post-translational modification governs a multitude of cellular processes including DNA repair. When interacting with the damaged DNA, PARP1 catalyzes the synthesis of a long branched poly (ADP-ribose) polymer (PAR) by using NAD^+ as a substrate. PAR can be attached to the acceptor amino acid residues of nuclear proteins or to PARP1 itself. This process leads to reorganization of the functional protein complexes involved in base excision repair (BER) and other key processes in cell. The aim of the present research was to investigate the role of poly (ADP-ribosyl)ation in regulation of BER and to search for new targets of PARylation catalyzed with PARP1 and PARP2. The protein–protein interactions in BER were analyzed and quantified in the presence of BER DNA intermediates. **Methods:** Fluorescence titration methods, atomic force microscopy (AFM), light-scattering technique, biochemical and immunochemical approaches. **Results:** PARP1 interacts with BER proteins as well as with DNA intermediates of BER containing breaks or apurinic/apyrimidinic (AP-sites) which appear in BER process. PARP1 interacting with the AP sites shows AP lyase and 5'-dRP lyase activities. Protein-protein interactions of PARP1 with APE1, Pol beta, XRCC1, tyrosyl-DNA-phosphodiesterase 1, YB-1 and other components of BER machine were investigated quantitatively by various methods. The strength of protein-protein interactions in BER was influenced by the structure of DNA repair intermediates. The specificity of PARP1 and PARP2 interaction with DNA structures was confirmed and estimated quantitatively. **Conclusion:** The results obtained show that the PARP1 and PARP2 PARylation activity is dependent on the affinity of PARP proteins to damaged DNA. The protein-protein interactions and their regulation were estimated quantitatively at the various stages of BER in the absence or in the presence of BER DNA intermediates. The data show the existence of preformed BER repairsome, the function of which is regulated by protein-protein, DNA- protein interactions as well as by PARylation stimulated by PARP1and PARP2. **Acknowledgements:** This work was supported by grant from RSF (14-24-00038).

Keywords: *PARP1, PARP2, poly(ADP-ribose), BER, protein-protein interaction*

5-ene-thiazolidinones as michael acceptors – not always "pains" – efficient tool in drug design

Roman Lesyk, Borys Zimenkovsky, Danylo Kaminskyy

Danylo Halytsky Lviv National Medical University;
Pekarska 69, Lviv 79010, Ukraine
dr_r_lesyk@org.lviv.net

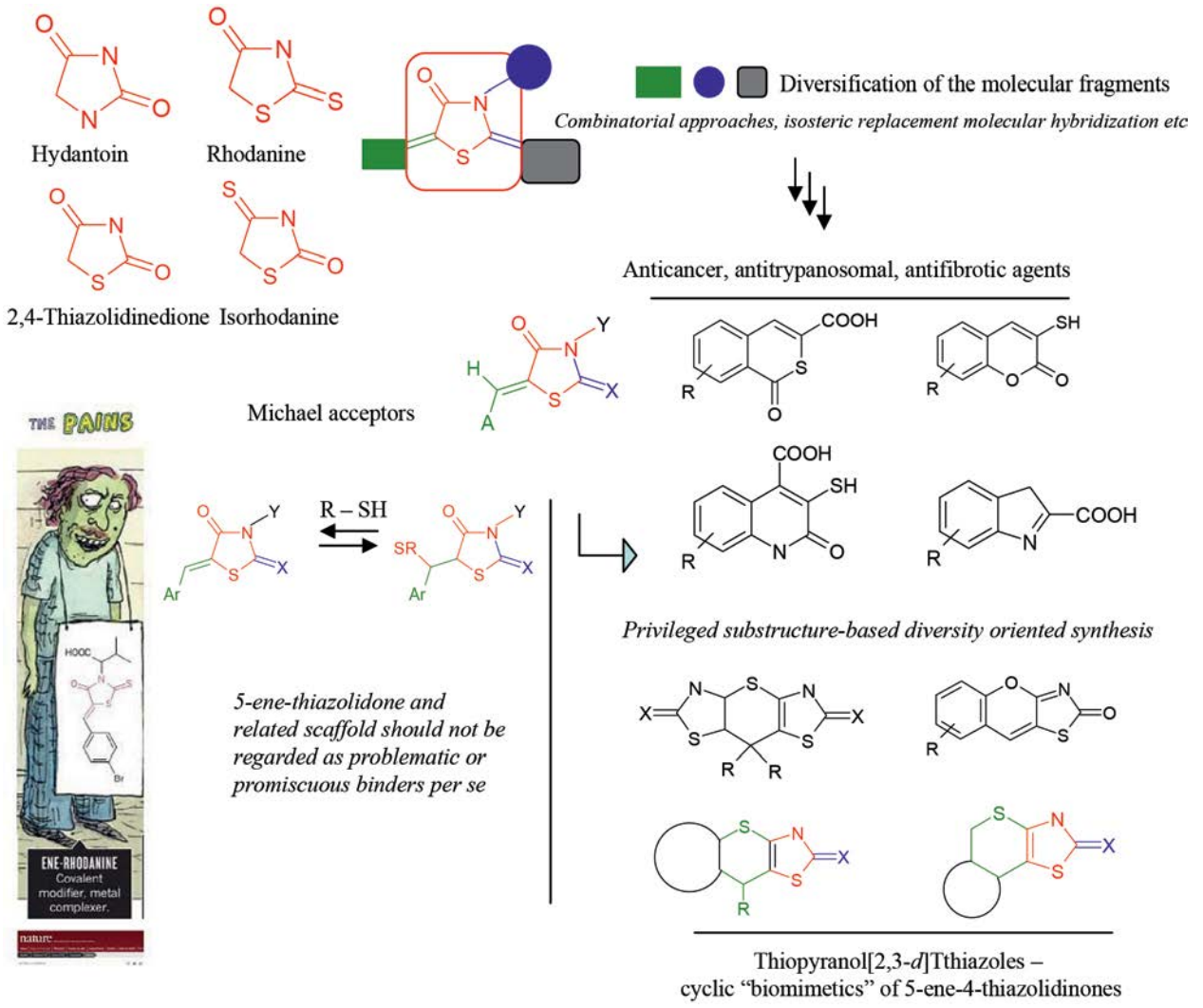
Thiazolidinones as a sourse for new drug-like molecules design are in focus from the 1960s. An interest in thiazolidinones is growing rapidly and is illustrated by the introduction of the set of thiazolidinone-based drugs to the medical practice. Among various thiazolidinones and related heterocycles (e.g. derivatives of rhodanine, 2,4-thiazolidinedione, hydantoin, *etc.*), 5-ene-4-thiazolidinones are of special interest. The compounds of mentioned sub-type as privileged heterocycles are the examples of high-affinity ligands of a number of biological targets, lead-compounds, and drug-candidates with antidiabetic, antimicrobial, antiviral and anticancer activities.

Conjugation of C5 double bound with C4 carbonyl group of thiazolidinones core allows considering them as potential Michael acceptors. This feature determines the belonging of 5-ene-4-thiazolidinones to the so-called «frequent hitters» or «PAINS» (pan-assay interference compounds), which is useless in modern medicinal chemistry because of the compounds interaction with thiolic groups of proteins, and apparently their low selectivity. This view, argued by J. Baell (*Nature, 2014*) is criticized by us because: *i*) data mainly concern *in silico* screening; *ii*) low selectivity within poly-pharmacological approach is treated as a benefit; *iii*) compounds are considered as a basis for further modifications and selectivity improving; *iv*) the ability to interact with the thiols often is not confirmed experimentally; *v*) Michael acceptors are one of the most effective activators of Nrf2, which opens new perspectives in the treatment of inflammation and cancer; *vi*) structural similarity to endogenous Michael acceptors leads to modulation of ROS-dependent regulatory pathways, *etc.*

Following the above, and based on the combinatorial approaches, bioisosteric replacement, molecular hybridization (combination of other pharmacologically attractive scaffolds with thiazolidinone frame), *etc.*, we experimentally confirmed the potency of 5-ene-4-thiazolidinones for designing anticancer, antiparasitic and antifibrotic agents with the confirmed ROS-dependent and proapoptotic mechanism of action. This allow us to put forward and confirm the thesis about a critical influence of the presence/nature of C5 fragment on the biological activity of this subtype of 4-thiazolidinones.

On the other hand, the combination of several reaction centers of 5-ene-thiazolidinones makes them an effective tool for the synthesis of other heterocycles (isothiocumarins, thiopyranothiazoles, chromenothiazoles *etc.*) especially in multicomponent reactions as well as a tool for the modification of other compounds. Based on the directed synthesis strategy within the rational privileged substructure-based diversity oriented synthesis we worked out various methods of synthesis of thiopyrano [2,3-*d*]thiazoles and related heterocycles. Study on their biological activities, including anticancer and antitrypanosomal, allow us to consider thiopyrano[2,3-*d*] thiazoles as cyclic isosteric mimetics of pharmacologically active 5-ene-4-thiazolidinones without Michael acceptor functionality.

Ref. and details: https://goo.gl/Ak3Z9F


Hydantoin
Rhodanine
2,4-Thiazolidinedione
Isorhodanine
Diversification of the molecular fragments
Combinatorial approaches, isosteric replacement molecular hybridization etc
Anticancer, antitrypanosomal, antifibrotic agents
Michael acceptors
THE PAINS
ENE-RHODANINE
Covalent modifier, metal complexer.
nature
R – SH
5-ene-thiazolidone and related scaffold should not be regarded as problematic or promiscuous binders per se
Privileged substructure-based diversity oriented synthesis
Thiopyranol[2,3-d]Tthiazoles –
cyclic "biomimetics" of 5-ene-4-thiazolidinones

Synthesis and anticancer activity of novel thiazolo[4,5-*b*]pyridine-5-carboxylic acid amides

A. V. Lozynskyi, G. M. Semenciv, R. B. Lesyk*

Danylo Halytsky Lviv National Medical University
Pekarska 69, Lviv, Ukraine, 79010
dr_r_lesyk@org.lviv.net, roman.lesyk@gmail.com

Background. Thiazolidinone-based molecules are attaractive targets in the rational design of «drug-like» compounds which possess anti-inflammatory, antioxidant, antitumor, antimicrobial, antiviral and other activities. A prominent place among biologically active 4-thiazolidinones belongs to their condensed derivatives, especially to thiazolo[4,5-*b*]pyridines. Thus, a considerable attention to thiazolo[4,5-*b*]pyridines is paid by the medicinal chemists owing to their capacity to mimic the biologically important 4-thiazolidinone fragment in a rigid fused system. Therapeutic applications of this template are very broad, and range from cardiotonic agents (including cAMP PDE III inhibitors) to antimicrobial and anti-inflammatory compounds. High affinity ligands have been obtained also for H_3-histamine, metabotropic glutamate 5 (mGluR5) and epidermal growth factor receptors. **Materials and methods.** All new thiazolo[4,5-*b*]pyridine derivatives were synthesized from 4-amino-5*H*-thiazol-2-one and arylidene pyruvic acids in the reaction of [3+3]-cyclocondensation. A number of thiazolo[4,5-*b*]pyridine-5-carboxylic acid amides were synthesized by interaction of corresponding acid chlorides with various amines in anhydrous dioxane. The synthesized compounds were selected by National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. **Results.** The synthesized compounds were evaluated at the single concentration of 10^{-5}M against the full panel; they displayed significant activity in the vitro screen on the cell lines tested. Thus, the compounds were highly active in the colon cancer COLO-205 (GP = –52.11 %), leukemia HL-60(TB) (GP = –53.72 %) and melanoma SK-MEL-2 (GP = –5.01 %) cell lines. Finally, several compounds were tested in an advanced assay against a full panel of about sixty tumor cell lines at 10-fold dilution of compounds of five concentrations (100, 10, 1, 0.1 and 0.01μM). The tested compounds showed the inhibition activity against 58 (GI_{50}< 10 μM) from 59 human tumor cells with average GI_{50}/TGI values 1.98μM/15.44μM. With regard to the sensitivity against some individual cell lines among several subpanels, the synthesized compounds demonstrated a certain sensitivity profile towards the Non-small cell lung cancer subpanel tumor cell lines with in the range of GI_{50} values 0.52 – 2.81 μM. **Conclusion.** The preliminary results allowed identifying the active compounds with promising anticancer activity, which demonstrated certain sensitivity profile towards the Non-small cell lung subpanel tumor cell lines.

Mechanisms of pulmonary alveolar proteinosis related to *MARS* gene mutations

Martine Comisso[1], Alice Hadchouel[2], Marc Mirande[1]

[1] Institute for Integrative Biology of the Cell (I2BC), CNRS, Universite Paris-Saclay, CEA, CNRS, Universite Paris-Sud, 1 avenue de la Terrasse, 91190 Gif-sur-Yvette, France

[2] Hopital Necker-Enfants Malades, Paris, and INSERM U955, Creteil, France

Pulmonary alveolar proteinosis (PAP) is a rare cause of chronic lung disease characterized by the alveolar accumulation of lipoproteinaceous material[1], leading to hypoxemic respiratory failure. Primary PAP includes auto-immune forms related to anti-GM-CSF auto-antibodies, representing the main cause in adults, and genetic forms, with isolated mutations in genes encoding the alpha and beta chains of the GM-CSF receptor. Recently, we identified mutations in the *MARS* gene by exome sequencing in a specific type of PAP prevalent on La Reunion and the nearby islands, and also in 3 patients originated from other geographic areas[2]. This disorder differs from other forms of PAP as it usually displays an early onset and a severe prognosis with frequent progression to pulmonary fibrosis (70 %), and also associates with a liver involvement (90 %), a systemic inflammation (90 %), failure to thrive (90 %) and arthritis in one case[3].

MARS encodes the cytosolic methionyl tRNA synthetase (MetRS). Aminoacyl-tRNA synthetases (ARSs) play a critical role in protein biosynthesis by charging tRNAs with their cognate amino acids. MetRS is also, together with other ARSs, a component of a cytosolic multi-protein complex (the MSC; Multi-aminoacyl-tRNA Synthetase Complex) with multiple roles described in immune response, inflammation, tumorigenesis, angiogenesis and neuronal homeostasis[4]. MetRS is ubiquitously expressed, consistent with the multi-systemic phenotype observed in patients. However, pathophysiological mechanisms of this disease are still unknown.

Mutations in *MARS* are also associated with neurodegenerative disorders, and in one case – with a multi-systemic phenotype that resembled *MARS* related PAP. The effect of these mutations on the catalytic activity of MetRS is generally unknown, and the putative direct functional correlation between a specific mutation and a given disease is also unknown. Because some ARSs contribute to the non-canonical activities unrelated to their primary function in protein synthesis[4], we shall consider that *MARS* variants might contribute to PAP pathology either through a loss-of-function (reduced aminoacylation or loss of interaction with normal cellular partners) or a gain-of-function (association with new cellular partners) mechanism. Therefore, deciphering the molecular mechanisms of *MARS* related PAP is essential to understand the pathophysiology of this specific disease and to develop therapeutic approaches, but could also have a major impact on other lung diseases in children and adults, and represent an original and innovative model of multi-systemic inflammatory disorder. Initial characterization of the *MARS* mutants will be described.

1. *Rosen SH,Castleman B, Liebow AA.* Pulmonary alveolar proteinosis. *N. Engl. J. Med.* 1958;**258**: 1123–42.
2. *Hadchouel A et al.* Biallelic Mutations of Methionyl-tRNA Synthetase Cause a Specific Type of Pulmonary Alveolar Proteinosis Prevalenton ReunionIsland. *Am. J. Hum. Genet.* 2015;**96**: 826–31.
3. *Enaud L et al.* Pulmonary alveolar proteinosis in children on La Reunion Island: a new inherited disorder? *Orphanet J. Rare Dis.* 2014;**9**: 85.
4. *Paul M, Schimmel P.* Essential nontranslational functions of tRNA synthetases. *Nat. Chem. Biol.* 2013; **9**: 145–53.

Translation elongation complex eEF1 in human cancer

L. M. Kapustian, M. V. Veremieva, B. S. Negrutskii

Institute of Molecular Biology and Genetics,
150 Zabolotnogo Str. Kiev 03680 Ukraine
negrutskii@imbg.org.ua

Translation elongation complex eEF1, comprising eEF1A and eEF1B, provides efficient elongation of nascent polypeptide chains on 80S ribosome. eEF1A-GTP contributes to correct codon-anticodon recognition by hydrolyzing GTP, with subsequent fixation of correct aminoacyl-tRNA in the ribosomal A site. eEF1B stimulates GDP/GTP exchange in eEF1A. eEF1B is a complex of three subunits, called eEF1Bα, eEF1Bβ and eEF1Bγ. The first two are active in nucleotide exchange, the role of eEF1Bγ is considered mostly structural. There are indications of non-translational functions of the subunits as well.

Our aims are: i) to find out whether eEF1B is a stable complex or it may lose its components in different human cancer tissues; ii) to decipher possible non-translational functions of the individual eEF1B subunits in cancer cells.

The independent, non-coordinated changes in the level and localization of different eEF1B subunits have been observed in the human cardioesophageal and lung cancer tissues. These data argue for the existence of some unknown cancer-related roles of different eEF1B subunits. To approach these roles we have determined the protein partners of eEF1Bβ and eEF1Bγ by co-immunoprecipitation and mass-spectrometry of the nuclear and cytoplasmic fractions of lung carcinoma cells A549 and analyzed a functional potential of resultant networks.

Short homologous repeats involvement into mtor gene isoforms mRNA formation

S. Palchevskyy, N. Nosachuk, O. Garifulin, A. Zhyvoloup, I. Gout, V. Filonenko

Department of Cell Signaling, Institute of Molecular Biology and Genetics,
150 Zabolotnogo st., 03680 Kyiv, Ukraine
s.s.palchevskyy@imbg.org.ua

The progress of NGS and RNA-sequencing technologies reinforced by improved methods of bioinformatics analysis allow to assess the organizational complexity of the transcriptome and diversity of post-transcriptional regulation processes more deeply. Alternative splicing (AS) remains the main provision mechanism of transcriptomic variety and proteomic diversity from a genome. The functional similarities and differences of constitutively expressed (so called reference) as well as alternatively spliced protein isoforms are gradually clarified and refined. The interactome hierarchy formed by those reference and alternative forms of protein are also a matter of continuous studies (Yang *et al.*, 2016). Also the information about the mechanisms the multitude of alternatively spliced transcripts is implemented keeps on accumulating. As an example for such processes we would like to draw attention to the mTOR kinase gene chimeric mRNAs formation. The existence, unique structure and possible aspects of functioning of alternative splicing isoform mTORbeta was postulated several years ago. It works as a protooncogene and contains large deletion of central part of mRNA protein coding sequence (Panasyuk *et al.*, 2009). The formation of this isoform occurs without usage of canonical splice sites and through the involvement of so-called short homologous sequences (SHSs) flanking the deletion. The further PCR analysis revealed additional multiple mTORbeta-like spliced isoforms with deletions of different size but in all cases bordered by SHSs. Also, the possibility of spliced variants (mTORd28-58, mTORd35-58) formation by similar to mTORbeta mode but with the involvement of 3'UTR region of mRNA was shown in our study. Subsequent analysis has showed potential common aspects of mTORbeta-like and mTORd28-58, mTORd35-58 spliced variants formation. The events we have observed may possibly occur with a low frequency. We can speculate that these events testify in favor of transcriptional slippage model. The theoretical possibility of such chimeric RNA formation broadens our understanding of the mechanisms of alternative splicing as well as functions of novel splice isoforms of mTOR.

Antioxidant activity of some 4-thiazolidinone derivatives

Andriy Pyrih[1], Danylo Kaminskyy, Gertjan den Hartog[2], Sergii Golota[1], Aalt Bast[2], Roman Lesyk[1]

[1] Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, Lviv, 79010, Ukraine;

[2] Department of Pharmacology & Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

The balance of the concentration of reactive oxygen species (ROS) is the pivotal rod of so called ROS depending signaling system, playing an important role in the processes of proliferation, differentiation, immune response, apoptosis, and senescence. The hyperproduction of ROS in amounts, much exceeding the ability to utilize them by the antioxidant systems of the cell, lies in the basement of oxidative stress – the key chain of a plethora of pathologies, conjugated with mentioned physiological processes: autoimmune, cardiovascular, neurodegenerative, chronic inflammatory diseases, cancerogenesis. Involving ROS and components of ROS dependent signaling to these crucial events makes ROS attractive targets for the development of new biologically active compounds. Perspective small molecules for this purpose are the compounds with 4-thazolidinone fragment and their derivatives, especially concerning the broad spectrum of bioactivity they exert as antidiabetic, antimicrobial, anticancer, anti-inflammatory agents. Such capacities assort with the conception of the multitarget drugs inferring different activities of a single compound, among them the antioxidant one. Moreover, it has been established the antioxidant potential of the agonist of PPARγ receptors bearing 2,4-thiazolidone core – troglitazone.

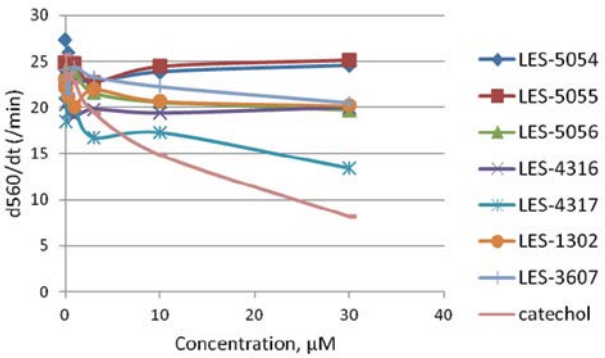


Fig. 1.

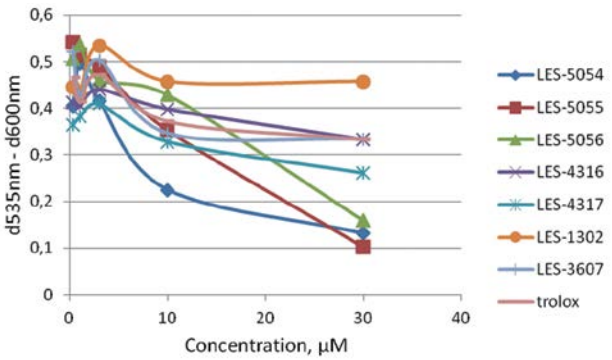


Fig. 2.

For the evaluation of the antioxidant properties, seven compounds have been selected from our in-home library containing 4-thiazolidinone moiety and their derivatives. The compounds have been explored using two approaches: capability of scavenging superoxide anion-radicals and inhibiting the lipid peroxidation (LPO). The level of scavenging potential has been estimated in the system, containing xanthine and xanthine oxidase and nitro blue tetrazolium (NBT) as indicator, the parameter to be measured –a decrease of NBT concentration as a rate (dA560/dt) of the linear part of the absorption vs. time plot. The lipid peroxidation has been assayed on the rat liver microsomes, with the initiation by iron sulfate and ascorbic acid and ceasing by trichloracetic acid; the parameter to be measured – the difference between the absorption at 535 nm and 600 nm of the products of interaction of the thiobarbituric acid (TBA) reactive materials with TBA. The results of the performed protocols showed the lack of the influence of the compounds on the scavenging of superoxide anion-radicals. Just only one compound, LES-4317, has revealed a moderate level of scavenging, comparing with the referent compound – catechol (Fig. 1). At the same time, the compounds, containing 3,5-ditert-butyl-4-hydroxybenzilidene fragment (LES-5054, LES-5055, LES-5056), have shown much better inhibition of LPO against the backdrop of the referent compound – trolox (Fig. 2). This fact witnesses the mechanisms of action of the compounds are not connected to the eradication of the superoxide as well as hydrogen peroxide, spontaneously emerging after superoxide disproportion. As for the compound LES-4317, its impact on LPO was not so remarkable, but stronger, than for trolox.

Apoptosis and eryptosis influence differently plasma membrane lipid phases

K. A. Pyrshev[1,*], S. O. Yesylevskyy[2], A. S. Klymchenko[3], A. P. Demchenko[1]

[1] Laboratory of Nanobiotechnologies, Department of Molecular Immunology, O.V. Palladin Institute of Biochemistry of the NAS of Ukraine, 1030, Kyiv

[2] Department of physics of biological systems, Institute of Physics of the NAS of Ukraine, 03028, Kyiv

[3] Laboratoire de Biophotonique et Pharmacologie UMR 7213 CNRS/Université de Strasbourg, Illkirch, France.

** e-mail: pyrshevka@gmail.com*

Plasma membrane lipid phases play an essential role in regulation of cell functioning. The activity of ion channels, pumps and receptors is proved to be associated with the lipid rafts stability and cholesterol levels. Indeed, programmed cell death is a complex process, which involves most of the structures and organelles, including nucleus, mitochondrion, nucleus and plasma membrane. The pathologies of apoptosis lead to systemic, autoimmune and oncogenic disorder states. According to erythropoiesis, the red blood cells provide programmed death limited by plasma membrane features as they lack in organelles. Thus, the research of apoptosis and eryptosis, focused on plasma membrane, could provide the evidence of cell surface specificity, which is important for cells interaction, stability and erythrocytes' circulation. In current research the fluorescent techniques were combined with molecular dynamics simulations to study the composition and properties of cell membranes during programmed cell death of erythrocytes, HeLa and Jurkat cells. The relatively new solvatochromic probes were applied in microscopy flow cytometry and spectrofluorometry to characterize the plasma membrane lipid order. To describe the possible local changes in features of the membranes influencing the probes' response, the molecular dynamics simulations were applied. The dynamical properties of the plasma membrane lipid phases were studied by the NR12S binding kinetics, analyzed by the maximum entropy method. The results suggest the strong lateral heterogeneity of the outer plasma membrane leaflet organization providing two observable lipid phases separated by different diffusion in both nucleated cells and erythrocytes. The results of current experiments showed higher sensitivity to programmed cell death activation in erythrocytes, which is in line with literature data. The transmembrane asymmetry of the plasma membrane decreased during both apoptosis and eryptosis. However, the lateral lipid order was influenced significantly only in nucleated cells. We showed that the plasma membrane of apoptotic cells, in addition to lipid transporters, was influenced dramatically by the mixing with internal membranes providing lower cholesterol content and lipid order. Thus, in contrast to nucleated cells, the plasma membrane of red blood cells demonstrates lower hydration dynamics and retains the lipid order during programmed cells death.

Molecular basis for mechanism of editing activity by D-aminoacyl-tRNA-deacylase from *Thermus thermophilus*

M. Yu. Rybak, O. P. Kovalenko, M. A. Tukalo

Department of Protein Synthesis Enzymology, Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680
mariia.rybak@gmail.com

Introduction. Quality control during protein biosynthesis provides the accurate flow of genetic information from mRNA to correct amino acid sequence. This process is controlled by several mechanisms: precise amino acid recognition by aminoacyl-tRNA-synthetases (aaRSs), proofreading mechanisms against appearing mistakes by *cis*- (aaRSs) and *trans*-editing factors (additional enzymes) and discrimination of mischarged substrates by elongation factors. Amino acids exist in D- and L-forms; and normally only L-amino acids are selected to incorporate into proteins. The biosynthetic machinery involves different enzymes for the prevention of such misincorporation. D-aminoacyl-tRNA-deacylase (DTD) belongs to such *trans*-factors. It is involved in the correction of mischarging tRNAs with D-amino acids by aaRSs, connected with the lack of L-stereospecificity in recognition process. DTD is specific only to D-aminoacyl-tRNA substrates (D-Tyr/D-Phe/D-Trp/D-Asp-tRNA) (Calendar & Berg, 1967, Soutourina *et al.*, 1999, Zheng *et al.*, 2009) and does not hydrolyse L-aminoacyl-tRNAs. TyrRS is not able to discriminate the stereospecificy of amino-acid, requiring additional checkpoint before elongation. **Aim.** To investigate the mechanism of aminoacylation by TyrRS from *Thermus thermophilus* (TyrRSTT) and the editing of misaminoacylated D-Tyr-tRNA^{Tyr} by DTD from *T. thermophilus* (DTDTT), we used molecular modelling, molecular dynamics (MD) simulations and site-directed mutagenesis of the enzyme. **Methods.** We performed comprehensive site-directed mutagenesis studies, based on molecular modelling and MD simulations of the proposed active site of DTDTT and amino- and deacylation assays with α-[^{32}P]-tRNA^{Tyr} from *T. thermophilus*. The structural model of DTDTT bound to the D-Tyr-A76 was generated by homology modelling using the reported crystal structure of *Plasmodium falciparum* DTD bound to this substrate (Ahmad, 2013). MD simulations were used to study the frames where the water molecules formed a necessary angle and distance to perform a nucleophilic attack at carbonyl group of the ester of the mischarged D-Tyr-tRNA^{Tyr} as well as for identification of H-bounds with protein environment. The assays with radiolabelled tRNA^{Tyr} have been applied as an experimental basement of proposed catalytic mechanism. **Results.** The kinetic parameters for the TyrRSTT aminoacylation reaction were determined from Michaelis-Menten plot. The results of MD simulations after 5ns were analysed to perform further in site-directed mutagenesis of the enzyme's active site. DTDTT and its 12 substitution mutants showed differences in activity, confirming the importance of some residues in the enzyme's selectivity and catalysis. **Conclusions.** In sum, (a) because of the absence of discrimination between L- and D-Tyr by TyrRS (discrimination factor is only 11 against 3300 in the literature data of the ratio cognate/noncognate amino acid), there is the necessity of additional checkpoint of D-aminoacyl-tRNA substrates by DTDTT, (b) the catalytic mechanism of DTDTT, based on MD stimulations and primary mutagenesis studies is proposed here.

The influence of anti-Hsp60 antibodies on cancer cell viability

L. Sidorik[1], L. Yakovenko[1], A. Tsisarenko[1], O. Pavliuk[1], I. Tykhonkova[1], V. Grygorenko[3], M. Vikarchuk[3], I. Kroupskaya[1], P. Pogribnyy[2]

[1] Institute of Molecular Biology and Genetics NAS of Ukraine, Kyiv, Ukraine

[2] Kavetskii Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kyiv, Ukraine

[3] SI «Institute of Urology NAMS of Ukraine», Kyiv, Ukraine

Background. The elevated levels of anti-Hsp60 antibodies were identified in sera of patients with osteosarcomas, breast cancer, ovarian cancer, oral cavity squamous cell carcinoma, prostate cancer, thyroid cancer *etc.* The role of anti-Hsp60 antibodies in cancerogenesis has not been established yet. It remains unknown whether the anti-Hsp60 antibodies serve only as a marker of cancerogenesis or they can negatively influence the cancer cell viability. Our aim was to investigate the effect of anti-Hsp60 antibodies on viability of cancer cells. **Methods.** LNCaP cells (androgen-sensitive human prostate adenocarcinoma cells) were treated for 24 h with polyclonal anti-Hsp60 antibodies, IgG antibodies affinity purified from donors' blood sera and highly reactive against Hsp60 in sera of patients with aggressive prostate cancer (final concentrations – 0,1; 1; 5; 50 μg/ml). The dehydrogenases activity was measured by MTT-test. The expression of molecular chaperons (Hsp60, Hsp90) and key protein kinases (P70S6K, Erk 1/2) involved in cell viability was determined by Western blotting. **Results.** A decrease in the dehydrogenases activity was observed in the cells treated with anti-Hsp60 antibodies purified from the sera of patients with aggressive cancer (5 μg/ml – 66% of control) and anti-Hsp60 antibodies purified from blood sera of healthy donors (1 and 50 μg/ml – 63% and 61% accordingly), $p<0,05$. A significant decrease in both kinases activity was observed only in thecells treated with donors' IgG antibodies (1 and 5 μg/ml). The heat shock proteins expression altered depending on the antibody source and concentration. **Conclusion.** IgG anti-Hsp60 antibodies affinity purified from the cancer patients and donors blood sera affect differently the viability of cancer cells.

Dual COX/LOX inhibitors capable of releasing H2S posses a decreased enterotoxicity in the small intestine of rats

Yu. Sklyarova, I. Fomenko

Danylo Halytskyi Lviv National Medical University, Lviv, Ukraine
Pekarska 69, Lviv, Ukraine, 79010

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the treatment of inflammatory states. The main side effects of their use are connected with an irritation in practically all organs of gastrointestinal (GI) system including small intestine. The development of new drugs that combine inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) and release H_2S is promising, because it is supposed, that combined inhibition allows avoiding some disadvantages of conventional COX-inhibitors whereas H_2S possesses the anti-inflammatory activity. **Aims & Methods:** The aim of the research was to compare the changes of parameters of oxidative stress in mucosa of small intestine of rats under application of COX nonselective inhibitor indomethacin and compounds that inhibit both COX and LOX (thiazol-4-one derivatives: Les-3985 and 2C3DHTA), which potentially release H_2S in tract. Inhibitors were introduced by an intraperitoneal injection in a single dose 10 mg/kg. In the mucosa of small intestine were determined: area and degree of destructive changes, alterations in H_2S and malonic dialdehyde (MDA) concentration, activity of mieloperoxydase (MPO), superoxide dismutase (SOD), catalase (CAT) and parameters of NO-synthase system. **Results:** Administration of indomethacin caused the development of ulcerative lesion localized mainly in the distal part of small intestine, whereas the injection of both studied COX/LOX inhibitors (Les-3985 and 2C3DHTA) did not interrupt the integrity of mucosa. Nonselective COX inhibition by indometacine was accompanied by a decrease in the H_2S concentration (by 30 %, $p \leq 0,05$) and an increase in the MDA content (by 42 %, $p \leq 0,01$); activity of MPO increased more than two fold ($p \leq 0,01$), activity of SOD and CAT was by 17 and 15 % higher than in the control group. Compounds Les-3985 and 2C3DHTA did not cause the significant change in the H2S and MDA concentrations as well as in the activity of MPO, SOD and CAT. **Conclusion:** Dual action of COX-2/5-LOX inhibitors Les-3985 and 2C3DHTA demonstrated decreased enterotoxicity as compared to conventional COX inhibitor indometacine.

Quality control of nonproteinogenic amino acids: tRNA-dependent mechanisms of the errors editing.

M. Tukalo, O. Kovalenko, M. Rybak, O. Gudzera, A. Rayevskiy,
M. Il'chenko, I. Dubey, I. Kriklivyi, G. Yaremchuk

Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine,
150, Zabolotny St., 03143 Kyiv, Ukraine,
mtukalo@imbg.org.ua

Accurate translation of mRNA into the corresponding amino acid sequence is an essential step during gene expression. Fidelity of translation is controlled by several mechanisms: precise amino acid recognition by aminoacyl-tRNA-synthetases (aaRSs), proofreading mechanisms against appearing mistakes by *cis*- (aaRSs) and *trans*-editing (additional enzymes) factors, discrimination of mischarged substrates by elongation factors and selection of correct aminoacyl-tRNA for decoding by a translating ribosome. Some nonproteinogenic amino acids are the naturally occurring metabolites and can potentially act as the substrates for the protein synthesis. Therefore, the editing of nonprotein amino acids is more important than the proofreading of genetic code amino acids under some growth conditions [1]. In this work we have studied molecular mechanisms of editing nonproteinogenic amino acids by two different editing enzymes in two different ways: by *Thermus thermophilus* leucyl-tRNA synthetase (LeuRS), in *cis* and by *trans*-editing domain, *Thermus thermophilus* D-aminoacyl-tRNA-deacylase (DTD), in *trans*. Our results support the notion that the editing activity of LeuRS is aimed at preventing the misincorporation of nonproteinogenic norvaline. DTD is involved in the correction of mischarging tRNAs with D-amino acids by aaRSs, connected with the lack of L-stereospecificity in recognition process. We have confirmed that at the aminoacylation level there was no discrimination between D- and L-tyrosine by *T. thermophilus* TyrRS. To understand the mechanisms of editing reaction for enzymes with absolutely different architecture of editing active sites [2, 3], we have used a number of approaches, including molecular modeling, quantum-mechanical calculations, site-directed mutagenesis enzyme and modification of tRNA. Our intensive alanine scanning mutagenesis of LeuRS and DTD editing sites has failed to identify catalytic residues for hydrolysis within the active site. On the other hand, modification of $tRNA^{Tyr}$ at the 2'-OH of A76 and $tRNA^{Leu}$ at the 3'-OH of A76 by replacing each of them with a proton, revealed an essential function for these groups in hydrolysis. On the basis of obtained experimental results and our QM calculations we suggest the tRNA-dependent mechanisms of post-transfer editing by LeuRS and DTD in which 2'- or 3'-OH group of the substrate plays a key role.

1. *Bullwinkle TJ, Reynolds NM, Raina M, Moghal A, Matsa E, Rajkovic A, Kayadibi H, Fazlollahi F, Ryan C, Howitz N, Faull KF, Lazazzera BA, Ibba M.* Oxidation of cellular amino acid pools leads to cytotoxic mistranslation of the genetic code. *Elife.* 2014;**3**.
2. *Cusack S, Yaremchuk A, Tukalo M.* The 2 A crystal structure of leucyl-tRNA synthetase and its complex with a leucyl-adenylate analogue. *EMBO J.* 2000;**19**(10):2351–61.
3. *Ahmad S, Routh SB, Kamarthapu V, Chalissery J, Muthukumar S, Hussain T, Kruparani SP, Deshmukh MV, Sankaranarayanan R.* Mechanism of chiral proofreading during translation of the genetic code. *Elife.* 2013;**2**:e01519.

HIV Tat remodels nuclear organization and activates aberrant transcription in human B-cells

Diego Germini[1], Tatiana Tsfasman[1], Rawan El Amine, Olga Iarovaia[1,2], Stephanie Bury-Moné[3], Marc Lipinski[1], Eric Oksenhendler[5], Yegor S. Vassetzky[1]

[1] UMR8126, Université Paris-Sud, CNRS, Institut de cancérologie Gustave Roussy, Villejuif, France

[3] Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia

[4] ENS, Cachan, France

[5] Service d'immunologie clinique, CHU Paris-GH St-Louis Lariboisière F-Widal - Hôpital Saint-Louis, Paris, France
vassetzky@igr.fr

Tat protein of human immunodeficiency virus (HIV-1) is released by infected T-cells and is able to penetrate into other uninfected cells, leading to the development of different pathologies, including cancer. AIDS patients, even those receiving highly active antiretroviral therapy (HAART) develop Burkitt's lymphoma and B-large cell lymphoma at high frequency. To elucidate the mechanisms of HIV Tat induced oncogenesis, we have treated primary human B cells with low doses of purified Tat protein. Tat induced DNA damage, profoundly rearranged nuclear architecture of B-cells, changing nuclear positions of gene loci and altering epigenetic marks. Tat also induced expression of several oncogenes and miRNA clusters. Our model for Tat-mediated oncogenesis will contribute to our understanding of the pathogenesis Burkitt's and B-large cell lymphomas at the molecular level and may be important in exploring novel therapeutic methods for lymphoma therapy and prevention.

The search for new anticancer agents among 5'-carboxy-7'-aryl-1-aryl-3',7'-dihydro-*2H*,*2'H*,*5H*-spiro[pyrolidin-3,6'-thiopyrano[2,3-*d*]thiazol]-2,2',5-triones

Nataliya Zelisko, Nataliya Finiuk, Roman Lesyk

Danylo Halytsky Lviv National Medical University
Pekarska 69, Lviv, Ukraine, 79010
dr_r_lesyk@org.lviv.net, roman.lesyk@gmail.com

Background. Cancer is the second of mostly widespread diseases in the world. Ukraine takes the second place in Europe in terms of the spread of cancer. One in four deaths in Ukraine is due to the cancer. Because of ineffectiveness of many cancer treatments, numerous side effects, difficulties in early cancer detection, new devices have the potential to change cancer therapy for the better, and to increase the number of highly effective therapeutic agents. Study on thiopyrano[2,3-*d*]thiazoles, as 4-thiazolidinone derivatives is an integral part of the chemical science and constitutes a considerable area of the modern research. There are anticancer, antitrypanosomal, antimycobacterial, antibacterial and antifungal agents among them. The most powerful instrument for the synthesis of thiopyrano[2,3-*d*]thiazoles is *hetero*-Diels–Alder reactions as it was described in our previous works. **Materials and methods.** Novel 5'-carboxy-7'-aryl-1-aryl-3',7'-dihydro-*2H*,*2'H*,*5H*-spiro[pyrolidin-3,6'-thiopyrano[2,3-*d*]thiazol]-2,2',5-triones were synthesized via *hetero*-Diels–Alder reaction in glacial acetic acid medium. As dienophile we used *trans*-aconitic acid. We also managed to obtain very interesting by-product, which proved the path of new triones formation. We have also reported the efficient methods for getting it. All synthesized compounds were tested towards various cancer cell lines *in vitro*. **Results.** The compounds showed different levels of activity towards various cancer cell lines (human breast adenocarcinoma cells of MCF-7 line, human ovarian carcinoma cells of Scov3 line, human melanoma cells of SK-Mel-28 line, human non-small-cell lung cancer cells of SW-1573 line, human acute T-cell leukemia cells of Jurkat line). There was no inhibition of cell viability observed in the case of testing MCF-7, Scov3, SK-Mel-28 and SW-1573 cells. Most of the tested compounds were shown to be potentially active as antitumor agents against leukemia cells. The most interesting fact is that by-product was found as the most active drug towards human acute T-cell leukemia cells of Jurkat line with significant antitumor potential (IC_{50} = 34.0 μM). Leukemia cells appeared to be more sensitive towards the studied derivatives. **Conclusion.** The synthesized compounds have been demonstrated to be a prospective source for the innovative anticancer agents. **Funding.** The publication contains the results of studies conducted by President's of Ukraine grant for competitive projects F-63 of the State Fund for Fundamental Research.

Evaluation of complex (cytogenetic and physiological) Biomarkers of individual radiosensitivity

Nadareishvili D, Zedginidze A, Namchevadze E, Ormotsadze G.

I. Beritashvili Center of Experimental Biomedicine. Departement of problem of radiation safety.
Tbilisi, Georgia

The results of already completed and present large scale radio-epidemiological studies ranked the late and very late tissue effects prognosis, prevent and mitigate issue among priority problems of fundamental and applied radiobiology [1,2]. Cellular radiosensitivity depend on cells ability to repair the radiation induced damages in DNA, late reactions, based on combined cellular, parenchymal, vascular and connective tissue changes, and the individual features of adaptive response of whole organism. Hence, the study of mechanisms of interindividual variability in the regulation of functional systems of the organism and determination of their possible casual relationship with the final outcome of radiation impact is a prospective way of the detection of individual radiation risk predictors Investigated functional state of red blood system (RBS) and cytogenetic status in 12 patients (6 mail, 6 female, 50-65 year) with head and neck cancer (II-IV stage) before Radiotherapy, after first irradiation and after last irradiation. Radiotherapy was performed on linear accelerator in conformal regimen 2 Gy per fraction (20-33 fractions) total 40-70 Gy. The functional state of RBS was determined by using the specially developed method [3] based on analysis of population spectrum of erythrocytes of peripheral blood (EPB) – EPB distribution according to their morphometric characteristics (volume, shape). Was determined chromosomal disorders (dicentrics and other chromosomal aberrations), level of buccal cell micronuclei and DNA damage by means of DNA-comet method. Statistical analysis was performed by nonparametric statistic methods Mann-Whitney U test and Wilcoxon Matched Pairs test. The study of chromosomal abnormalities, DNA-comets and buccal micronuclei has showed a statistically significant correlation between initial cytogenetic indices in cancer patients and their change dynamics during and after radiation exposure. Also correlation trend between initial cytogenetic parameters and functional stage of red blood system (RBS) was revealed. Our results allow us to conclude that there is an importance of further complex research to estimate the individual radiation risk

Keywords: radiation impact, red blood system, chromosome aberrations, comet assay, micronuclei,

Evaluation of cytogenetic and physiologica biomarkers of individual radiosensitivity

D. Nadareishvili, A. Zedginidze, E. Namchevadze, G. Ormotsadze

I.Beritashvili Center of Experimental Biomedicine. Departement of problem of radiation safety.
Tbilisi, Georgia

Prognaosis and prevntion are essential problems of fundamental and applied radiobiology [1, 2]. Radiosensitivity depends on the ability of cells to repair the radiation-induced DNA damages in DNA, induced chages in cellular, parenchymal, vascular and connective tissues, and the individual adaptive response of an organism.Hence, the study of mechanisms of individual variability in the regulation of functional systems of the organism and determination of their possible relationship to the final outcome of radiation impact is essential for prediction of individual radiation risk.

Here we have studied the functional state and cytogenetic status of the red blood system (RBS) in 12 patients (6 male, 6 female, 50–65 year) with head and neck cancer (II-IV stage) before radiotherapy, after the first irradiation and after the last irradiation. Radiotherapy was performed using the linear accelerator in regimens of 2 Gy per fraction (20–33 fractions) and a total of 40–70 Gy.

The functional state of RBS was determined by analysis of erythrocytes of peripheral blood (EPB) according to their morphometric characteristics (volume, shape) as describerd elsewhere [3]. We followed chromosomal aberrations, .level of buccal cell micronuclei and DNA damage Statistical analysis was performed by nonparametric statistic methods Mann-Whitney U test and Wilcoxon Matched Pairs test.

The study of chromosomal abnormalities, DNA damage and buccal micronuclei priduced a statistically significant correlation between the initial cytogenetic indices in cancer patients and the the dynamics of their change during and after radiation exposure. This correlated with the functional state of RBS.

K e y w o r d s: radiation impact, red blood system, chromosome aberrations, comet assay, micronuclei.