New cytotoxic agents and their combinations for the treatment of chemoresistant glioblastoma and mantle cell lymphoma

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Background. Glial tumors are driven by multiple molecular aberrations, chromosomal instability and tumor heterogeneity; therefore combined multitarget anti-cancer therapy aimed simultaneously at different elements of tumor formation mechanisms might be more effective and will promote the extension of patients' life. **Results.** Several bradykinin antagonists (BA) and 4-thiazolidinones (TD) were analyzed for their cytotoxic effect using different glioblastoma (GB) and mantle cell lymphoma (MCL) cells. BKM-570 appeared to be the most effective agent with IC_{50} 4 μM and 3,3 μM in rat glioma C6 and human glioblastoma U251 cell lines, correspondingly. ERK1/2 and AKT1 phosphorylation was suppressed in U251 cells after treatment by this compound, thus, growth-repression effect of BKM-570 could be mediated by the modulation of MAPK- and PI3K-signaling cascades. Temozolomide (TMZ), a first-line anti-gliomic drug used in clinics, has only a temporary positive effect and severe side effects in GB patients. We have shown that combination of 1 μM BKM-570 with only 10 μM temozolomide (TMZ), led to about 80% growth reduction of C6 and U251 cells, compared to temozolomide used alone. Thus, BKM-570 significantly potentiates TMZ cytotoxicity. Screening of 4-thiazolidinones revealed ID28 to be the potent suppressor of C6 and U251 cells growth (IC_{50} 4 μM and 15 μM, correspondingly). ID4523 demonstrated the highest activity in C6 cells with IC_{50} 0.13 μM. Treatment of MCL cells by this compound and its chemical produced IC_{50} values of 0.27 μM for ID4526 and 0.16 μM for ID4527 as compared to 0.37 μM for doxorubicin. Recombinant proteins with cytotoxic properties are promising therapeutic when used with chemotherapy and immunotherapy. Glioma-associated protein CHI3L2 down-regulates proliferation of U251, as well as 293 cells. Furthermore, CHI3L2 protein inhibits cell viability of U251 cells more effectively than TMZ in therapeutic concentrations. Combination of CHI3L2 and BKM-570 resulted in additive cytotoxic effect in U251 cells. CHI3L2-mediated decrease of cell viability is associated with G1/S transition arrest. We also analyzed the impact of CHI3L2 on key components of cell cycle machinery, namely pRb, cyclin D, p53, and p21. CHI3L2 provoked a dramatic reduction of pRB phosphorylation and a significant decrease of cyclin D1 expression. Moreover, p53 expression level was substantially increased. We also demonstrated the upregulation of cyclin-dependent kinase inhibitor p21, thus G1/S cell cycle arrest in CHI3L2 treated cells could be due to activation of pRB and p53 and downregulation of cyclin D. **Conclusions**. BKM-570 has a potent cytotoxicity against glioma cells and significantly potentiates anti-gliomic drug TMZ. TD treatment suppresses of human and rat gliomas and MCL cells growth. CHI3L2 protein, which is overexpressed in human gliomas, inhibits glioma cells viability and enhances the cytotoxic properties of chemotherapeutic agents, thus, could be considered as a potential component for complex chemotherapy. Reduced cell viability after CHI3L2 treatment could be due to activation of pRB and p53, an downregulation of cyclin D1. **Acknowledgments.** This work was supported in part by a travel grant of the Boehringer Ingelheim Fonds 'Multitargeted complex therapy of human gliomas and lymphomas' and FP7 Project 294932 'COMBIOM'.

DNA polymerase Iota participates in clustered damage repair

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Multiple DNA lesions occurring within one or two turns of the DNA helix known as clustered damage are a source of double-stranded DNA breaks, which represent a serious threat to the cells. Repair of clustered lesions is accomplished in several steps. If a clustered lesion contains oxidized bases, an individual DNA lesion is repaired by the base excision repair mechanism (BER). One of the BER stages involves specialized DNA polymerases after excising of DNA damage. Here, we investigated DNA synthesis catalyzed by DNA polymerase iota using damaged DNA templates. Two types of DNA substrates were used as model DNAs: partial DNA duplexes containing breaks of different length, and DNA duplexes 5-formyluracil (5-foU) and uracil as a precursor of apurinic/apyrimidinic sites (AP) in opposite DNA strands. For the first time, we shown that DNA polymerase iota is able to catalyze DNA synthesis using partial DNA duplexes having breaks of different length as substrates. In addition, we found that DNA polymerase iota could catalyze DNA synthesis during repair of clustered damage via the BER system by using both undamaged and 5-foU-containing templates. We found that hPCNA (human proliferating cell nuclear antigen) acting here as a replication protein increased efficacy of DNA synthesis catalyzed by DNA polymerase iota.
This study was supported by the Russian Scientific Foundation (Grant No. 14-24-00038) and the Russian Foundation for Basic Research (Grant No. 14-04-00268).

Modulation of GluN2B subunit-containing NMDA receptors expression and Spatial long-term memory in medial septal immunolesioned rats

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The hippocampus is important in the formation of spatial memory in both humans and animals. The N-methyl-D-aspartate (NMDA) type of glutamate receptors in the hippocampus has been reported to be essential for spatial learning and memory as well as for the induction of synaptic plasticity. Evidence accumulated from recent studies suggests that GluN2A and GluN2B subunit-containing NMDA-Rs preferentially contribute to the induction of hippocampal LTP and LTD. Using a Morris water maze (MWM) task, the LTP-blocking GluN2A antagonist had no significant effect on any aspect of performance, whereas the LTD-blocking GluN2B antagonist impaired spatial memory consolidation.
The present study was designed to investigate the effect of selective immunolesions of cholinergic and GABA-ergic SH projection neurons [using 192 IgG-saporin (SAP) or GAT-1 saporin (GAT), respectively] on spatial memory assessed in MWM and NMDA receptor GluN2B subunit expression in the rat hippocampus. We used MWM training protocol with eight training trials. One day after training, probe test with the platform removed was performed to examine long-term spatial memory retrieval. We found that immunolesion of medial septal cholinergic or GABAergic neurons did not affect spatial learning as exhibited by a decreased latency to find the hidden platform across the eight training trials. Trained control and SAP treated rats spent significantly longer than chance (15 s) performances such as swimming time in test sector (where the hidden platform was located). Moreover, they spent significantly longer in test sector than in the opposite sector, confirming the establishment of long-term memory. In contrast, the preference for test sector was abolished in medial septal GAT treated rats. Because GAT treated rats learned the location of the hidden platform during training, the results suggest that GAT treated rats could not remember the training a day later. We found that the expression level of NR2B subunit of NMDA receptor in the hippocampus was decreased significantly in the GAT treated group compared with the control and SAP treated groups.
In conclusion, our findings suggest that immunolesion of medial septal GABAergic neurons can interrupt hippocampus dependent spatial memory, possibly through modulation of NMDA receptor subunit expression in the hippocampus. Moreover, our finding that selective lesions of medial septal GABAergic neurons affects probe-test performance but not spatial learning, suggests that septohippocampal GABAergic projections are involved specifically in the consolidation or retrieval, but not in the acquisition of long-term spatial memory.

Radiobiological effects modeling by using plant test-systems

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One of main feature of radiobiological effects at various structural-functional level of the organization is their cascading formation. In the formation of radiobiological reactions each level of the organization of living organism is characterized by specific mechanisms of post–radiation recovery.

While with respect to cells, tissues and organs we face the realization of reparative, regenerative and other compensative mechanisms, with respect to population, population recovery rates are the leading criteria.

Various types living organisms differ from each other by radiosensitivity. The mentioned feature varies even within one species (individual radiosensitivity). Hence, in order to estimate the results of irradiation correctly, radiosensitivity study with respect to organization level of living organisms will be reasonable. Although there is modern equipment determining the damaging influence of various physical-chemical factors, adequate estimation of injury level is practically impossible. At the same time the structures that are widely used in identification of quantitative parameters of radiation hazards are found in living organisms. The inhibition of cell proliferation activity or entire inactivation is one of the most visible effects among the processes that are detected in the result of damaging impact of radiation exposure. It is only natural that exploration of specific bio-subjects targeted for modeling and the study of their individual radiosensitivity enables to form general radiobiological concept. The diversity of plant organisms and unique structures existence in their organization enable to use them as bio-models. From this point of view it is essential to find such subjects that reveal radiobiological effects even when their reaction is not adequate to damage level. In the present work we have discussed the effectiveness of the use of plant test-systems in order to study such radiobiological processes as: transmission of radiation damage in to cellular generations, regulations of detection of somatic embryogenesis, and radio-resistance of separate parts of secondary metabolism.

Key words: plant test-system, ionization radiation, radiobiological effects

Evaluation of the spatial prevalence of cancer in conditions of an inadequate data of population

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We have introduced the concept of the spectral coefficient of the incidence rate, allowing to assess the incidence rate under the circumstances of uncertain population data. The quantitative relationship of this indicator with a standard (population) incidence rate has been displayed and the main qualities of this connection have been studied.
The independence of the ratio between the standard and spectral coefficients of the incidence from the tumor site induces has been determined. It has been shown that this ratio is equal to ratio between signed numbers of cancer cases in area to be study and region in whole.
It has been determined that the value of the standard (population) and the spectral coefficients for the large populated areas are approximately equal, so their ratio is approximately equal to one.
It's estimated that the time series of standard, as well as spectral coefficients of the incidence for specific populated areas can't be distributed by Poisson random variables.
The algorithm for calculation of the Cochran-Student confidence interval from the extension of the initial array has been built. The confidence intervals have been calculated for the mean values of the spectral coefficients of the incidence of all sites of tumors in all districts of Georgia.
The clearly pronounced spatial heterogeneity of the spectral coefficients of the incidence of some localization of tumor has been shown, reaching the value of 5 – 6, allowing us to suggest the existence of cancer-causing agents in a number of areas.

Key words: cancer spatial prevalence, evaluation of incidence spectral rate, estimation of confidence intervals.

Antimicrobial peptides as anticancer agents

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There is a considerable interest in developing anticancer agents with new mode of action. Many natural or synthetic cationic peptides have been reported to show anticancer activity. They are united in a separate class of anticancer peptides (ACP). Compared with the traditional cancer treatments such as chemotherapy or radioactive treatment, peptides with high specificity against cancer cells may present the way of killing cancer cells while protecting normal cells and helping patients to recover rapidly. Since ACPs are not directed to a specific extracellular or intracellular receptor, some mechanisms of resistance can be impaired. The number of approved peptide-based drugs has been increasing from the last few decades, which reflects the potential of peptides as therapeutics. In order to assist the scientific community engaged in developing anticancer drugs, information on ACPs and anticancer proteins scattered in the literature have been collected and stored in the "CancerPPD" database (http://crdd.osdd.net/raghava/cancerppd/).

Antimicrobial Peptides (AMP), produced by the innate immune system in response to infectious agents, are currently considered for drug development. Many AMPs show broad-spectrum toxicity against both bacteria and cancer cells. The electrostatic interaction of ACPs with negatively charged components of plasma membrane of cancer cells is believed to play a crucial role in the cancer-selective toxicity of ACPs. AMPs and most ACPs have similar characteristics: small length, positive charge, amphyphaticity. The mechanism of their action is not fully understood so far. Most ACPs, like AMPs, exhibit membrane-lytic mode of action. However, some ACPs and AMPs induce apoptosis via disruption of mitochondrial membrane; consequently, AMP and ACP can be united in the same class of membrane-active peptides.

Anticancer /Antimicrobial drug design has focused on peptides with desired properties through the set of known or predicted peptide sequences. Effective predictive methods will allow investigators to conduct task-oriented design of new peptides, and decrease costs of new drug production. This requires data regarding the peptide's chemical structure and antimicrobial/anticancer activities. We have developed a Database of structure and Antimicrobial/Cytotoxic Activities of peptides (DBAASP) (http//: dbaasp.org). DBAASP is the depository of information for structure/activity study providing the information and analytical resources to the scientific community in order to develop new peptide drugs. The "Prediction" utility allows to reveal a potential antimicrobial activity for the queried peptides based on amino acid sequence. This utility is based on the machine learning algorithm and peptide parameters such as hydrophobic moment, charge density, depth-dependent potential, etc. The predictive model was optimized by the positive and negative training set of peptides. Positive set was formed by AMPs from DBAASP (Training-AMP). The negative one consisted of randomly selected fragments of non-secretory proteins from UniProt (training-UniProt). Two different positive sets were used for testing : test set from DBAASP (test-AMP) and test set from CancerPPD database (test-ACP). The accuracy of predictions for both testing was 92% for test-AMP and 90% for test-ACP.). The predictive model developed on the basis of AMP is successfully working in the case of anticancer peptides. These results give additional proof that the data on AMP can be used for the design of anticancer drug.

PARP1 and PARP2 catalyze poly(ADP-ribosyl)ation of DNA strand break termini.

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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. Here, we investigated interactions of PARP enzymes with the DNA repair intermediates of base excision repair (BER) and nucleotide incision repair (NIR) pathways. Our results revealed that both mammalian PARP1 and PARP2 can covalently modify DNA oligonucleotide duplexes by addition of multiple poly(ADP-ribose) units to 3' extremities of DNA. Therefore, DNA can be regarded as a substrate for PARP1 and PARP2 which catalyze a post-replication 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-replication modification of DNA mediated by PARPs provides an heuristic insight into molecular mechanisms involved in DNA repair, transcription and chromatin dynamics in eukaryotic cells.

Mechanisms of heat stress-induced cell senescence.

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The **aim** of this study was to investigate delayed effects of heat stress on mammalian cells.
Methods*:* Comet assay and modified PFGE were used to measure DNA damage. Indirect immunofluorescence was applied to track DNA damage response events. FISH was used to quantify the levels of hyper-replication of certain genomic loci. Gene expression analysis was performed using qRT-PCR and Western blot hybridization.
Results*:* In the present study we demonstrated that acute heat stress (HS) induces cell senescence in human cells – HS results in a robust G2/M cell cycle arrest within approximately one cell cycle. Heat-treated human cells acquire most of the cell senescence marks (proliferation arrest, senescence-associated (SA) beta-gal activity, changes in cell size/morphology, etc.) in few days after the treatment. Interestingly, HS does not lead to formation of SA heterochromatin foci (SAHF) – only ring-like heterochromatin domains are formed. We demonstrated that HS-induced cell senescence state is maintained by p21. We found that persistent DNA damage response, which depends on DNA hyper-replication, is the trigger of HS-induced cell senescence.
Conclusion*:* Heat stress induces p21-dependent cell senescence.
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Mechanism of suppression of limbic motor seizures by activation of the thalamic reticular nucleus

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At present, a number of forms of epileptic attacks are intractable. The search for alternative possibilities for therapy of such disorders motivated ones to study such “antiseizure” approaches as electrical stimulation of profound structures of the brain. Several studies have indicated that thalamic nuclei are involved in seizure development and expression. For example, it is well-established that thalamocortical systems participate in the generation of pathological rhythms with 3-4 Hz frequency. Neurons in the thalamic reticular nucleus (TRN) are involved in the mechanisms of synchronization and generation of this type of seizure activity. The TRN forms a shell around the dorsal thalamus and consists of GABA-ergic neurons, which provide a strong inhibitory input on thalamic relay cells.

Because our recent data [Exp. Neurol. 2003.] provide the first evidence that stimulation of TRN can act to suppress limbic motor seizures, objective was to explore a possible mechanism of blocking the limbic motor seizure reactions induced by activation of the TRN.

Experiments were carried out on adult cats (n = 4). Simultaneous recordings were made expra- and intracellularly in the thalamic ventrolateral nucleus and TRN.

Our experiments support the hypothesis that the activity of the TRN neurons is potentiated in the course of activation of this structure and inhibition of generalization of the seizures can be based on this phenomenon. Thus, stimulation of the TRN appears to be a rather valuable methodical tool that can open up prospects in the development of new anticonvulsive strategies in the treatment of temporal lobe epilepsy.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) interacts with apurinic/apyrimidinic sites in DNA

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Aim. Apurinic/apyrimidinic (AP) sites are some of the most frequent DNA damages and the key intermediates of base excision repair. Certain proteins can interact with the deoxyribose of the AP site to form a Schiff base, which can be stabilized by $NaBH_4$ treatment. The aim of this study was to identify the human cell extract protein specifically interacting with AP DNA containing single-stranded regions. **Methods.** The Schiff base-dependent crosslinking of proteins with 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 the target protein and to purify the protein. **Results.** We performed borohydride trapping of human cell extract proteins with several types of AP DNA. In the case of single-stranded AP DNA and AP DNA duplex with both 5 and 3 dangling ends, the major crosslinking product had apparent molecular mass of 45 kDa. Using peptide mass mapping based on mass spectrometry data, we identified the protein forming this adduct as an isoform of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is a glycolytic enzyme with many additional putative functions, such as interaction with nucleic acids, including damaged DNA, and DNA repair enzymes. We investigated interaction of GAPDH purified from HeLa cells and rabbit muscles with different AP DNAs. In spite of the ability to form a Schiff-base intermediate with the deoxyribose of the AP site, GAPDH does not display the AP lyase activity. In addition, along with borohydride-dependent adducts with AP DNA containing single-stranded regions, GAPDH was shown to form also stable borohydride-independent crosslinks with these DNA. NAD^+ inhibits GAPDH–AP DNA adduct formation. GAPDH was proven to crosslink preferentially to AP DNA cleaved via β-elimination mechanism (spontaneously or by AP lyases) as compared to DNA containing the intact AP site. The level of GAPDH–AP DNA adduct formation depends on oxidation of the protein SH-groups; disulfide bond reduction in GAPDH leads to loss of its ability to form adducts with AP DNA. **Conclusion.** Formation of stable adducts with AP sites by GAPDH may be involved in regulation of DNA metabolism. One can assume that under the oxidative stress GAPDH undergoes disulfide bond formation that results in enhancement of its DNA binding capacity and translocates to the nucleus. When the pool of NAD^+ is exhausted due to the PARP-1 activity, GAPDH can bind with DNA and, if the unrepaired AP site is present, GAPDH can be trapped in the stable covalent complex that would hamper DNA repair.

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mRNA and DNA selection via protein multimerization:
YB-1 as a case study

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Translation is tightly regulated in cells for keeping adequate protein levels, this task being notably accomplished by dedicated mRNA-binding proteins recognizing a specific set of mRNAs to repress or facilitate their translation. However the binding of isolated mRNA-binding proteins to short mRNA sequences/structures requires a high protein affinity to these specific sites, which is not a common feature among mRNA-binding proteins. Indeed many of them rather display a weak specificity to short and redundant sequences. Here we examined an alternative mechanism by which mRNA-binding proteins inhibits the translation of specific mRNAs, using YB-1, a major translation regulator, as a case study. We found using a single molecule approach that YB-1 triggers a homo-multimerization process based on a cooperative binding to mRNA. By doing so, YB-1 may inhibit translation of the mRNAs on which it has formed multimers. This novel mechanistic view on mRNA selection may be shared by other proteins considering the elevated occurrence of multimerization among mRNA-binding proteins. Interestingly, we also demonstrate how, by using the same mechanism, YB-1 can form multimers on specific DNA structures, which could provide novel insights into YB-1 nuclear functions in DNA repair and multi-drug resistance.

The Study of Structure and Function of Muscle Giant Proteins

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Besides the main contractile proteins the muscle contains the giant proteins with high molecular mass - Titin (skeletal muscle) and Smitin (smooth muscle)with molecular weights 3000 kD and 2000 kD accordingly.
Smitin, likewise Titin, has similar molecular morphology and location within the contraction apparatus, but its role in the smooth muscle tonic contraction is unexplained (there, in contrast to the striated muscle, the sarcomere is not distinctly formed and contraction character is different).
By means of Reverse sieve super pure preparations of Titin and Smitin have been received.
By the method of sedimentation analysis it was shown that speed of sedimentation of Smitin (C-titin) is less than that of Titin (Smitin $S_{20,\omega}$ =9.3S;Ttitin $S_{20,\omega}$ =13.8S) which is also due to the higher molecular mass of Titin.
The analysis of viscosimetrical data shows that the characteristic viscosity for Titin and Smitin is not high (Smitin ρ_{charac}=0.21; Titin ρ_{charac}=1.32). The apparent low viscosity obtained during the experiment, is typical for globular proteins but we believe that this fact is explained by the elastic properties of the investigated proteins.
Calorimetric studies show that thermal denaturation of Titin starts at 40^0C and ends at 80^0C. Transition temperature is T=59.4^0C and for Smitin is starts at 50^0C and ends at 80^0C. Transition temperature is T=66.1^0C.
The influence of Smitin (C-titin) on Mg^{2+}-activated ATPase activity of chicken smooth muscle (stomach) Actomyosin in different area conditions (ionic strength, pH, different concentrations of Smitin) was studied. It was shown that Smitin, likewise Titin, causes the increasing of Mg^{2+}-activated ATPase activity of Actomyosin. Obtained results confirm that in smooth muscle Smitin has the same effect on Actomyosin ATPase activity as Titin has on skeletal muscle ATPase activity. Smitin stipulates muscle elastic properties, on the one hand, and on the other hand it is the “scaffold” for the proteins participating in muscle contraction, forming the supermolecular complex with these proteins.
Our work is the first step for explanation of a role of those proteins in cell cytoskeleton formation and their participation in the process of muscle contraction.

Human tyrosyl-DNA phosphodiesterase 1: new activities and development of enzyme inhibitors as anticancer drugs

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Tyrosyl-DNA phosphodiesterase 1 (TDP1) is responsible to process topoisomerase 1 (TOP1) – DNA adducts as well as to hydrolyze a variety of other DNA 3′-substituents. We have shown recently that TDP1 can initiate repair of apurinic/apyrimidinic (AP) sites located in the internal positions of DNA generating breaks with the 3′- and 5′-phosphate termini [1-3]. This activity was not observed for TDP1 SCAN mutant responsible for neurodegeneration. Polynucleotide kinase phosphatase, Polβ and DNA ligase coordinated with XRCC1 are completing repair of the AP sites. The AP site cleaving activity of TDP1 is shown to be stimulated by the key modulator of base excision repair (BER) – poly(ADP-ribose)polymerase 1 (PARP1). The data suggest a role of TDP1 in the new APE1-independent BER pathway in mammals. This activity can contribute to repair of AP sites particularly in ssDNA structures or in the context of cluster-type lesions. The specific real-time fluorescent detection of the AP-site cleaving activity of TDP1 was developed, which is not sensitive to AP site cleavage by APE1 and useful to evaluate a biological significance of new TDP1 function to initiate cleavage of AP sites. TDP1 is a promising target for antitumor therapy based on TOP1 poison-mediated DNA damage. The row of novel benzopentathiepins and other compounds were synthesized and tested as TDP1 inhibitors using an original oligonucleotide-based fluorescence assay [4]. The benzopentathiepines showed IC_{50} values in the range of 0.2–6.0 μM. The specificity of these inhibitors was investigated by using other target DNA repair proteins such as PARP1, PARP2, AP-endonuclease 1 and DNA polymerase beta. The study of cytotoxicity of these compounds revealed that their action leads to the apoptotic cell death of cancer cells. Therefore the new class of very effective inhibitors of TDP1 was elaborated, which are very prominent to improve cancer therapy based on TOP1 poison–mediated DNA damage.

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Postsynaptic reactions of cat somatosensory cortex neurons in response to painful stimualation and analgesia

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We studied effects of electrical stimulation of the *substantia nigra* (SN), *locus coeruleus* (LC), *raphe nuclei* (RN), *substantia innominata* (SIn), *nucleus caudatus* (NC) and *central grey* (CG) on postsynaptic processes evoked in neurons of the cat somatosensory cortex by excitation of nociceptive and non-nociceptive afferent inputs (intense stimulation of the dental pulp and moderate stimulation of the thalamic ventroposteromedial nucleus, VPMN, respectively). We analyzed intracellularly recorded activity of cortical cells activated exclusively by stimulation of nociceptors and cells activated by both nociceptive and non-nociceptive influences ("nociceptive" and "convergent" neurons). In neurons of both groups, stimulation of both nociceptive afferents and thalamic VPMN resulted in the development of successions of EPSP – action potential (AP) or their series – IPSP (IPSP duration 200–300 msec). Conditioning electrical stimulation of the above-mentioned nuclei induced suppression of synaptic reactions that occur in cortical neurons in response to stimulation of nociceptive inputs. The maximum decrease in the amplitude of the IPSP was observed at test intervals of 600 to 800 msec when stimulated nuclei containing biogenic amines and 100-150 msec at conditioning electrical stimulation of cholinergic structures. We observed certain parallelism between conditioning influent ions of CG activation and effects of systemic injections of morphine. Discusses is the physiological significance of presumebly dendritic action potentials observed in our experiments. Decrease in the amplitude or complete postsynaptic inhibition of IPSP in cortical neurons under different treatments associate with the occurrence of convulsive epileptic activity, and at the painful action with analgesic effect. Discussed are the mechanisms for modulatory influences exerted by conditioning stimulation of SN, LC, RN, NC, SIn and CG on the somatosensory neurons, activated upon excitation of high-threshold (nociceptive) afferent inputs. Such modulation is probably based on changes developing in both pre and post-synaptic intracortical mechanisms.

Broken proto-oncogenes AML1 and MLL leave the inherent chromosome territories in human lymphoid cells treated with DNA topoisomerase II poison etoposide

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Aim: Today it is known that treatment of cells with DNA topoisomerase II poisons (such as etoposide) leads to the formation of double stranded DNA breaks in breakpoint cluster regions (BCRs) of known proto-oncogenes such as *MLL* or *AML1*. And that incorrect repair of these breaks may cause chromosomal rearrangements which in turn may induce the so-called "treatment-related" leukaemias. But the exact mechanisms of these rearrangements remain unclear. Our experimental data support the "breakage-first" theory supposing that DNA breaks formed at distant locations might be brought together and produce inter-chromosomal translocations. *Methods:* We have treated cultured human lymphoid cells (Jurkat) with etoposide or camptothecin and then visualized the chromosome territories and proto-oncogene parts upstream and downstream of corresponding BCR by using 3D-FISH technique. In order to increase statistical significance of results, we have analyzed hundreds of cells in each experiment. To facilitate the task we have wrote a computer software in order to analyze three-dimensional confocal images automatically. The first step of analysis is the detection of cells by positioning of uniform cylinders overlapping the cells in optimal places within image. Next step is the detection of signals as groups of the brightest pixels within each cell. Visual control of random subsample of images confirmed the correctness of the automatic detection of both cells and signals. Final step is the measuring the distances between determined signals. *Results:* New software allowed us to obtain number of data and reliable statistics. We have found that exposure of Jurkat cells to etoposide resulted in frequent cleavage of *MLL* and *AML1* genes, with the flanks of the break located distant from each other and are often found outside the inherent chromosome territories. Therewith it was no differences between 5'- and 3'- flank of the breaks. It was also observed that spatially separated flanks of proto-oncogenes are more frequently located outside chromosome territory than non-separated ones. Treatment of cells with camptothecin does not affect the integrity of *MLL* and *AML1* genes. *Conclusion:* Our findings demonstrate that flanks of the breaks introduced in BCR of *MLL* or *AML1* gene under inhibition of DNA topoisomerase II acquire additional mobility inside the nucleus and tends to be on the edge or even outside the inherent chromosome territory, which might allow the meeting and incorrect joining with its translocation partners.

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Squalenoylated-siRNA for the treatment of thyroid and prostate carcinomas

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We aim at introducing a personalized treatment for patients affected by cancers harbouring fusion oncogenes (FO). FO are the products of chromosomal rearrangements that are responsible for the development of more than 20% of cancers. In our laboratory, we focused on two pathologies harbouring FO: papillary thyroid carcinomas (PTC) where RET/PTC is found in 70% of cases and prostate cancers (PCa) harbouring TMPRSS2-ERG present in 50% of biopsies. Because FO are only present in cancer cells, they represent a promising target for personalized therapies based on small-interfering RNA (siRNA). siRNA, short oligonucleotides able to knockdown genes at mRNA level, are highly specific and present low toxicity; however their administration is a major challenge because the biological efficacy is hampered by their poor stability in biological environments. It is therefore necessary to implement methods for their protection, such as vectorization. *In vitro*, siRNA designed across the RET/PTC and TMPRSS2-ERG sequences, inhibited specifically the expression of the corresponding oncogenes, and impaired cell viability of both PTC and PCa cells, respectively. Induction of apoptosis was also observed. To deliver and protect siRNA, we covalently linked them to squalene (SQ) and the resulting bioconjugate was able to self-assemble into stable nanoparticles. The siRNA-SQ nanoparticles injected intravenously in mice were able to reduce drastically tumour growth in PTC and PCa xenografts following inhibition of RET/PTC and TMPRSS2-ERG oncogenes and oncoproteins, respectively. This strategy opens new therapeutic perspectives for the use of siRNAs-squalene nanoparticles as an innovative nanomedicine for patients affected by cancer pathologies harbouring FO.

The primer RNA packaging complex of HIV-1: understanding its assembly and disassembly

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The replication phase of HIV-1 viral RNA involves reverse transcription, which converts the viral RNA into corresponding double stranded DNA. This step is performed by reverse transcriptase using the $tRNA_3^{Lys}$ of the host cell as a primer. We previously showed that tRNA3 Lys packaging into HIV-1 particles involves the assembly of the packaging complex GagPol:mLysRS: $tRNA_3^{Lys}$. The mLysRS, or mitochondrial form of lysyl- tRNA synthetase, is hijacked from the host cell by GagPol polyprotein of HIV-1 and is selectively incorporated into the budding virion particle. It serves as a carrier for $tRNA_3^{Lys}$. The catalytic domain of mLysRS interacts with the TF (Transframe) and IN (Integrase) domain proteins of Pol domain of the GagPol polyprotein. A better understanding of the packaging complex may lead to the design of inhibitors at this stage of the HIV-1 replication. The precursor of Pol protein is very difficult to express and purify because of its tendency to aggregate and undergo proteolysis. This study was designed to reconstruct the packaging complex in vitro with a surrogate of Pol protein containing only the TF and IN domains separated by an artificial linker. The purpose is to analyze the binding affinity of Pol surrogate to mLysRS in the presence or absence of tRNA3 Lys and to further analyze if the binding of $tRNA_3^{Lys}$ to mLysRS is affected in the presence of the Pol surrogate. The interaction between the Pol surrogates containing spacers of different lengths was quantified using a FRET-HTRF assay. This should help in determining a minimum distance between the TF and IN domains which should be sufficient for a proper binding with the mLysRS. The interaction of tRNA3 Lys with mLysRS was then quantified in the presence of a suitable Pol surrogate, using a fluorescence polarization assay. We determined that the binding of $tRNA_3^{Lys}$ to mLysRS is strengthened in the presence of Pol surrogate. This analysis allowed us to express and purify a derivative of Pol that can form a binding complex with mLysRS and $tRNA_3^{Lys}$, and that can substitute to GagPol in further biochemical and structural studies of the GagPol:mLysRS: $tRNA_3^{Lys}$ packaging complex. This surrogate of Pol will be a powerful tool in the understanding of this essential process in HIV-1 replication.

Sigma-receptor-1 and mGluR5 may participate in Rac-dependent oncogenesis through modulation of macrophage activity

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A defining feature of tumor inflammation is the polarization of M1 into M2 macrophages which promotes tumor growth, angiogenesis, invasion, and metastasis. The mechanism(s) that control the M1-M2 transition remain to be determined. During inflammatory states, activated innate cells, as well as tumor cells, release amino acid glutamate that induce innate cell migration by activating class I/5 metabotropic glutamate receptors (mGluR1/5). Mutation of mGluR1/5 detected in several tumors triggers a downstream signaling cascade leading to activation of Rho family GTPase, Rac1, and Rac2. Rac overexpression in tumors may play a role in cancer progression. Targeting the Rac pathway is an approach to cancer treatment.The sigma1R (Sig1R) is an endoplasmic reticulum (ER)-resident molecular chaperone expressed at the mitochondria-associated ER membranes (MAM). Cell treatment with sigma antagonists causes rounding, detachment and growth inhibition of several cancer cells, however whether Sig1R protects cancer cells from death remains to be elucidated.To investigate the interplay between SigR1, Rac, and mGluR5, immunoprecipitation experiments were performed. Sig1R interacted with the Rac1 and Rac2. This interaction was increased in the presence of sigma-agonists, decreased by sigma-antagonists. Sig1R-Rac interaction was enhanced by GppNHp, suggesting that active Rac interacted with Sig1R. Specific association/dissociation of Sig1R with an inositol-3-phospate receptor (IP3R) and BiP was also found. p21-activated kinases (PAK) downstream effectors of Rac. PAK can phosphorylate various regulatory proteins, including pro-apoptotic Bad. Sigma-agonists increase PAK-dependent phosphorylation, causing dissociation of Bad from mitochondria, thus sigma-ligands could operate through SigR1/Rac complex by changing the PAK activity. Rac1 is be involved in the assembly and activation of ROS-generating enzyme - NADPH oxidase (NOX). ROS are increased in the presence of sigma-agonist (pentazocine), and sigma-antagonist (haloperidol) eliminated this effect, suggesting that that sigma-ligands could operate through SigR1/Rac complex by changing the NOX activity. Sigma 1-receptor induces immunosuppression through modulation of IP3R and Rac in macrophages. In activated macrophages, the binding of SigR1 to IP3R sigma ligands was shown to be decreased, and binding of SigR1 to Rac2 was increased. SigR1 was immunoprecipitated with mGluR5 suggesting that intracellular glutamate receptor may be part of a macromolecular complex in MAM. We next transfected mGluR5 cDNAs into macrophages. This decreased the binding of SigR1 to IP3R. Immunostimulants, like LPS, induced the association of SigR1 with Rac only in non-transformed cells., whereas sigma-agonists increased the binding of SigR1 to Rac in mGluR5-overexpressing macrophages that had a decreased sensitivity to the immunostimulants. Our data suggest that Sig1R dissociates from IP3R, and then associates with Rac1 and Rac2, and through this association/dissociation, sigma ligands can regulate Rac activity. The intracellular glutamate receptor may participate in induction of SigR1-Rac association. This association could lead to activation of downstream effectors (Pak and NOX) of Rac, to initiation of actin rearrangement and production of ROS. This may induce the M1→M2 transition and immunosuppression contributing to progression of tumorigenesis.

Translation elongation factor eEF1A1 and its oncogenic variants

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Aim. To find specific features of oncogenic proteins eEF1A2 and PTI-1 which make them different from very similar non-oncogenic translation factor eEF1A1 and, thus, to predict possible ways of their involvement in cancer development.
Methods. Complexes of translation elongation factor eEF1A1 and its very similar proto-oncogenic or oncogenic analogs eEF1A2 and PTI-1 correspondingly, with both translational and non-translational partners were studied by crystallography, atomic force microscopy, confocal microscopy, low-speed sedimentation and pull-down assays.
Results. Different shape of actin bundles produced by eEF1A1 or eEF1A2 is shown. PTI-1 protein is not capable of forming actin bundles due to its inability to dimerize, so it cannot be sequestered by actin compartments in cell.
eEF1A1 rather than oncogenic eEF1A2 is able to interact with Ca2+calmodulin and other regulatory proteins. Ca2+-calmodulin interferes with eEF1A1 binding to translational (tRNA) and non-translational (actin) partners.
PTI-1 protein retains similar to eEF1A1 translation elongation activity as well as ability to bind nucleotide exchange factor eEF1B. Surprisingly, eEF1B cannot stimulate nucleotide exchange in PTI-1. Based on our recent crystallographic data we built a molecular model of nucleotide exchange in eEF1A explaining inability of PTI-1 to be regulated by eEF1B.
Conclusion.
eEF1A1 and its oncogenic analogs can fulfill similar functions but the oncoproteins are suggested to perform these functions in non-controlled way, that may be one of specific features of cancer cells.

Transient receptor potential channel inactivation by non-steroidal anti-inflammatory drugs

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Transient receptor potential (TRP) cation channels have been extensively investigated as targets for analgesic drug discovery. These channels are the largest group of sensory detector proteins expressed in nerve terminals and pain receptors, and are activated by temperature and chemicals that elicit hot or cold sensations. Stimuli include menthol, cinnamaldehyde (CA), gingerol, capsaicin, allyl isothiocyanate (AITC) (a natural compound of mustard oil), camphor, and eugenol. The thermal thresholds of many TRP channels are known to be modulated by extracellular mediators, and released by tissue damage or inflammation, such as bradykinin, prostaglandins, and growth factors. Antagonists of these channels are likely promising targets for new analgesic drugs at the peripheral and central levels. Because some non-steroidal anti-inflammatory drugs (NSAIDs) are structural analogs of prostaglandins, we examined three widely used NSAIDs (clodifen, ketorolac, and xefocam) on the activation of TRPA1 and TRPV1 channels using thermal paw withdrawal (Hargreaves) test and mechanical paw withdrawal (von Frey) test in male rats. Thermal withdrawal latencies and mechanical thresholds for both hind paws were obtained with 5, 15, 30, 45, 60, and 120 min intraplantar post-injection of CA and AITC or vehicle. Twenty minutes prior to the start of the experiment, clodifen, ketorolac or xefocam were pre-injected in the same hindpaw and animals were examined by these two tests. After pretreatment of all three NSAIDs in the ipsilateral (injected) hindpaw that produced strong antinociceptive effects, CA, AITC, and capsaicin caused significant decreases in latency of the thermal withdrawal reflex compared with vehicle or the contralateral hindpaw. The same findings were observed for the paw withdrawal threshold. In approximately 30 min the effects of CA, AITC, and capsaicin returned to baseline. The findings in this study are different from our previous results, where TRPA1 agonists CA and AITC and TRPV1 agonist capsaicin produced hyperalgesia for nearly 2 h and resulted in facilitation of these withdrawal reflexes. Thus, we show for the first time an inactivation of TRPA1 and TRPV1 channels by NSAIDs to channel agonists in two behavioral assays.

Topokaryotyping – a novel approach to study nuclear organization

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The **aim** of this study was to develop a new approach to study nuclear organization at the single cell level.

Methods: Proximity Utilizing Biotinylation was used to label chromatin proximal to nuclear envelope, by coexpressing BirA-emerin (NE protein emerin fused to Biotin ligase BirA) and BAP-H2A histone (H2A fused to Biotin acceptor domain BAP, targeted specifically by BirA for biotinylation). After biotin was removed from the medium and cells were allowed to enter mitosis, mitotic spreads were generated and the location of biotinylated chromatin was visualized via streptavidin-Cy3 staining. The quality of the spreads was sufficient for partial identification of the chromosome in the spread by karyotyping; moreover, the streptavidin labelling can be combined with FISH for more conclusive chromosome identification.

Results*:* In the present study we demonstrated that the chromatin domains that are proximal to nuclear envelope appear as discrete bands on mitotic chromosomes. Importantly, the band patterns for individual chromosomes differ from one mitotic spread to another, pointing to variability of chromosome domain localization in the interphase nuclei and thus to importance of the study of nuclear organization at the single-cell level. Treatment of cells with various stressors affects the pattern distribution in the cell population.

Conclusion*:* We have developed a new approach to study nuclear organization at the single cell level.

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Functional state of red blood system as a possible predictor of individual radiosensitivity

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The results of already completed and present large scale radio-epidemiological studies ranked the late - stochastic and tissue effects prognosis, prevent and mitigate issue among priority problems of fundamental and applied radiobiology.

As it is known, that if early radiobiological tissue effects are mainly dependent on absorbed dose, individual features of adaptive response of organism have an important role in the formation of late and very late effects of radiological impact.

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

There were studied red blood system functional state in physiological norm and early postradiation period .

The functional state of RBS was determined by using the specially developed method based on analysis of the dynamics of peripheral blood erythrocytes spectra (PBE PS) - distribution of PBE according to their spherulation degree (Q) and volume (V)). Spherulation degree is considered as biological age and along with the volume determines the probability of their elimination from circulating bed. In connection with these positions distribution P=P(V,Q) express mechanism of production-aging-elimination of PBE and according to their dynamics we can discuss about the character of these processes.

In animals exposed with lethal and sublethal doses of ionizing radiation, at the initial stage of post -radiation period (6-10 days) PBE co-operative (massive) elimination and the involvement of young red blood cells with short life span are observed. Right short life span factor is considered as one of the main mechanisms in the development of radiation anemia.

On the other hand, it was detected that massive involvement in circulation of immature red blood cells that have diminished living status, elimination of the old fraction of PBE and erythropoiesis tension are observed during the further period of stress influence on animals.

From the position of the obtained results, the role of PBE PS regulation mechanisms in the development of radiation anemia is of great interest, while elucidation of inter- individual mechanisms of functional state regulation of RBS lays out new ways for prognosis of late effects of radiation impact.

Keywords: radiation impact, red blood system, erythrocytes of peripheral blood .

Roles of RNA and RNA-binding proteins in TDP-43 and FUS aggregation

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The formation of insoluble TDP-43 and FUS cytoplasmic inclusions are critical for the onset and progression of devastating neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD). These proteins are predominantly nuclear but, as many other proteins involved in mRNA metabolism and transport, they actively shuttle in and out of the nucleus under physiological conditions. However, in affected neurons, they are found enriched in the cytoplasm and form cytoplasmic inclusion.
If the mechanisms driving the onset of ALS and FTLD remain unknown, one can assume that the loss of normal TDP-43 and FUS functions is probably involved in neuron degeneration. First, these proteins bind to a huge number of mRNAs and, by doing so, regulate the translation efficiency. Second, TDP-43 and FUS are components of RNA transport granules which have important functions in neurons. For example, ALS linked mutations of TDP-43 impair the transport of RNA granules to distal neuronal compartments. Finally, due to their RNA recognition motif and their prion-like domain, both proteins are recruited in cytoplasmic stress granules upon stress conditions in a reversible manner. However, in pathological conditions, stress granule dynamics is deregulated, with the appearance of RNA granules of different morphologies and which, in contrast with stress granules, can no longer be dissociated after stress.
The role of TDP-43 and FUS in devastating neurodegenerative disorders explains the numerous studies on this subject both at the animal and cellular level but strikingly, structural analysis of the protein-RNA complexes has received little attention except for the potential amyloid characteristic of the filaments observed in inclusions of ALS or FTLD patients. To that end, we propose here a structural analysis at the single molecule level using Atomic Force Microscopy (AFM) of TDP-43-RNA and FUS-RNA complexes. We will take advantage of the high resolution imaging possibilities of AFM to detect potential differences in the binding mode of these proteins to RNA and the influence of RNA-binding proteins present in RNA granules on the stability of the TDP-43- and FUS-RNA complexes.

Reconfiguration of extended DNA fragment harboring alpha-globin gene domain in chicken erythroid cells.

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The chicken domain of alpha-globin genes represents a useful model to study regulation of globin genes expression. Current evidence suggests that spatial organization of genomic domains plays and important role in regulation of gene expression. We have previously found that activation of globin gene transcription correlates with a spatial reconfiguration of the alpha-globin gene domain resulting in an assembly of the active chromatin hub. Here, in order to get more information on a possible relation between transcription and large-scale chromatin folding, we have studied the spatial configuration of an extended (2.5 Mb) segment of chicken chromosome 14 harboring the domain of alpha-globin genes in cultured lymphoid cells, cultured erythroid cells, and cultured erythroid cells stimulated to a terminal differentiation resulting in activation of globin gene transcription. The results obtained demonstrate that active transcription of globin genes correlates with a limited decompaction of a relatively large area harboring the alpha-globin gene domain, although the domain itself became more compact apparently due to the formation of an active chromatin hub, ACH. Importantly, organization of the area under study into topologically-associated domains and spatial separation of active and repressed chromatin compartments remain virtually the same in all three cell lines studied.

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Macromolecular complexes in invadopodia formation.

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Aim. Invasive cancer cells form membrane protrusions, invadopodia, that degrade extracellular matrix and facilitate cell invasion and metastasis. Invadopodia contain an actin-rich core surrounding by adhesion and scaffolding proteins which presence correlates with invasive behavior of human cancer cells. Key players include the adaptor proteins Tks4 and Tks5, the actin regulators cortactin and N-WASP, the tyrosine kinase Src and the transmembrane metalloprotease MT1-MMP. In spite that in the last two decades significant advances in our knowledge of the structure and development of invadopodia have been made, detailed mechanisms by which they facilitate cell migration is not yet available. **Methods.** We used pull-down assay and Wester blot analysis, immunofluorescence and confocal microscopy, invadopodia gelatin degradation assay. **Results.** We have identified a series of new Tks4 binding partners including adaptor proteins of the ITSN family (ITSN1 and ITSN2) and small adaptor molecules Crk and Grb2, the Amphiphysin protein family (Amph1 та Amph2), kinase Src and phospholipase C gamma 1 (PLCg1) and also another member of the Tks family - Tks5. It may indicate the possible role of Tks4 in transport and sorting of cell vesicles. Current data is supported by interaction with the proteins of Amphiphysin family (Amph1 та Amph2), as their main functions are membrane trafficking and remodeling. Adaptor proteins Crk, Grb2 and ITSNs are important for the actin cytoskeleton rearrangements and signal transduction. Tks4 also may participate in these processes as we found that Tks4 formes complexes with above mentioned proteins. Moreover, we have identified and characterized new Tks4 isoform - Tks4-beta. We suggested that an active state of Tks4 is regulated via intramolecular interactions between its proline-rich motifs and own SH3-domains. We have shown the interaction between ITSNs and other prominent component of invadopodia WIP. Data from immunofluorescent analysis revealed co-localization of ITSN1 and WIP at the sites of invadopodia formation and in clathrin-coated pits. We have also demonstrated that the key endocytic protein ITSN1 and WIP and N-WASP can form a complex in cells. Together, these findings provide insights into the molecular mechanisms of invadopodia formation and identify ITSNs as scaffold proteins involved in this process.

Disassembly of interphase prenucleolar bodies are driven by the maturation of pre-rRNA

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Aim*:* Cell nucleus is a complex structure, harboring a variety of discrete subnuclear organelles, collectively referred to as nuclear bodies (NBs). Recent studies are beginning to elucidate the molecular mechanisms responsible for the assembly and maintenance of several NBs, but little is known regarding the mechanisms of NB disassembly.
Methods*:* Here, we have used the model of interphase prenucleolar bodies (iPNBs), nucleoplasmic bodies which form from the material of destroyed by hypotonic treatment nucleoli after return of cells to isotonic culture medium. The iPNB disasembly occurs during several hours after the formation and synchronously in all cells, which makes the system convenient for studying the mechanisms of NB disassembly.
Results*:* The nucleoplasmib B23-containing bodies – iPNBs, were easily seen in cells during 5 hours after return from hypotonic buffer to isotonic medium. During this time, iPNB number and size were gradually reduced. iPNBs demonstrated preferentially constrained diffusion, and there was no directed motions of iPNBs to the nucleoli. The major nucleolar protein B23 was transferred from iPNBs to nucleoli by diffusion. The maturarion of pre-rRNA occured inside iPNBs, and inhibition of pre-rRNA maturation led to the stabilization of iPNB structure and inhibition of B23 transfer to the nucleoli.
Conclusion*:* It seems that molecules of pre-rRNA played the 'seeding' role inside iPNBs, and their maturation and subsequent delivery from iPNBs led to the disassembly of these ***structures.***
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The peculiarities of anti-HSP60 antibodies at thyrod cancer progression

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Background. The study of anti-Hsp60 antibodies levels in several tumors is a topic of growing interest. It was demonstrated that levels of circulating anti-Hsp60 antibodies and Hsp60 could be used as biomarkers for carcinogenesis, and prognosis factor for survival and treatment susceptibility. The nature of the anti-Hsp60 immune response in malignant transformation and role of antibodies in that process need to be elucidated.
Our aim was to investigate the possible biological activity of anti-Hsp60 antibodies (both polyclonal and autoantibodies, purified from thyroid patients sera on Protein G Sepharose) on different continuous cell lines.
Methods. 25 patients sera (20-57 years old, 18 – follicular adenoma, 6 – papillary and 1 follicular carcinoma) and 12 healthy donors (control) were used for determination of anti-Hsp60 antibodies level by ELISA.
The anti-Hsp60 antibodies from highly reactive sera were purified by affinity chromatography method. Polyclonal anti-Hsp60 developed in our Laboratory were used in the study of biological activity of such antibodies in MTT-test.
Results. The increased anti-Hsp60 autoantibodies level was detected by ELISA in sera more than 50% of patients with follicular adenoma and autoimmune thyroiditis, and in 86% of thyroid cancer patients sera, respectively. IgG antibodies purified from highly reactive sera recognized target antigen (human Hsp60) in Western-blot analysis. Increased anti-Hsp60 antibodies level and Hsp60 expression in thyroid cancer tissue were associated with lymphoid infiltration and sclerotic changes of thyroid tissue.
First we investigated the influence of polyclonal anti-Hsp60 antibodies on different continuous cell lines (WRO, KTC-2, TPC-1, A431 and 3LL) vitality. We established dose-dependent and time-dependent anti-Hsp60 antibody effect on dehydrogenises cell activity. We also determined decreased level of THF-α production in MAEC supernatant and IL-8 production in total lyzates of continuous cell lines (Namalva, A549, A431W) after 24 hours incubation with polyclonal anti-Hsp60 antibody in vitro. However, polyclonal Hsp60 antibodies didn’t affected IL-10 production in MAEC.
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. The biological (cytokine-induced and vitality-suppressed) activity of anti-Hsp60 antibodies was observed firstly on different continuous cell lines. The results obtained could serve as a base for new diagnostic tool development at thyroid tumors and cancer diagnosis verification.

Regulation of DNA-repair, nitrosative stress-related and proteasomal gene expression by 1,4-dihydropyridines in diabetic animals

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DNA damage by endogenous free radicals and ineffective repair of these lesions is one of the etiological factors of diabetes mellitus (DM). Oxidative stress and abnormal production of nitric oxide lead to increased DNA breakage in DM. In DM production of NO by inducible NO synthase (iNOS) is dramatically increased, but endothelial NO synthase (eNOS) is uncoupled and produces superoxide radical. One of treatment strategies of DM can be aimed on decrease of production of reactive oxygen species with consequent DNA damage prevention, normalization of NO production and DNA repair enhancement. 1,4-dihydropyridine (1,4-DHP) derivatives appear to be prospective as novel drugs with above spectrum of effects. The aim of our work was to evaluate the impact of a group of novel 1,4-DHP on expression of genes involved in NO production, DNA repair and proteolysis. Induction of DM model by injection of streptozotocin to rats increased expression of *iNOS* gene in kidneys and blood cells, decreased expression of *eNOS* in kidneys cells; DNA-repair related genes *PARP1* and *γH2AX* were up-regulated in blood cells, DNA breakage level was increased in nucleated blood cells. **NO synthases**. Three of the tested compounds - AV-153, metcarbatone and etcarbatone increased *eNOS* gene expression in kidneys of diabetic animals, the effect seems to be favourable in DM conditions. Some of the tested compounds (etcarabatone, glutapyrone and J-9-125) enhanced *iNOS* gene expression in kidneys of intact animals, and further enhanced the increased *iNOS* expression in kidneys of diabetic animals, except etcarbatone. Etcarbatone, metcarbatone and glutapyrone normalized iNOS expression in blood cells of diabetic animals. **NOS-independent NO production**. The enzyme xanthine deoxygenase (XD) is involved in NOS-independent production of NO from nitrite in diabetic animals, several 1,4-DHP inhibit this process. Most of the studied compounds except etcarbatone increased the level of *XD* gene expression both in intact and STZ-treated animals. This apparent contradiction might be due to feedback arising when XD enzyme is inhibited by a compound. **DNA repair**. We have observed increased DNA breakage in animals treated with AV-153; it also enhanced effects of DM on DNA integrity and expression of DNA repair-coupled genes (*PARP1* and *γH2AX*). Etcarbatone decreased expression of these genes in blood of diabetic animals, although it increased the DNA breakage induced by diabetes. Glutapyrone *per se* increased expression of *PARP1* and *γH2AX* in kidneys, but decreased in blood. J-9-125 and metcarbatone increased expression of DNA repair genes in kidneys of both intact and diabetic animals, however metacarbatone was the only compound able to reduce DNA breakage in diabetic animals. **Proteasomes**. Ubiquitin-proteasome system dysfunction is an important factor in DM pathogenesis. In our model we did not detect modification of the proteasomal *PSMA6* gene expression by DM conditions. Some of 1,4 DHP (etcarbatone, glutapyrone) enhanced expression of the gene in intact animals, however almost all compounds produced increase of its expression in diabetic animals. **Conclusions**. 1,4-DHP can interfere with expression of genes involved in DNA-repair, nitrosative stress-related and ubiquitin-proteasome system. Effects of the compounds are dependent on structure and organ-specific. Some of the 1,4-DHP appear to be prospective for normalization of DM-induced metabolic abnormalities.

Extracellular ubiquitin regulates regeneration of leucopoiesis

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Deregulation of protein catabolism mediated by ubiquitin-proteasome system is involved in pathogenesis of different malignancies. Dividing cells are more sensitive to defects in protein degradation. Therefore direct control of ubiquitin-proteasome system components and its targets opens new potential ways for cancer therapy. Behaviour of extracellular ubiquitin in mammal cells is poorly presented in literature. Our earlier experiments revealed that *in vivo* injected extracellular ubiquitin inhibits mitotic activity of hepatocytes in healthy rats, but significantly stimulates it in alcoholic liver after partial hepatectomy. Moreover, we showed that extracellular ubiquitin caused significant changes in dynamics of regeneration of leucopoiesis. Mitotic activity in bone marrow of test group animals injected by ubiquitin was decreased by about 53% as compared with the intact animals' of control group. In the presented work we exposed the influence of in vivo injected extracellular ubiquitin on spontaneous regeneration of leucopoiesis in modelled leucopenia in Wistar female rats with weight about 120–150gr. Calculation of nuclear cell ratio in bone marrow smears stained with azure-eosin was performed. Animals were divided into two groups. In the first, control group of animals inhibition of haematopoiesis was achieved by means of 100mg/kg cyclophosphamide LD_{50} 50-200mg/kg injection. Bone marrow samples from the first group of rats had been taken at 0h (intact), 24h, 48h, 72h, 96h and 168h time points after injection of cytostatic. The second, test group was injected by 200 µg/ml Ubiquitin 72 hours later after cytostatic induction. Bone marrow samples from the second test group of rats had been taken at 6h, 24h and 96h points after injection of ubiquitin. 5000 cells per sample were counted. Calculation of number of nucleic figures per 1000 cells (‰) was carried out. Animals were anesthetized by ether before decapitation. Treatment of animals performed in accordance with regulations established by the Centre animal's ethic committee (Protocol N06/13.10.2014). The most noticeable cytological effect induced by ubiquitin in bone marrow is a significant increase of per mille at 6 and 24 hour points after ubiquitin injection. Ratio of nuclear cell to total cell count is increased 2 times in both cases as compared with the same (72h and 96h) hour points of control group. Finely, per mille reaches the value of intact animals cell count at 7^{Th} day after cyclophosphamide injection. So, after cytostatic injection clearly marked cytopenia in peripheral blood and depletion of nuclear cell index was achieved through the next 24 hours that was followed by elevation of nuclear cell per mille at 48h point. The second depletion of nuclear cells at 72h point has been removed by means of injected ubiquitin. Supposedly, extracellular ubiquitin enhances proliferative activity of stem cells and acts as a cell cycle regulator in bone marrow after chemically induced cytopenia, retains bone marrow cells in compartments of differentiation and maturation and prevents their early passage to peripheral blood. Farther investigation of extracellular ubiquitin effect on regeneration of leucopoiesis may elucidate new possible pathways of ubiquitilation in hematopoietic cells, mechanisms of regulation of leucogenesis in norm and pathology and provides excellent perspectives to create new methods of targeted therapy for hematological disorders.

Detection the specificity of poly(ADP-ribose) polymerase 1 and poly(ADP-ribose) polymerase-2 interaction with DNA strand breaks at single molecule level

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The **aim** of this study was to investigate the interaction of PARP1 and PARP2 with single DNA damage site in the context of long DNA substrates.
Methods: The interaction PARP1 and PARP2 with long DNA fragments containing DNA breaks was studied using atomic force microscopy (AFM) and fluorescent titration.
Results: PARP1 and PARP2 are eukaryotic nuclear proteins which are implicated in synthesis of poly(ADP-ribose) (PAR) after DNA damage detection. We directly visualized and compared the binding of PARP1 and PARP2 to long DNA fragments (1200-bp) containing only double-strand breaks (DSBs) or DSB along with a unique nick (nicked 1200-bp). AFM data show that PARP1 specifically binds to both nick and DSBs. At the same time, this protein binds to undamaged DNA, but to a lower level than to breaks. In contrast to PARP1, PARP2 binds weakly to undamaged DNA and to DSBs, and localizes mainly to nick site. These results correlate with fluorescence measurement data revealing that the binding affinity of PARP2 to 1200 dsDNA is 5-fold weaker in comparison to the same DNA fragment containing a nick. Interestingly, AFM assay clear demonstrate that PARylated PARP1 and PARP2 are still able to interact with DSB and nick.
Conclusion: Our AFM observations at single-molecule level implicate PARP2 in the recognition of nick and suggest its role in single-strand break repair. It is appears that the length of the PAR polymer formed by PARP1 and PARP2 proteins is influenced by the initial binding affinity of the proteins for the damaged DNA site.
Funding: The work was supported by INSERM, the Genopole Evry, program GDRI of CNRS and Russian Foundation for Basic Research (project no. 15-54-16003).

Nature of hyperpolarizing afterpotentials accompaning epileptic discharges

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The aim of present investigation was to explain the inhibitory mechanisms in neocortical neurons leading to the arrest of epileptic ictal activity.

Experiments were performed on adult cats. All surgical procedures were conducted under pentobarbital (36 mg/kg) anaesthesia. Ictal activity was induced by repetitive electrical stimulation of the sensory-motor region of cerebral cortex, or local application of penicillin on the surface of sensory-motor region of cerebral cortex. Glass microelectrodes filled with 2M potassium citrate were used for intracellular recording of neuronal electrical activity.

Hyperpolarizing after-potentials of penicillin – induced paroxysmal depolarizing shifts in neocortical neurons of the cat were investigated. On the bases of studying the membrane conductance changes at different points of hyper-polarization and the role of various ionic currents in its generation the conclusion was drawn that paroxysmal depolarizing shifts of hyperpolarizing after-potentials is a composite nature. The membrane resistance value was measured by recording the increase in membrane potential induced by injecting hyper-polarizing current pulses. At the beginning of the post-ictal hyper-polarization the resistance was markedly reduced to 45±10 % (n=30) of its ictal value, suggesting that this phase of post-ictal inhibition was caused by an IPSP with increased conductance. The initial component conditioned by increased membrane permeability to chloride, presumably a synaptic GABA-A response, the next component conditioned predominantly by potassium current, representing presumably a GABA-B response and the final component comprising mainly a calcium-activated potassium current. However, the reduced membrane resistance gradually returned to the initial level (90±10%, n=30), while the hyper-polarization persisted for a long time. It is possible that the activation of an electrogenic pump might be responsible for this continuing hyper-polarization.

Aminoacyl-tRNA synthetases as biomarkers for cancer diagnostics

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Recent evidence suggests that aminoacyl-tRNA synthetases (ARSs) exhibit remarkable functional versatility and their noncanonical functions have been pathologically linked to cancers. ARSs seem to have critical mechanistic roles in a variety of cellular processes that are relevant for disease development and pathology, and these roles may be used as one possible avenue for improvement of diagnostics.

To uncover the biomarkers related with tumorigenesis and behavior of cancer we have studied of differently expressed genes of four or six aaRSs in tissues of colon and kidney cancers by the quantitative polymerase chain reaction (Q-PCR) method.

In the case of kidney cancer from 18 samples of tumor tissue (ccRCC), increased expression (more than 2 fold) of seryl-tRNA synthetase (SARS) was observed in 10, and only two were noted its slight decrease. The tendency for co-expression of SARS, leucyl-tRNA synthetase (LARS) and histidyl-tRNA synthetase (HARS) in ccRCC has also been shown.

In the case of human colon cancer we have observed the expression profile for LARS, HARS, SARS and lysyl-tRNA synthetase (KARS) in 16 primary cancer samples. We have found that genes of LARS, SARS and KARS underwent most changes and tendency for expression with mutual exclusivity of KARS and SARS and LARS in colorectal cancer has been shown The LARS and SARS expression decreased significantly (from 2 to 60 fold) in 38 % of samples (6 of 16) in comparison with surrounding normal tissue according to Q-PCR data. The KARS expression was increased significantly (from 2 to 3000 fold) in 50 % (8 from 16) and was decreased in 25% of cancer samples. These genes might be used for diagnosis of colon tumors.

Nuclear organization, oncoviruses and cancer

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Background: Environmental factors play an important role in most human cancer. Burkitt's lymphoma (BL), a rare B-cell lymphoma caused by specific chromosomal translocations resulting in the juxtaposition of the CMYC oncogene with an immunoglobulin gene locus, is a typical example of cancer strongly affected by environmental factors. BL is associated with the Epstein Barr virus, human immunodeficiency virus (HIV), malaria and exposure to a Euphorbiaceae plant. The molecular mechanisms of these environmental factors remain largely unknown.
Results: One of the major enigmas to solve is why all these factors specifically induce Burkitt's lymphoma, and no other malignancies? Our data indicate that this these factors perturb the nuclear organization of B-cells inducing the prolonged colocalization of potential translocation partners, the IGH and CMYC loci.
Conclusions: HIV- and EBV induce changes in the nuclear architecture of B cells. This may specifically provoke BL-specific translocations.

How to become famous and popular in science

(*All scientometric tricks in 5 minutes for everyone)*

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Impact-factor and h-index are more often used on the pre expertise phase of scientist' activity estimation or evaluation of the represented projects and grants. The formula for the indexes calculation is so simple and it is used by different scientometric databases.
For the correct presentation the achievements, a scientist must have only one profile in Scopus, ResearcherID, Researchgate, Google scholar and Orcid. Check you visibility at
http://www.scopus.com/ for profiles merging go to http://www.scopusfeedback.com/
http://www.researcherid.com
https://www.researchgate.net
https://scholar.google.com.ua/
http://orcid.org/
Calculation of Impact Factor and h-index at http://biopolymers.org.ua/I_b.pdf

The study of nuclear localization of amh and sox9a genes in male and female gonadal tissues of zebrafish

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Zebrafish (Danio rerio) is widely used model organism in numerous research fields. Different RNA FISH techniques have been optimized for zebrafish, however DNA FISH on tissue cryosections was never adapted for this organism. The main goals of present research were to optimize DNA FISH on tissue cryosections of zebrafish testis and ovaries for the study of nuclear positioning of *amh* and *sox9a* genes in gonadal tissues.
DNA FISH was successfully optimized for cryosections of gonadal tissues using BAC clones. Positioning analysis of *amh* and *sox9a* genes reveals that both loci are found predominantly on the nuclear periphery in male germ cell nuclei. Along with this we evaluated the interallelic distances for both genes and showed that mostly homological loci are localized together at the nuclear periphery. Similar distribution however was not observed in ovarian tissues. However it should be mentioned that only oocytes on early stages of development were suitable for FISH analysis in our experimental conditions.

Some cellular and molecular mechanisms of radiation damage in animal and cellular model systems

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Far tissue effects of ionizing radiation on living organisms represents primary radiation damages and the body's whole hierarchy responses adaptive-regulatory mechanisms interference, which outcome is importantly determined by the the individual features of the metabolism. We have studied the metabolism of post-irradiation changes in the irradiated rats (disposable irradiation 6 Grey) and Jurkat cells culture (2 Grey during 1 minute).
After 24 hours incubation of radiated Jurkat cells population amount of gaploid (apoptotic G 0 stage) cells was increased by 400 %, diploid cells (G 0/G1 stage) – increased by 17 %, tetraploid cells (G2/M) decreased by 73 %, amount cells in S phase decreased by 35 % in comparison to the control group. It was revealed intensive production of oxygen and nitrogen free radicals and inactivation of antioxidant enzymes (superoxidismutasa, catalasa and glutathione peroxidase). Statistically significant dependence between intensification of cellular apoptosis level, amount of tetraploid cells and intensity of oxidative stress was revealed.
In irradiated rats blood alteration in the antioxidant enzimes activity, parameters of lipid metabolism, NO-s content and its modulatory activity, also increase proapoptotic factor p-53 in rats hepatocytes were revealed on the different stages after radiation.
We concluded that free radical oxidation plays important role in the radiation-induced cell and tissue damage.

Mitochondria-targeted antioxidants prevent TNFα induced endothelial cell damage

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Using mitochondria-targeted antioxidants made of plastoquinone and penetrating cations (SkQ family), we investigated the role of mitochondrial reactive oxygen species (ROS) in the TNF-induced cytoskeleton reorganization and apoptosis in endothelial cells.

SkQ, as well as the classic antioxidant N-acetylcysteine and Trolox significantly suppressed apoptosis induced by TNF [1]. Their action was directed at the suppressing the release of cytochrome c. We showed that SkQ treatment led to an increased level of Bcl-2, and reduced levels of Bax and p53 [1]. Nevertheless, SkQ had no on Bcl-2 mRNA expression, but it enhances Bcl-2 phosphorylation thus contributing to the inhibition of protein ubiquitination. We have also shown that SkQ prevents Bcl- 2 proteolysis induced by TNF. We assume that SkQ may affect activation of the redox sensitive stress kinases that are involved in the phosphorylation of the Bcl-2 family proteins.

In our system we plan to evaluate the influence of mitochondria-targeted antioxidants on DNA damage induced by TNF. The results will be presented at the conference.

We showed that SkQ as well as N-acetylcysteine and Trolox inhibited TNF-induced monolayer endothelial permeability for dextran with a molecular weight of 65–85 kDa. Endothelial monolayer permeability induced by TNF is accompanied by cytoskeleton reorganization. We observed that SkQ prevented TNF -induced release of VE- cadherin and β- catenin from the contact area, as well as disassembly of the annular peripheral beam of actin microfilaments. TNF-activated matrix metalloproteinases (MMP) cleaved extracellular fragment of VE- cadherins. We observed MMP-dependent decrease in the overall VE- cadherin level in the cells and the appearance of its cleavage product in cell medium induced by TNF. These effects were markedly suppressed by SkQ.

Thus, it was shown that TNF-dependent endothelial cell damage was largely dependent on ROS production in mitochondria, thus indicating promising angioprotective action of SkQ.

Reference

[1] I.I. Galkin, O.Yu. Pletyushkina, R.A. Zinovkin, V.V. Zakharova, I.S. Birjukov, B.V.Chernyak, and E.N.Popova, Mitochondria-targeted antioxidants prevent TNFα-induced endothelial cell damage. Biochemistry (Moscow), 79(2), (2014),169–177.

Detection of genetic disorders during the radiation impact on human organism

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Being a strong mutagen, Ionizing Radiation first of all causes changes in genetic apparatus of the living organisms . That's why genetic indices are excellent biomarkers for detection of the effects of Ionizing Radiation. The actual problem is to find the optimal combination of different biomarkers. We used the genetic methods (chromosomal aberrations, comet assay and level or micronuclei in lymphocytes and buccal exfoliative cells) for assess the mutagenic effect on the organism individuals in different conditions exposed to ionizing radiation. Irradiation of individuals is possible not only in nuclear disaster, or environmental contamination, but also during routine diagnostic procedure (e.g. computed tomography scanning, etc). In recent years the use of ionizing radiation for treatment and diagnostic purposes has significantly increased during recent years, Although , the general radiobiologic principles underlying external beam and radionuclide therapy are the same , there are significant differences in the biophysical and radiobiological effects.. The existence of genetic disorders is important to sort out the risk groups, because radiation as the mutagen has cancerogenic features. We revealed the heterogeneity of different organism response to irradiation.

Determination of absorbed dose, identification of various genetic disorders in individuals exposed to identical doses of radiation, offers the opportunity to judge the individual biological effect and is very important for individual preventive activities.

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Key words irradiation. ,biomarker, radiotherapy

Neuronal porosome complex in norm and pathology. Electron microscopic study

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In all cells, cellular cargo destinated for secretion is packaged in membranous vesicles that transiently dock and establish continuity at the base of cup-shaped membranous structures called "porosomes" and neurons are not exception. Via porosomes vesicles release intravesicular contents to the outside of the cell during secretion. It is suggested that in each type of secretory cell special content of secretory vesicles, different speed of release and different volume of content release dictates specific size of porosomes. In earlier atomic force microscopic and electron microscopic studies, it was shown that in the neurons, reprensenting fast secretory cells, 40–50 nm synaptic vesicles are docked at roughly 10 nm in diameter neuronal porosomes. Recent EM 3D tomography in rat brain also reveals the presence of 12–17 nm permanent presynaptc densities in which 35-–50 nm synaptic vesciles are found docked. Moreover, the inside-out ultrahig-resolution atomic force microscopic study of presynaptic membrane preparations of isolated synaptosomes displays the presence of the inverted cup-shaped 10–17 nm neuronal porosomes. Neuronal porosomes possess a central plug, that is absent in porosomes in other kinds of secretory cells. This plug interacts with proteins at the periphery of the structure, conforming to an eightfold symmetry; each of them is connected with spoke-like elements to the central plug that is involved in the rapid opening and closing of the neuronal porosome to the outside. The central plug has been further examined in various conformations: fully pushed outward, halfway retracted, and completely retracted into porosome cup – has been demonstrated. Recently we described the morphology of porosome in different brain structure of various mammals (rat, cat, dog). In this electron microscopis study we evaluate if rat porosome structure/size is altered as a result of pathological and other conditions, specifically as a result of chronic restraint stress. Finally, to further understand the structure of the neuronal porosome complex, and the bare protein backbone of the complex for future single-particle cryo-EM studies, we evaluated the size of this complex from high-detegent solubilized synaptosome membrane preparations. For to assess the effect of hypokinetic stress on porosome structure, conventional electron microscopic methods were used. For to evaluate the isolated neuronal porosome complex, it was immunoisolated from synaptosomes, using SNAP-25 specific antibody conjugated to protein A-sepharose. For all cases the morphometric analysis of porosome diameter and depth was made. The one-way ANOVA was performed on the diameter and depth. The results revealed that the parameters of porosome, especially diameter, are very heterogenous. However despite the dynamic nature of neuronal porosome, the ranges of dimension (diameter 12–16 nm, depth – 5–20 nm) remain the same in normal conditions and after influence of chronic restraint stress or white noise. Results of studies of isolated complex demonstrate, that althouight the eigth – fold symmetry of the immunoisolated porosome is maintained, and the central plug is present, there is a loss in the average size of the porosome, possibly due to a loss of lipids, proteins or both from the complex. In view of this, proteomics and lipidomics on the isolated neuronal porosome using our current procedure using elevated detergent for synaptosome solubilization, will be carried out to determine whether there is loss of lipids, proteins, or both from the structure

Scientific Program. VIII International meeting "From Molecular to Cellular Events in Human Pathologies", 18–20 October 2015, Tbilisi, Georgia

MONDAY 19 OCTOBER

9:00–9:30 Opening of the meeting.

I. Beritashvili Center of Experimental Biomedicine,
David Nadareishvili, Olga Lavrik, Patrick Curmi

9:30–9:50 Keynote lecture **David Nadareishvili** (*Georgia*) Functional state of red blood system as a possible predictor of individual radiosensitivity

9:50–11:30 Session 1**. Chromatin and nuclear organization.**

9:50–10:10 **Sergey Razin** (*Russia*) Reconfiguration of extended DNA fragment harboring alpha-globin gene domain in chicken erythroid cells

10:10–10:30 **Yegor Vassetzky** (*France*) Nuclear organization, oncoviruses and cancer

10:30–10:50 **Evgeny Sheval** (*Russia*) Disassembly of interphase prenucleolar bodies are driven by the maturation of pre-rRNA.

10:50–11:10 **M. Karapetian** (*Georgia*) The study of nuclear localization of amh and sox9a genes in male and female gonadal tissues of zebrafish

11:10–11:30 **Vasily Ogryzko** (*France*) Topokaryotyping –a novel approach to study nuclear organization

11:30–11:35 **Iryna Tykhonkova** (*Ukraine*) How to become famous and popular in science (All scientometric tricks in 5 minutes for everyone)

11:30–11:50 Coffee Break

11:50–13:00 Session 2 **Cellular mechanisms of physiological processes and disorders**

11:50–12:10 N. Gachechiladze (*Georgia*) The study of structure and function of muscle giant proteins

12:10–12:25 Vlada Zakharova (*Russia*) Mitochondria-targeted antioxidants prevent TNFα induced endothelial cell damage

12:30–12:50 N. Khizanishvili (*Georgia*) Mechanism of suppression of limbic motor seizures by activation of the thalamic reticular nucleus

12:45–13:00 Dmitry Kretov (*France*) mRNA and DNA selection via protein multimerization: YB-1 as a case study

13:00–13:50 Lunch

13:50–16:00 **Session 3. Radiobiology, DNA Damage and Repair**

13:50–14:10 **Olga Lavrik** (*Russia*) Human tyrosyl-DNA phosphodiesterase 1: new activities and development of enzyme inhibitors as anticancer drugs

14:10–14:30 **M. Gogebashvili** (*Georgia*) Radiobiological effects modeling by using plant test-systems

14:30–14:45 **Ekaterina Belousova** (*Russia*) DNA polymerase Iota participates in clustered damage repair

14:45–15:05 **A. Zedginidze** (*Georgia*) Detection of genetic disorders during the radiation impact on human organism

15:05–15:25 **Alexander Ishchenko** (*France*) PARP1 and PARP2 catalyze poly(ADP-ribosyl)ation of DNA strand break termini

15:25–15:40 **Anastasiya Kosova** (*Russia*) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) interacts with apurinic/apyrimidinic sites in DNA

15:40–16:00 **Nikolajs Sjakste** (*Latvia*) Regulation of DNA-repair, nitrosative stress-related and proteasomal gene expression by 1,4-dihydropyridines in diabetic animals

TUESDAY 20 OCTOBER

9:00–12:00 Session 4 **Cellular mechanisms of physiological processes and disorders II**

9:00–9:20 **R. Sujashvili** (*Georgia*) Extracellular ubiquitin regulates regeneration of leucopoiesis

9:20–9:40 **Loic Hamon** (*France*) Roles of RNA and RNA-binding proteins in TDP-43 and FUS aggregation

9:40–10:00 **G. Beselia** (*Georgia*) Modulation of GluN2B subunit-containing NMDA receptors expression and Spatial long-term memory in medial septal immunolesioned rats

10:00–10:20 **T. Labakhua** (*Georgia*) Postsynaptic reactions of cat somatosensory cortex neurons in response to painful stimualation and analgesia

10:20–10:40 **Alla Rynditch** (*Ukraine*) Macromolecular complexes in invadopodia formation

10:40–11:00 **M. Tsagareli** (*Georgia*) Transient receptor potential channel inactivation by non-steroidal anti-inflammatory drugs

11:00–11:20 **Mzia Zhvania** (*Georgia*) Neuronal porosome complex in norm and pathology. Electron microscopic study

11:20–11:40 V. **Tsomaia** (*Georgia*) Nature of hyperpolarizing afterpotentials accompaning epileptic discharges

11:40–12:00 **Marc Mirande** (*France*) The primer RNA packaging complex of HIV-1: understanding its assembly and disassembly

12:00–12:20 Coffee Break

12:20–13:15 Session 5 Cancer and Pathologies I

12:20–12:40 **H. Grebenchuk** (*Georgia*) Evaluation of the spatial prevalence of cancer in conditions of an inadequate data of population

12:40–13:00 **Boris Negrutskii** (*Ukraine*) Translation elongation factor eEF1A1 and its oncogenic variants

13:00–13:15 **Nikolai Lomov** (*Russia*) Broken proto-oncogenes AML1 and MLL leave the inherent chromosome territories in human lymphoid cells treated with DNA topoisomerase II poison etoposide

13:15–14:00 Lunch

14:00–15:35 Session 5 Cancer and Pathologies II

14:00–14:20 **M. Grigolava** (*Georgia*) Antimicrobial peptides as anticancer agents

14:20–14:40 **Liliane Massade** (France) Squalenoylated-siRNA for the treatment of thyroid and prostate carcinomas

14:40–14:55 **Stanislav Avdieiev** (*Ukraine*) New cytotoxic agents and their combinations for the treatment of chemoresistant glioblastoma and mantle cell lymphoma

14:55–15:15 **N. Natsvlishvili** (*Georgia*) Sigma-receptor-1 and mGluR5 may participate in Rac-dependent oncogenesis through modulation of macrophage activity

15:15–15:35 **Mikhail Tukalo** (*Ukraine*) Aminoacyl-tRNA synthetases as biomarkers for cancer diagnostics

15:35–16:30 Coffee Break + **Posters**

16:30–19:00 Round Table and Closing ceremony