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## Comprehensive treatment of locally malignant tumors using radiomodifications

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In recent years the search for ways to improve the treatment of malignant tumors is one of the main problems of today. The problem of treating cancer is particularly relevant for locally advanced tumors, in which the possibility of surgery is limited criterion for resectability and operability, and radiation and medication – a moderate radio- and chemosensitivity these tumors, particularly when widespread, large-obemu neoplasms. In the problem of finding more effective treatment of locally advanced tumors of the focus is on developing methods combined and complex therapy. In developing methods of pre-exposure are important searches of factors determining the maximum damage of the tumor, preoperative treatment tolerability, and optimal conditions for postoperative course. In this direction, a very effective is the use of various chemical and physical radiomodifiers selectively modifying the radiosensitivity of tumor cells. When treating widespread malignant tumors rectum and colon, breast, and alternative agent for other locations (only 158 pationts) preoperative combined treatment was carried out using differents radiomodifications – sinchronization cell cycle Xeloda and 5-FU (monoradiomodification) and in combination with short-term artificial hyperglycemia (polyradiomodification). Assessment of results of preoperative combined treatment with different radiomodifiers testifies to the effectiveness of the treatment: when using the effect monoradiomodification resorption and tumor regression by 50 % or more was found in 61 patients (59.8 %), small – less than 50 % – an effect was observed in 37 patients (36.3 %) and no effect – in 4 (3.9 %) patients. When using the effect polyradiomodification resorption and tumor regression by 50 % or more identified in 37 patients (75.5 %), small (less than 50 %) effect was observed in 10 patients (20.4 %) and no effect – in 2 (4.1 %) patients. We have developed a comprehensive approach to treatment with preoperative irradiation effects in the synchronization of cell cycle Xeloda and short-term artificial hyperglycemia, not yielding to the effectiveness of other well-known methods with radiomodifiers, compares favorably with simplicity in clinical use, requires no special skills of medical personnel and special equipment and equipment, which is very important in economic terms.



## PARP-9: a newcomer in the cell response to DNA damage

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The PARP-9/BAL1 encoding gene was reported to be overexpressed in some high-risk B cell lymphomas [1]. It is regulated by an IFNγ-responding bidirectionnal promoter [2]. By *in situ* hybridization in mouse adult organs and during mouse development, we have revealed that the *PARP-9* gene is developmentally and differentially expressed in lymphoid organs, brain and intestine [3]. The PARP-9 protein contains two macro domains, a domain present in the histone variant macroH2A and described as an ADP-ribose binding module [4]. PARP-9 macro domains were shown to efficiently bind poly(ADP-ribose) [4], supporting the idea that PARP-9 could be recruited to sites of intense poly(ADP- ribose) synthesis such as DNA strand breaks. Using live-cell imaging, we have examined whether GFP-tagged human PARP-9 could be recruited to DNA damaged sites locally introduced by laser microirradiation. Our results showed that PARP-9 is recruited via its two macrodomains to DNA damaged sites, in a PARP-1 and poly(ADP-ribose)-dependent manner. To gain insights into the function of PARP-9 in the DNA damage response, we have generated animal and cel lular models deficient in PARP-

9, by genetic ablation of the *Parp-9* gene in mouse and by shRNA-mediated silencing of PARP-9 expression in human tumour cells. We have examined the consequence of PARP-9 deficiency on the cellular response to DNA damages introduced by alkylating agents and ionizing radiations. Results will be presented, describing PARP-9 as a newcomer in the cell response to DNA damage. Supported by Centre National de la Recherche Scientifique, Universite de Strasbourg and Ligue Nationale contre le Cancer (Equipe Labellisee).

1. *Aguiar R. C., Yakushijin Y., Kharbanda S., Salgia R., Fletcher J. A., Shipp M. A*. BAL is a novel risk-related gene in diffuse large B-cell lymphomas that enhances cellular migration // Blood.–2000.–**96**, N 13.–P. 4328–4334.
2. *Juszczynski P., Kutok J. L., Li C., Mitra J., Aguiar R. C., Shipp M. A*. BAL1 and BBAP are regulated by a gamma interferon-responsive bidirectional promoter and are overexpressed in diffuse large B-cell lymphomas with a prominent inflammatory infiltrate // Mol. Cell Biol.–2006.–**26**, N 14.–P. 5348–5359.
3. *Hakme A., Huber A., Dolle P., Schreiber V*. The macroPARP genes *Parp*-9 and *Parp*-14 are developmentally and differentially regulated in mouse tissues // Dev. Dyn.–2008.–**237**, N 1.–P. 209–215.
4. *Karras G. I., Kustatscher G., Buhecha H. R., Allen M. D., Pugieux C., Sait F., Bycroft M., Ladurner A. G*. The macro domain is an ADP-ribose binding module // EMBO J.–2005.–**24**, N 11.–P. 1911–1920.

## Present-day state of paediatric oncology service in Armenia

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Considerable progress was achieved in diagnostics and treatment of malignant tumors at children during the past decade. The use of contemporary medical technologies, in particular radiotherapeutic, also elaboration of the new methods of medicinal antineoplastic therapy makes it possible to cure up to 70–80 % of the sick children. As well as in other countries, pediatric oncology in Armenia is one of the youngest branches of clinical oncology. The first and unique Pediatric Oncology Department (POD) with 20 beds was opened on the base of National Centre of Oncology in 1994. Diagnostics and treatment of all kinds of benign and malignant tumors except for leukemia is carried out in POD. The mostly occurring pathologies are: non-Hodgkin's and Hodgkin's lymphomas (15–18 %), bone sarcomas and PNET (10–12 %), Wilms' tumor (7–8 %), germ cell tumors (7 %), neuroblastoma (5–6 %). Among the benign tumors hemangiomas are uppermost and criotherapy is widely applied at their treatment. Medicinal therapy is the basic method in complex treatment of malignant neoplasms. The chemotherapy of tumours at children has frequently intensive character that demands applying multicomponent supportive care – one of the scientific directions of POD. Thus the frequency of such complications of chemotherapy as thrombocytopenia and leucopenia has considerably decreased. Limb salvage operations, specifically endoprosthesis replacement are widely applied in surgery. The weak point in pediatric oncology service in our country is absence of the unit of stem cells and bone marrow transplantation which necessity appears at resistant and relapsing tumors. It should be mentioned that there are cases of treatment of malignant solid and system tumors of childhood in non-oncological hospitals that leads to tactical mistakes. Leukemia is traditionally and rather successfully treated at pediatric department of Hematology Center. Analyzing and comparing the achievements of pediatric oncology in developed countries during the past 10 years we consider that there are a number of serious problems hindering the further progress in treatment of childhood cancer in Armenia. First it is necessary to resume teaching of the subject «children's oncology» to the students of Yerevan State Medical University on the base of National Centre of Oncology and to raise prestige of the profession. We consider essential and vital mobilization of all available resources and building of the Republican Center of Pediatric Oncology and Hematology.

## Molecular mechanisms involved in anti-cancer effect of hemorphins

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Earlier we have demonstrated that hemorphins *in vitro* modulate $Ca^{2+}$/calmodulin (CaM)-dependent protein phosphatase calcineurin activity by binding to CaM, exhibiting a concentration-dependent biphasic response on enzyme activity. It is well known that calcineurin controls gene expression of several cytokines, including IL-2, tumor necrosis factor $\alpha$ and others via dephosphorylation and nuclear translocation of NFATc (nuclear factor of activated T cell) family members. Calcineurin/NFAT pathway is involved in pathophysiology of cancer as well (Padma S. et al., Cancer Cell Int., 2005). The immunosuppressive drugs cyclosporin A (CsA) and FK506 are inhibitors of calcineurin. Both these compounds exert their inhibitory action on calcineurin activity by binding to immunophilins (cyclophilin A and FKBP12, respectively) (Liu J. et al., Cell, 1991). It has been found out that *in vivo* inhibition of calcineurin activity by CsA and FK506 induces apoptosis of leukemic cells and rapid tumor clearance in lymphoblastic leukemia (Medyouf H. et al., Nature Medicine, 2007). The goal of present study was to determine if hemorphins may affect tumor growth and regulate plasma and brain calcineurin activity *in vivo* in rats bearing sarcoma-45 (S-45). In the experiments were used 3 synthetic hemorphins: LVV-hemorphin-7, hemorphin-7 (H-7) and LVVYPW. Results obtained demonstrated that intraperitoneal (ip) administration of rats bearing S-45 with synthetic hemorphin (1 mg/kg) recovered plasma and brain calcineurin activity reduced in pathophysiology of S-45. It is proposed that hemorphins act as homeostatic agent. Moreover, 8 days treatment of rats bearing S-45 with hemorphins (single ip injection of hemorphin daily) resulted in the inhibition of

tumor growth. The most pronounced inhibition of tumor growth was induced by ip injection of H-7 (by 44.9 %). Recently, by using proteomics approaches in collaboration with Dr. F. Lottspeich (Department of Protein Analysis of Max-Planck Institute of Biochemistry, Martinsried, Germany), peptidyl-prolyl cis-trans-isomerase A (cyclophilin A) was identified as regulated (1.8 fold down regulation) by hemorphins protein in healthy mouse brain (Barkhudaryan N. et al., Neurochem. Res., 2010). It should be noted that by using cDNA microarray, among genes up-regulated in the colon cancer was peptidyl-prolyl isomerase-like 1 encoding PPIL1, a cyclophilin-related protein. It was suggested that development of drugs to antagonize the function of PPIL1 may be a good strategy for therapy of colon cancer (Obama K. et al., Clin Cancer Res., 2006). Importantly, it has been shown that H-7 regulates DNA-binding activity of NF-AT, AP-1 and NF-κB transcription factors in stimulated Jurkat T cells (Barkhudaryan N. et al., Blood (RA), 2010). It is very likely that the ability of hemorphins to regulate transcriptional activity and change the expression level of some proteins involved in pathophysiology of cancer (e. g. regulation of the expression of cyclophilin A) may explain their anticancer effect.

## Autoimmune processes in pathogenesis of complex polygenic diseases

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A growing amount of data has indicated the involvement of autoimmune reactions in etiology and pathogenesis of many neuropsychiatric disorders. However, for a majority of the diseased conditions the molecular pathomechanisms responsible for the development of autoimmune reactions and their relationships with other molecular and cellular level changes associated with the diseased condition remain unclear. The present report summarizes our recent investigations on the involvement of autoimmune processes in pathogenesis of complex polygenic neuropsychiatric diseases including stroke, schizophrenia and posttraumatic stress disorder [1–7]. Patients with the above mentioned diseased conditions as well as sex- and age matched healthy volunteers were involved in the studies. Among autoimmunity markers, different subpopulations of circulating immune complexes, including pathogenic immune complexes and cryoglobulins were studied. Special attention was focused on the immune complexes containing complement cascade initiating C1q protein and opsonins derived from C3 component protein during the activation of the complement cascade. Both blood levels and molecular composition of the immune complexes have been evaluated, and disease-specific antigens have been revealed for each diseased condition. In addition, the expression profiles of proinflammatory and immunoregulatory cytokines as well as blood levels of neuron-specific proteins (neuron-specific enolase and S-100b) were assessed. Cytokines profile includes both interleukins (tumor necrosis factor-α, interleukine-1-β, interleukine-6) and chemokines (monocyte chemoattractant protein-1, and cytokine induced neutrophil chemoattractant protein). Methodological design was mainly based on enzyme-linked immunosorbent assays (ELISA), protein chemistry, immunochemistry, and bioinformatics methods. The results obtained indicate that all studied diseases are characterized by the development of autoimmune processes. Correlation analysis between the measured parameters and those indicating pathogenesis of diseased conditions reveals positive correlation between the intensity of autoimmune reactions disease progression and severity, on the on hand, and between the intensity of autoimmune processes and blood-brain barrier dysfunction, on the other. The data obtained allow identifying two types of autoimmune reactions developed in targeted diseases. One type is developed due to pathologic changes in structural and functional integrity of blood brain barrier, and another, due to increase in the level of acute phase proteins. In both cases a low grade inflammatory response (neuroinflammation and low-grade systemic inflammation) and aberrant apoptosis have been identified as triggering factors.

1. *Manukyan L., Boyajyan A., Arakelyan A., Ayvazyan V., Arakelova E., Sim R., Grigoryan G*. Immunochemical composition of cryoglobulins generated in stroke // J. Clin. Immunol.–2009.–**29**, N 3.–P. 274–281.
2. *Boyajyan A., Khoyetsyan A., Tsakanova G., Sim R. B*. Cryoglobulins as indicators of upregulated immune response in schizophrenia // Clin. Biochem.–2008.–**41**, N 6.–P. 355–360.
3. *Boyajyan A. S., Arakelova E. A., Ayvazyan V. A., Manukyan L. A.* Interleukins and chemokines in acute ischemic stroke complicated and non-complicated with diabetes // Cytokines and Inflammation.–2008.–**7**, N 1.–P. 40–43.
4. *Khoyetsyan A. G., Boyadzhyan A. S., Tsakanova G. V., Sim R. B*. Abnormal immune complexes in schizophrenia // Neurochemical J.–2008.–**2**, N 4.–P. 329–330.
5. *Hovhannisyan L. P., Mkrtchyan G. M., Boyajyan A. S., Sukiasian S. H*. Immune complexes and complement classical cascade in posttraumatic stress disorder // Immunology (Moscow).–2008.–**9**, N 23.–P. 269–274.
6. *Boiadzhian A. S., Arakelova E. A., Arakelian A. A., Avetisian G. V., Aivazian V. A., Manucharian G. G., Mkrtchian G. M., Sim R. B., Willis A. K*. Circulating immune complexes in families with positive history of ischemic stroke // Zhur. Nevrol. Psikhiat. Im. S. S. Korsakova.–2007.–Suppl. 21.–P. 43–46.
7. *Mailian K. R., Boiadzhian A. S., Sogoian A. F., Sim R. B., Manukian L. A.* Concentration and protein composition of circulating immune complexes in the blood of patients with schizophrenia and subjects with positive familial history of disease // Zhur. Nevrol. Psikhiat. Im. S. S. Korsakova.–2005.–**105**, N 4.–P. 55–60.

## Hypothalamic cytokine Galarmin is an inhibitor of mesenchymal tumors

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Activation of the PI3K-Akt-mTOR pathway is implicated both in the establishment of tumors and as well as a target for

therapy in many types of solid malignancy, its blockade represents an opportunity to improve outcomes in patients with tumors that are associated with poor prognosis. The mammalian target of rapamycin (mTOR) is an intracellular serine/threonine protein kinase that has a crucial role in a nutrient sensitive signaling pathway that regulates cell growth. Our experimental data indicates that proline rich polypeptide-1 (PRP-1, Galarmin), immunomodulator cytokine, produced by hypothalamic neurosecretory cells, exerts its antiproliferative effect on the tumor cells of mesenchymal origin such as chondrosarcoma, giant cell tumor, lymphosarcoma, and MDA 231 high metastatic breast carcinoma (in an epithelial-mesenchymal transition) via inhibiting mTOR kinase activity and repressing cell cycle progression. The goal of these investigations was to elucidate the antiproliferative action of PRP-1 on the breast carcinoma cell line MDA 231 (ER–) and to compare PRP-1 action previously reported on other mesenchymal tumors. These experiments confirmed maximum inhibition of cell growth at 0.5 and 1 μg/ml PRP-1 (71 % and 63 %, respectively) and inhibition at 10 μg/ml of 44 %. There was no inhibitory effect observed on luminal T47-D (ER+) cells. Videomicroscopy results demonstrated dividing cells in the cytokine-treated MDA 231 (ER–), suggesting that the cells were not in the state of dormancy. The flow cytometry synchronization experiments confirmed that PRP-1-treated cells were accumulated in S phase. No apoptosis, caspase activation, or senescence was detected after treatment with this cytokine. Experiments with mTOR with PRP-1 (10 μg/ml) indicated statistically significant 40 % inhibition of mTOR kinase activity in immunoprecipitates of the MDA 231 (ER–) cell line. PRP-1 is a novel mTOR inhibitor with strong antiproliferative action in mesenchymal tumors mostly resistant to radiation and chemotherapy. One can speculate that PRP-1 chooses the same signaling routes for the tumors of mesenchymal nature, which presents interesting challenge for the future exploration on whether or not the genetic signatures defining the neoplasm also seal the niche for the metastasis for mesenchymal tumors. More experiments are needed for further understanding of the cytostatic effect of PRP-1 on cell cycling *in vivo* on the animal model. PRP-1 has compelling molecular antitumor properties that may make it a candidate for new adjuvant therapy trials alone or in combination with other chemotherapeutic agents for the treatment of mesenchymal tumors.

## The incidence and mortality of malignant tumours in Armenia

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The fight against malignant tumours is not only a health system issue, it has a state significance due to annual increasing incidence rate in almost all countries of the world (2–4 % on average), high levels of health problems and the absence of decrease in mortality rate. Now, how is the situation in Armenia for last 10 years. In 2000 the incidence rate was 142.3 patients per 100.000 population (5413 primary patients – new cases), in 2010 it composed 233.0 (7584 patients), the increase was 90.7 unit. In 2000 amongst primary patients the early (I and II) stages were diagnosed in 56.0 % cases, in 2010 – in 50.0 % of cases. Thus, there was no progress in the fight against negligence of malignant tumours. The mortality from malignant tumours was the following. During last 10 years the rate has increased by 74.1 unit (7.1 unit per year), while the increase in incidence rate during that period composed 90.7. Therefore the incidence rate has increased faster than mortality rate. In general structure of malignant tumours during last decades lung cancer was in the leading position, the intensive rate of which during last 5 years has increased by 3.6 unit, in 2010 it composed 36.7 per 100.000 population. The second prevalent malignancy was breast cancer, the intensive rate of which has increased by 3.1 unit, in 2010 it composed 58.5. By frequency next tumours are stomach, colon, cervical, prostate cancers, leucaemias and lymphomas, bladder, endometrial, ovary, rectal, laryngeal carcinomas. Exploring the incidence rate of separate tumous in Armenia by mapping we determined a quite mosaic picture. The highest level of incidence was estimated in Lori region: 290.7 per 100.000 population, then in Kotayk and Shirak regions – 254.0, in Yerevan – 241.2. In the rest regions incidence ranged between 200.0 and 221.0 limits. The lowest incidence rate was established in Gegharquniq region – 166.8.

## Carcinoma *in situ* of the breast: riddles and controversies

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Carcinoma *in situ* is the earliest histologically recognizable form of malignancy and such provides an opportunity to treat the disease in a curative way. Because of widespread screening for breast cancer, noninvasive (*in situ*) cancer of the breast is diagnosed with increased frequency. There are 2 types of breast cancer *in situ* – lobular cancer *in situ* (LCIS) and ductal cancer *in situ* (DCIS). Both LCIS and DCIS are forerunners of invasive breast cancer but with different behaviour. The management of *in situ* cancer (especially, DCIS – intraductal carcinoma) is one of the most complex and controversial topics in the treatment of breast cancer. There are widely disparate philosophies concerning diagnosis, classification and treatment. LCIS is a marker for increased risk of invasive cancer in both breast (in homolateral breast in 18 % and in heterolateral breast in 14 % of cases after 20 years), it is likely to be bilateral. Treatment options of LCIS include observation (after surgical excision) without sentinel lymph node biopsy (SLNB) or axillary lymph node dissection. Some patients with high risks of breast cancer should be potential candidates for bilateral mastectomies. DCIS is defined as a carcinoma of ductal epithelial origin, without any evidence of stromal invasion. Laboratory and patient data suggest that DCIS is a precursor lesion for invasive cancer. The appropriate classification of DCIS has provoked much debate; a number of classification systems (Van Nuys, AFIP classification systems) have been developed, but there is a lack

of uniformity in the diagnsosis and prognostification of this disease. Further investigation of molecular markers should improve the classification of DCIS and our understanding of its relationship to invasive disease. Controversy also exists with regard to the optimal management of DCIS patients. In the past, mastectomy was the primary treatment for patients with DCIS, but as with invasive cancer, breast-conserving surgery has become the standard approach. The incidence of axillary lymph node metastases in DCIS is negligible (up to 4 %, 3.5 % – in our study). According to ASCO recommendations, SLNB is acceptable when a mastectomy is indicated or when immediate breast reconstruction is planned. In patients who undergo breast conserving therapy, SLNB is recommended in cases of large or high-grade DCIS. Three randomized trials have reported a statistically significant decrease in the risk of recurrence with radiation therapy in combination with lumpectomy alone, but there was no survival advantage with the addition of radiotherapy. Two randomized trials have suggested an additional benefit, in terms of recurrence, with the addition of adjuvant tamoxifen therapy, although in one trial the benefit was not statistically signi ficant. Current data suggest that tamoxifen use should be restricted to patients with estrogen-positive DCIS (ER-positive in 70 % – lower in high grade; HER2/neu positive in 50 % – higher in high grade). Neither trial demonstrated a survival benefit with adjuvant tamoxifen.

## Co-temporal replication and transcription in long genes induces common fragile site breakage that can be prevented by inhibiting R-loop formation

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**Background**. Almost all eukaryotic genes are transcriptionally silent during the S-phase of the cell cycle to prevent collisions between the RNA polymerase II transcription apparatus and the replication machinery. When transcription and replication machineries encounter in bacteria or yeast, replication forks have been shown to stall and genomic recombination rates increase. It was also demonstrated that cotranscriptionally arising RNA:DNA hybrids (R-loops) contribute to genome instability. Human cells contain specific genomic regions, which are prone to breakage and rearrangement formation. These chromosomal regions are called common fragile sites (CFS) and can be detected in metaphase spreads as gaps and breaks in the chromatin. However, the mechanism of CFS formation remains largely unknown. **Aim**. We asked whether the transcription of genes would par ticipate in CFS formation. We show that (i) CFSs are often found in very long coding regions and that (ii) the breakage of CFSs depend on the active transcription of these very long genes underlying the CFS regions. By analyzing the transcription timing of these CFS-associated human genes, we found that the corresponding extremely long transcripts are produced over more than one cell cycle, consequently also in S-phase. Moreover, our experiments demonstrate that the genomic regions coding for these long CFS-associated genes replicate in late S-phase, independently whether they are transcribed or not. Thus, CFS breakages appear in very long genes (about 1 Mb or more) that are transcribed and replicated about the same time in late S-phase. Furthermore, we were able to reduce the breakage of chromosomes in the CFS regions when we eliminated the co-transcriptionally arising R-loops by overexpression of RNase H1 that is known to hydrolyse R-loops. **Conclusions**. Thus, our results reveal that CFS instability is based on the formation of R-loops on very long genes, but only when replication and transcription occur at the same time.

## Oxidative modifications of biomolecules upon neurodegenerative processes

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The Parkinson's disease (PD) is a neurodegenerative disorder accompanied by movement-related, neuropsychiatric and vegetative problems. Progressive disease development, inadequate therapy, heavy disablement of the majority of patients turn the PD into a serious social issue. The objective of the present work was the study of the oxidative destruction of proteins and lipids in mitochondrial fraction of the brain in animals with experimentally induced syndrome of PD. The experimental PD syndrome was induced in rats by 1-methyl-4-phenyl-1,2,3,6-tetrapyridine. It was shown that the development of PD leaded to increase of hydroperpxides and malone dialdehyde in mitochondrial fraction of the brain. Analysis of oxidative damage of proteins at induced PD shown the statistically reliable increase of protein carbonyl derivatives. The rate of oxidative protein modification is known to be determined by the amino acid composition of proteins. The levels of aliphatic aldehyde and ketone dinitrophenyl hydrasones increased, that suggested the acceleration of oxidative destruction of proteins. It was concluded that the oxidative modification of lipids and proteins occurs at PD and, probably, plays an essential role in pathogenesis and development of this disease.

## Oncogenic and anti-oncogenic properties of genes with significantly changed expression in brain tumors

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An important task in understanding oncogenesis is the identification of those genes whose copy number and expression

increase during tumorigenesis. In an effort to identify molecular markers for glial tumors, we compared gene expression in glioblastoma, the most aggressive form of brain tumors to the normal brain cells. Among the genes with the most pronounced increased expression in tumors was *CHI3L1*, encoding the secreted chitinase 3-like 1 protein. CHI3L1 can decrease the doubling time of 293 cells, allows the anchorage independent growth in soft agar and in addition, stable *CHI3L1* expression made 293 cells tumorigenic: these cells stimulated the initiation of tumors after their transplantation into the rat brains. The activation/inactivation kinetics of the signaling pathways has been associated with specific outcomes. 293_CHI3L1 cells activated extracellular signal-regulated kinases (ERK1/2) MAPK and AKT-mediated phosphoinositide 3-kinase (PI3K) pathways; phosphorylated ERK1 and ERK2 were localized in both cell cytoplasm and nuclei while AKT only in cytoplasm. 293_CHI3L1 cells differed from 293 cells transfected by an «empty» vector in their size and ability to adhere to the culture plate. Chitinase 3-like 2 (CHI3L2) most closely related to human CHI3L1 also showed increased expression in glial tumors at both the RNA and protein levels and stimulated the activation of the MAPK pathway through phosphorylation of ERK1/2 in 293 and U373 cells. In contrast to the activation of ERK1/2 phosphorylation by CHI3L1 that lead to a proliferate signal (similar to the EGF effect in PC12 cells), activation of ERK1/2 phosphorylation by CHI3L2 (similar to NGF) inhibited cell mitogenesis and proliferation, thus possessed anti-oncogene properties. In addition we found one more antagonist to oncogene *CHI3L1*, the tumor suppressor gene *TSC22* with decreased expression on mRNA and protein levels in glial brain tumors. TSC22 inhibited cell proliferation and may serve as a mediator of apoptosis stimulated signal from TGF-β1. Thus, the overexpression of *CHI3L1* is likely to have an important role in tumorigenesis and orthotopic implantation of transformed human cells with overexpressed human oncogene *CHI3L1* to the rat brain presents a new model of human brain tumor which can be used as a target for anticancer drug development. Address influence on oncogenes is becoming a clinical reality, however the therapy aimed against one singular gene will be not really effective because of huge bio logical heterogeneity of tumors. At present it is understandable that the establishment of specific signature of gene expression for every tumor type is obligatory for their more effective therapy. Two years ago we elaborated sample of «gene expression prediction», namely a 10-genes glioblastoma signature. More precise signature was obtained by so called self-organized Kohonen's map (SOM) using in this case twenty genes significantly changed their expression in about two hundred glioblastoma and seventy normal brain samples. Obtained data clearly show the clusterization of glioblastoma and normal brain samples. Moreover, self-organized Kohonen's mapping of the data obtained with same twenty genes significantly changed expression in more than 400 samples of different grade astrocytomas and normal brain allowed to separate clusters of samples from patients with pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma, glioblastoma, and samples of normal brain.

## High frequency stimulation of the subthalamic nucleus impacts adult neurogenesis in a rat model of Parkinson's disease

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**Aim**. Dopamine loss in Parkinson's disease (PD) affects adult neurogenesis in the two main neurogenic regions (the subventricular zone-rostral migratory stream-olfactory bulb continuum and the dentate gyrus of the hippocampus), with presumed involvement in non-motor symptoms, such as hyposmia and depression. This study addresses the impact of prolonged subthalamic nucleus high frequency stimulation (STN-HFS), an efficient therapeutic option for the treatment of motor symptoms in advanced PD, on adult neurogenesis in a rat PD model. Knowing that neurogenic changes can be involved in antidepressant action, we also looked for its effect on depressive-like behavior. **Methods**. Unilateral lesion of the nigrostriatal dopamine neurons was performed by intranigral 6-hydroxydopamine injection. STN-HFS was applied continuously during 8 days in freely moving rats through an implanted electrode on the same brain side with the following parameters: frequency 130 Hz, pulse width 80 μs, intensity 80 μA. The forced swim test was used to assess the antidepressant action of STN-HFS. Two steps of neurogenesis were investigated, proliferation (Ki-67-immunopositive cells) and survival (cells immunopositive to BrdU, a thymidine analogue that labels dividing cells *in situ* by incorporation in the DNA, given by systemic administration at the first day of HFS), in the two above-mentioned neurogenic regions. **Results**. Dopamine lesion has a negative consequence on both cell proliferation and survival through different mechanisms, the effect on proliferation being correlated to the level of striatal dopamine denervation whereas the effect on survival is not. In the two neurogenic zones, STN-HFS does not act on cell proliferation but promotes the survival of newly formed cells both in the ipsi- and contralateral sides, suggesting the involvement of a complex neural network in the control of this critical phase of neurogenesis. Regarding behavior, in contrast with a previous report showing that acute STN-HFS impairs performance in the forced swim test, we found improved performance in this test following prolonged STN-HFS. **Conclusions**. Altogether, these data suggest that chronic STN-HFS has a positive action on adult neurogenesis that is not directly linked to dopamine tone and might have an antidepressant-like potential. They strongly support the view that this neurosurgical treatment can largely impinge brain function and neuroplasticity. We are now using an implantable microstimulator allowing chronic HFS in behaving rats to identify the phenotype of newly formed cells and further investigate the motor and non-motor behavioral outcomes of the long term neurosurgical treatment.

## Iatrogenic aplasia induced by chemotherapy and treatment with «proline rich peptide»

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The patients with breast cancer of the 4th degree (according to the international classification) with metastasis injury of the bone, and unable to independent movement, as well as without surgical intervention on breast, received a full rate of irradiation treatment and chemotherapy by doxorubicin and cyclophosphamide solution. On the 3–4 days after chemotherapy and definition of quantity of leukocytes, the patient was treated with subcutaneous (before sleeping) application of Proline Rich Peptide (PRP), which is a polypeptide from bovine hypothalamus, with quantity of a single doze of 70 mcg with an interval in one day within 5 days, i. e. three injections in a total doze of 210–220 mcg. Study of the spectrum of leukocytes' condition shows that during the irradiation the following changes have been registered: The 1st fraction of leukocytes 30–100 fl (fl = mcm$^3$), representing a picture of fraction lymphocytes in a population of leukocytes, is not exposed to special changes. Their quantity changes from 1.0 K/μl at pretreatment up to 1.2 K/μl at post-treatment with PRP. Thus, the quantity of lymphocytes reduces up to 30 % after chemotherapy, and rises after PRP application. Monocytes (medium-sized blood cells), as well as basophils and eosinophils settle down on the histogram in the field of 100–150 fl and remain on the former level irradiation, and do not vary after the application of PRP (0.3 K/μl). Granulocytes or neutrophils, representing the largest blood cells with polysegment nucleus in the populations of leukocytes, are reduced after chemotherapy up to 0.17 K/μl, and grow up to 1.9 K/μl after PRP application. After therapy the indicative condition of granulocytes increases after PRP application up to 155–350 K/μl. The quantity of granulocytes under influence of PRP grows by 950 %. According to the histogram both the quantity and also the volume of granulocytes increase, thus indicating their full physiological value. A cytogenetic observation of the lymphocytes culture after chemotherapy and 3-fold administration of PRP (by 75 μg) is carried out starting from the 5th day after finishing the chemotherapy course. The analyses are made from the 6th day after the final administration of PRP. The caryotype 44/XX is defined, the level of chro- mosome aberrations being 7.4 %. The quantity of tetraploid cells (4 p) was 0.55 per 100 cells. The opportunity of application of a preparation in clinic is discussed.

## Modeling of parkinsonism in mice: mechanisms of neuroplasticity

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A degradation of the nigrostriatal dopaminergic (DA-ergic) system is the key component of pathogenesis of Parkinson's disease (PD). Initial clinical symptoms appear 20–30 years after the onset of neurode-generation, at a 70 % DA depletion in the striatum and a 50 % loss of nigral DA-ergic neurons. Low efficacy of the therapy might be improved if preclinical diagnostics and preventive therapy are developed. The development of appropriate experimental models should precede clinical trials. This multidisciplinary study first managed to model in mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) all together the following stages of parkinsonism: (a) the early presymptomatic stage manifested by a subthreshold degeneration of axons and DA depletion in the striatum without loss of nigral cell bodies; (b) the advanced presymptomatic stage manifested by a subthreshold degeneration of striatal axons and DA depletion and by a subthreshold loss of nigral cell bodies; (c) the advanced presymptomatic stage characterized by threshold depletion of striatal DA and a loss of DA-ergic axons and nigral cell bodies resulting in motor dysfunction. The degeneration of axons proceeds and prevails that of cell bodies suggesting higher sensitivity to MPTP of the former. Compensatory processes were developed in parallel to neurodegeneration that was manifested by the increase of: (i) the DA content in individual nigral cell bodies and DA turnover in the striatum, (ii) the tyrosine hydroxylase gene expression and activity, (iii) DA release, (iv) DA synthesis in non-dopaminergic neurons. The above processes appear to prevent a development of motor disorders at the presymptomatic stage. A transition from the presymptomatic stage to the symptomatic one appears to be provoked by a decrease of DA release and an increase of DA uptake apparently resulting in a drop of the intercellular concentration of DA. The developed models might be exploited for: (a) an examination of pathogenetic mechanisms not only in the nigrostriatal system but also in other brain regions and in the periphery; (b) a study of the compensatory mechanisms under DA deficiency; (c) a search of precursors of motor disorders and peripheral biomarkers in presymptomatic parkinsonism; (d) the development of preventive therapy aiming to slow down the neurodegeneration and strengthen compensatory processes. Thus, the models of the early and advanced presymptomatic stages and of the early symptomatic stage of parkinsonism were developed in mice with MPTP.

## The nucleic acid binding properties of CSD of human YB-1

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The nucleic acid binding properties of CSD of human YB-1 has been investigated with molecular dynamics (MD) simulation method. It has been shown that CSD can significantly determine the specificity of the whole YB-1 protein to both RNA and DNA. MD simulation revealed a higher affinity of CSD to RNA sequences as compared with similar

DNA sequences. This is directly related to the contacts formed by OH-groups of ribose residues. A cap-binding site on the surface of CSD has also been proposed. Although we have not observed very stable interactions between CSD and the cap-structure of mRNA, these interactions should increase the affinity of YB-1 to capped mRNAs. Finally, we performed simulation of the phosphorylated CSD and its complexes with nucleic acids. Phosphorylation of CSD on Ser102 leads to a slightly decreased affinity of CSD to nucleic acids especially DNA sequences. The next question to be addressed is how this phosphorylation may influence the interaction of YB-1 with RNA cap-structure.

## Apurinic/apyrimidinic (AP) site recognition by poly (ADP-ribose) polymerase 1 and other key proteins of human cells

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**Aim**. DNA repair is a central mechanism to keep integrity of cell genome. Several pathways are involved in repair of DNA damages arising under the action of endogenous or exogenous stress among them base excision repair (BER). One of the most abundant lesions in DNA are the apurinic/apyrimidinic (AP) sites arising spontaneously or as intermediates in BER at a frequency of 10.000 to 50.000 lesions per mammalian cells per day. AP sites can appear also under the action of ionizing radiation and can be components of cluster lesions in DNA. AP sites are unstable and cytotoxic. AP sites within nucleosome produce significant amounts of DNA-protein cross-links and generate double-strand breaks, the most deleterious form of DNA damage. Repair of these lesions is the central task for protecting DNA structure. The aim of this work was to identify proteins which can interact with AP sites to regulate repair of these abundant DNA lesions. **Methods**. We applied DNA derivatives containing AP sites as tool to cross-link target proteins in the extracts of mammalian cells. The target proteins were identified by MALDI-TOF mass spectrometry after borohydride trapping of Schiff bases in DNA-protein adducts and purification of DNA-protein adducts on the affinity column. Interaction of proteins with AP site-containing DNA was analyzed by biochemical methods and proved by atomic force microscopy (AFM). **Results**. The capacity of human poly(ADP-ribose) polymerase-1 (PARP-1) to interact with intact AP sites in DNA has been demonstrated. In cell extracts, sodium borohydride reduction of the PARP-1/AP site DNA complex resulted in covalent cross-linking of PARP-1 to DNA; the identity of cross-linked PARP-1 was confirmed by mass spectrometry. Using purified human PARP-1, the specificity of PARP-1 binding to AP site-containing DNA was confirmed in competition binding experiments. PARP-1 was only weakly activated to conduct poly(ADP-ribose) synthesis upon binding to AP site-containing DNA, but was strongly activated for poly(ADP-ribose) synthesis upon strand incision by AP endonuclease 1 (APE1). By virtue of its binding to AP sites, PARP-1 could be poised for its role in base excision repair, pending DNA strand incision by APE1 or the 5'-dRP/AP lyase activity in PARP-1. Interaction of PARP-1 with DNA, containing AP-sites was detected using AFM-imaging of protein-nucleic acid complexes. Another member of PARP family – poly(ADP-ribose) polymerase 2 – shows interaction with AP site-containing DNA. Further characterization of PARPs interaction with AP site-containing DNA and its implications will be discussed. It was revealed that other cofactor proteins of the BER system – XRCC1 and HMGB1 – are also able to interact with AP sites. **Conclusions**. Therefore, it is likely that one of the functions of PARP proteins consists in regulation of AP site repair. One can expect contribution to this process of other protein factors of DNA repair complex. This work was supported by RFBR grants nos 09-04-93106, 10-04-01083, 11-04-00559, State contract 16.512.11.2241 and Program of RAS on Molecular and Cellular Biology.

## Embryonal proteoglycans and counteraction to a tumour growth

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The cancer epidemic has captured the modern society. According to the World Organization of Public Health, 13–15 million inhabitants of our planet are affected by cancer every year. The cancer morbidity increases annually by 3 percents in Armenia, while 390,000 new cases are registered in the Russian Federation. Each fourth and now even the third inhabitant of our planet dies of cancer. The National Cancer Institute of the USA, which is one of the leaders of the fundamental oncology, recommends turning the vector of the anticancerogenic struggle aside the preventive maintenance and considers this orientation as priority from many points of view. Particular importance is given to the earliest revealing of a neoplastic primordium before steady community of cells and tumoral angiogenesis form. The etiology of cancer is much wider than separately taken causal agent and hinges on the most complicated interrelation and hierarchy of many external and internal influences. Our researches with use of fluorescing antibodies to AFP, CEA and Ca-19-9 have shown that they are intensively precipitated on the cultivated malignant cells' surface. Envelopment of cancer cells by oncofetal antigens and fibrin (antifibrinogenic luminescing whey, raster electronic microscopy) creates exclusive conditions for their adaptation and growth. It is the basic mechanism of

tolerance and immunological escape, similarly to pre gnancy when «the maximal immunological most-favored status» to developing fetus (semiallogenic transplant) is provided. The necessity of the specific compensation of the weakened antineoplastic resistibility of people from high oncological risk groups is substantiated. With that end in view we have created Embryonic anti-tumor modulator (EATM), which comprises a wide pool of fetal proteins and proteoglycans of normal embryonic origin. EATM possesses also an antimutagen, interferonogenic, immune-response modulating and antiviral influence in experiment. The EATM injected once a year in extremely low doses brings on a specific sensitization of cells of the mononuclear-macrophage system aimed at devitalization of first cancer cells, permanently originating in adults and older persons. Vaccinal prevention of adults and older persons, who are amenable to becoming of malignant tumour, is argued.

## Electrophysiological study of the spinal cord motoneurons on the model of Parkinson's disease induced by rotenone

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Parkinson disease (PD) is a progressive, age-related neurodegenerative disorder, second in frequency only to Alzheimer's disease. It affects tens of millions of people worldwide. The disease is characterized by poverty of voluntary movements (akinesia), slowness and impaired scaling of voluntary movement (bradykinesia), muscle rigidity and tremor of the limbs at rest. The core, but not exclusive pathology is the degeneration of the dopaminergic neurons in the substantia pars compacta (SNc) of the midbrain that project to the striatum (Hammond et al., 2007). Recent studies focused on the contribution of non-genetic and environmental factors to the development of sporadic form of Parkinson disease. The action of pesticides is of interest among the pointed factors. Particularly the chronic administration of herbicide rotenone is leads to PD-like pathology in rats (Hanan et al., 2004). The study of mechanisms of motor symptoms developmed on spinal level provided by form of synaptic potentiation and depression, caused by unbalance of dopamine (DA) and acetylcholine and prevalence of cholinergic activity (Calabresi et al., 2006). There are arguments presented in favour of the secondarity of pathophysiology of motor complications, based on decrease of DA neurons number (Linazasoro, 2007). The aim of the project was to investigate whether parkinsonism have severe effect on the spinal cord neurons activity. Our main interest was how the motoneurons reveal in condition of rotenone induced parkinsonism, as SNc neurons activity has been studied in the same conditions (Sarkissian et al., 2007). Experiments were carried out on intact rats ($n = 4$), bilateral injected by rotenone into medial forebrain bundle per coordinates of stereotaxic atlas (Paxinos, Watson, 2005). The mathematical programmed analysis of single spike activity of spinal cord (SC) motoneurons (MN) under high frequency stimulation (HFS) SN and sciatic nerve (S) 12 weeks ago was carried out with special software. The activity was expressed as tetanic potentiation (TP) and depression (TD) with subsequent posttetanic potentiation and depression. TP MN of the SC evoked by HFS of SN in norm reached 3-fold increase as compared with prestimulus level. TD expressed as 3-fold decrease. TP on stimulation S revealed in the form of 7-fold increase, and TD in 4-fold decrease. In conditions of rotenone administration poststimulus activity on HFS SN and S ranged as TP 1.8-fold increase, TD 2.3-fold decrease and TP – 3.4-fold and TD – 4-fold, respectively. Thereby, the effects of HFS of S in norm were more significant as compared with those of SN. As regards the excitatory effects of SN and S stimulation in pathology, they appeared almost 2 time decreased. Depressive manifestation of activity in pathology turned out approximately about to norm. There was expressed burst activation of MN, preserved after HFS. We can suppose the preservation of inhibition as a protection and formation of burst activity on base of release of locomotor centers of spinal cord resulted from inhibition of suprasegmental control.

## Treatment with a BH3 mimetic overcomes the resistance of latency III EBV (+) cells to p53-mediated apoptosis

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Inactivation of the p53 tumor suppressor is often observed in Burkitt's lymphoma (BL) cells, due to mutations in the *TP53* gene or overexpression of its negative regulator, MDM2. This inactivation is now considered an essential part of the oncogenic process. On this other hand, Epstein-Barr virus (EBV) is strongly associated with BL and is a cofactor in its development. In previous studies, we showed that nutlin-3, an antagonist of MDM2, activates the p53 pathway in BL cell lines harboring wild type p53, regardless of EBV status. However, nutlin-3 strongly induced apoptosis in EBV (–) or latency I EBV (+) cells, whereas latency III EBV (+) cells were much more resistant. We now show that this resistance to apoptosis is also observed in latency III EBV (+) lymphoblastoid cell lines (LCL), a recognized model for the study of post-transplantation lympho-proliferative disorders (PTLD). We also show that, in latency III EBV (+) cells, Bcl-2 is selectively overproduced, interacts with Bax and thereby prevents Bax activation. The treatment of these cells with the BH3 mimetic ABT-737 disrupts Bax/Bcl-2 interaction and allows Bax activation by nutlin-3. Furthermore, treatment with these two compounds strongly induces apoptosis. Thus, a combination of Mdm2 and Bcl-2 inhibitors might be a useful anti-cancer strategy for diseases linked to EBV infection.

## The ITSN1 interactom and its functions

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**Aim**. Adaptor/scaffold proteins serve as platforms for the assembly of multiprotein complexes and regulate the efficiency and specificity of signalling cascades. Intersectins (ITSNs) are an evolutionarily conserved adaptor protein family engaged in endo- and exocytosis, actin cytoskeleton rearrangements and signal transduction. Abnormalities of ITSN1 expression were associated with the endocytic anomalies reported in Down syndrome brains and early stages of Alzheimer's disease. Overexpression of ITSN1 was shown to enhance huntingtin aggregation and neurodegeneration in Huntington's disease. Moreover, ITSN2 was proposed to be a predictive marker for breast cancer. Although significant progress has been made toward understanding ITSN functions, the role of ITSNs in disease development and regulation of ITSNs function in different cell processes remains unclear. The aim of present study was to uncover novel ITSN binding proteins, to elucidate the ITSN interactom functions and their regulation in a view of ITSN dysfunction under pathological conditions. **Methods**. Immunoprecipitations, pull-down experiments, determination of subcellular localization by direct and indirect immunofluorescence, mass-spectrometry. **Results**. Using mass spectrometry analysis and *in silico* prediction we identified 11 novel protein partners of ITSN1 and ITSN2, among them adaptor proteins Ruk/CIN85, Reps1 and SHB as well as ubiquitin protein ligase Cbl-b and negative regulator of receptor tyrosine kinases Sprouty 2, implicated in regulation of cellular signal transduction. ITSN1 and its shortest alternatively spliced isoform ITSN1-22a form complex with membrane-deforming proteins SGIP1 and amphyphisin, respectively. An interaction of ITSN1 with WASP-interacting protein WIP suggests a possible role of ITSN1 in the regulation of protein complexes during invadopodia formation in cancer cells. We also identified a new neuron-specific protein partner MAP6/STOP involved in microtubule stabilization and generation of synaptic plasticity. Our results demonstrated that ITSN1 could be regulated by ubiquitylation. The Nedd4-like E3 ubiquitin ligase AIP4 is involved in post-translational modification of ITSN1 isoforms. Using *Xenopus* animal model we have demonstrated the role of ITSN2 in the coordinated changes of actin cytoskeleton during early embryonic development. Overexpression of functional domains of ITSN2 in embryos resulted in aberrant phenotypes. The strongest phenotype was produced by the DH-PH tandem, a nucleotide exchange factor for the small GTPase Cdc42. Embryos displayed hyperpigmentation and inhibition of gastrulation movements that were incompatible with survival. **Conclusions**. These findings expand the role of ITSNs as a scaffolding molecules bringing together components of endocytic and signalling complexes. Identified ITSN interacting proteins are involved in signal transduction, actin and tubulin cytoskeleton formation, endocytosis, cell adhesion and migration. The functional characterization of novel established links and their contribution to the pathogenesis is a challenge for future investigations.

## Comparative protective and regenerative effects of hypothalamic proline-rich peptide – PRP-1, cobra venom Naja Naja Oxiana, cerebellar and hypothalamic inhibition on dynamics of vestibular compensation following unilateral labyrinthectomy

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Programming mathematical analysis of Deiters' lateral vestibular nucleus (LVN) ineurons impulse activity to high frequency stimulation (HFS) of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei and cerebellar anterior lobe (lobules I-V) was carried out. The proline-rich peptide (PRP-1) and cobra venom Naja Naja Oxiana (NOX) influence on Deiters' neurons upon dynamics of vestibular compensation after unilateral labyrinthectomy (UL) was tested. Early and late tetanic (TP), – post tetanic potentiation (PTP) and depression (TD and PTD) of neurons to bilateral HFS of PVN and SON was studied. The analysis of spike activity by on-line selection and special program as well as the construction of complex averaged peri-event time and frequency histograms were realized. The increasing of inhibitory and excitatory reactions of Deiters' neurons at early stage of vestibular compensation following PRP-1 and NOX injection reaching the norm at late stage was revealed. In histochemical study revealing of the activity of $Ca^{2+}$-dependent acidic phosphatase (AP) was carried out. In UL animals' the delay of central chromatolysis of Deiters' neurons, leading to deep neurodegenerative pattern of cellular shade, up to its total disappearance was shown. The AP activity after UL and PRP-1 injection exerts more favorable influence in comparison with expressive venom effects. In intact rats HFS of the cerebellar lobules I-V showed predominantly depressor tetanic manifestations of activity (TD), more pronounced at 100 Hz, but with twice smaller prestimulus frequency level of activity, as compared with that at 50 Hz. On $3^{d}$ day after UL at 50 Hz HFS of cerebellar cortex occurred in TD LVN neurons, similar to that of intact rats, but in a more than twice expressed background frequency of activity and usually accompanied by posttetanic inhibitory and excitatory effects. Along with this, there was a profound scarcity of TP to HFS of the cerebellar vermis with a stationary of activity in the norm and after the UL followed by posttetanic potentiation. At $9^{th}$ day after the UL in conjunction with the PRP-1 injection, both on intact and damaged sides depression was not completely reached the level of the norm, but was relatively

greater on the injured side. In general at 3$^{d}$ day after the UL on the injured side TD did not differ from that of 9$^{th}$ day, and TP was slightly lower than that of the 9$^{th}$ day on the intact side. In other words, in conditions of UL activity of the LVN neurons to HFS of cerebellum was characterized by deepening of depression and joining to the tetanic effects also the posttetanic manifestations of activity. The increased depressive effects at 9$^{th}$ day after the UL may not be assumed without the protective effects of PRP-1. The presence in LVN neurons almost equally excitatory and depressive effects, realizing by HFS of hypothalamic paraventricular and supraoptic nuclei at 3$^{d}$ day after the UL, in contrast to the cerebellar effect, indicate their regulatory impact. Moreover, when HFS of both hypothalamic nuclei TP at LVN level, in pathology and the norm is actually was the same, in contrast to depression, which in the pathology was more pronounced than in the norm, indicating the greater expression of the hypothalamic depression compared with cerebellar effects. The inhibitory protection of LVN neurons during UL is suspected. In conclusion, it is possible that in respect of autonomous and motor manifestations of the multiple functioning of the vestibular complex, the mentioned hypothalamic regulation predominantly forms inhibitory output of the cerebellar cortex at the level of LVN.

## Nitric oxide and GLUT1 transporter: targets for prevention of diabetes mellitus complications

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Diabetes mellitus (DM) and its complications cause numerous health and social problems throughout the world. Pathogenic action of nitric oxide (NO) is responsible to a large extent for development of complications of the disease. Abnormal NO production causes blood vessel anomalies in DM patients, as well as diabetic nephropathy, retinopathy, myocardiopathy and hypertension. Thus search for compounds modifying NO production in DM patients appears to be important for development of pharmacological remedies for treatment of DM complications. Dihydropiridines (DHP) appear to be prospective compounds from this point of view. DHP are known to be potent regulators of $Ca^{2+}$ transport, both $Ca^{2+}$ blockers and agonists of $Ca^{2+}$ channels have been identified between these compounds. Numerous novel DHPs were synthesized in the Latvian Institute of Organic Synthesis (IOS), several novel drugs were developed. One of these compounds, cerebrocrast, manifests pronounced antidiabetic activity. The goal of the present work was to study alterations of NO production in streptozotocin model of DM in rats and ability of several DHPs and to normalize NO synthesis. Mildronate, an inhibitor of fatty acid beta-oxidation developed in IOS, which also manifests antidiabetic activity was used as a reference drug. Production of nitric oxide was monitored by means of ESP spectroscopy of Fe-DETC-NO complex. Development of streptozotocin diabetes was followed by increase of NO production in the liver, kidneys, blood and muscles. Cerebrocrast treatment was followed by normalization of NO production in the liver, kidneys and blood. Another DHP etaftoron administered in similar dose was effective in kidneys, blood and muscles. Fenaftoron decreased NO production in the liver of diabetic animals and also kidneys, blood and muscles. Mildronate did modify much NO production in diabetic animals; however it normalized the overexpression of the iNOS gene in kidneys. Elucidation of the DHP action site in the mechanism of the NO production will be the goal of our future studies. As the studied DHP are $Ca^{2+}$ channel blockers constitutive NO synthases seem to be most the likely candidates for targets of these drugs. Glucose transport *via* GLUT1 protein could be one of additional mechanism of the drug anti-diabetic action. To test this hypothesis we have performed a study of GLUT1 gene and protein expression in the course of the streptozotocin diabetes model development and under treatment with glibenclamide, metformin and mildronate. Induction of streptozotocin diabetes provoked increase of both GLUT1 gene and protein expression in kidneys, tretment with glibenclamide, metformin and mildronate treatment produced normalization of the GLUT1 expression levels. This indicates that decrease of GLUT1 in kidneys is a common feature of anti-diabetic activity of different drugs. This effect prevents development of nephrosclerosis in diabetics.

## *PSMC6* gene associations with autoimmune diseases in Latvian population

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**Introduction**. Development of autoimmune diseases is complex process dependent on genetic predisposition, stress and environment. Proteasome dysfunction plays an important role in pathogenesis of autoimmune diseases, thus we found interesting to find out possible associations of proteasomal gene polymorphisms with autoimmune diseases. Eight proteasomal genes (*PSMB5*, *PSMB11*, *PSME1*, *PSME2*, *PSMA6*, *PSMC6*, *PSMA3*, *PSMC1*) are located on human Chromosome 14, distributed in loci14q11, 14q13, 14q23-24, 14q42. *PSMC6* gene is one of representatives of this cluster, it encodes proteasome 19S complex ATPase subunit 6, and possibly it is associated with autoimmune disease pathogenesis. **Goal**. To analyze possible *PSMC6* gene c.86–104A > G and c.86–46C > T SNP associations with autoimmune diseases in Latvian population. **Methods**. DNA samples were obtained from three groups of individuals: healthy controls, juvenile idiopathic arthritis patients and bronchial asthma patients. Samples were amplified, using primer specific polymerase chain reaction, amplification products digested with *DdeI* restrictase, size of digestion products was evaluated by means of electrophoresis. **Results**. Our studies bring evidence that several polymorphisms of *PSMC6* gene are associated with autoimmune diseases or their specific subtypes. Analyzing c.86–104A > G SNP we found correlations with

juvenile idiopathic arthritis and oligoarthritis subtype. In c.86–46C > T SNP case no similar correlations were found. Our data demonstrate, that a linkage disequilibrium exists in Latvian population between c.86–104 A > G and c. 86–46C > > T SNP, this is not the case in other European populations. This indicates a possible recombination event. Work with bronchial asthma patient DNA is on line. **Conclusions**. Our results indicate and association between c.86–104 A > G and autoimmune diseases in the Latvian population. *PSMC6* gene is perspective for further association studies.

## Blood lymphocyte lipids in diverse forms of cancer

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The involvement of cell plasma membrane (PM) lipids in the regulatory mechanisms of various important membrane-bound processes is well documented. These compounds by changing their composition and structure (microdomain) organization respond rapidly to different environmental perturbations, especially leading to the pathologies. However, the role of lipids and enzyme systems of their modification in cancer is not studied in detail. It is well known that compared to normal individuals, patients with several types of cancer have an increased prevalence of regulatory T cells ($T_{reg}$), coexpressing CD4, CD25 and $T_{reg}$ marker FOXP3, in the peripheral blood, tumor microenvironment and tumor draining lymph nodes. The increase in $T_{reg}$ that suppresses autoimmunity may also inhibit the immune response against cancer, as evidenced by improved tumor rejection and survival of tumor-bearing mice that have undergone $T_{reg}$ depletion. It was hypothesized by us earlier that in such intricate system diseases, as human tumors, alterations in PM lipid homeostasis in the peripheral blood crude lymphocytes may possibly represent some information useful for detection and state value of diverse cancers as well as for the personalized correction of chemotherapy treatment modes. We investigated early (5 s) and long-term (60 min) acylation of membrane lipids by different exogenous [$^{14}$C]fatty acids (FA) in lymphocytes isolated from the peripheral blood of patients with diverse forms of leukemia and solid tumors in comparison with the healthy people. Changes in the endogenous activities of some lipid-modifying enzymes in purified PM fraction as well as the mechanisms of diverse lipid second messengers (LSM) formation in [$^{14}$C]arachidonic acid-prelabelled intact lymphocytes were also investigated at different time points (5, 10, 30 and 60 s) following T cell costimulation by anti-CD3/CD28 antibodies. The data obtained provide evidence for reproducible defects in lymphocyte lipids FA content modification and different LSMs generation/utilization processes in studied forms of cancer. Importantly, identical alterations were revealed in all forms of disease mainly at the early (5 s) membrane-bound stage of cell stimulation. We also found that $Ca^{2+}$-dependent activities of phospholipases $A_1$, $A_2$ and C detected in purified PM fraction of lymphocytes were significantly or completely inhibited in all cancer types. Notably, abnormally high lysophosphatidylcholine acylhydrolase activity, which was distinctly individual for each patient, was observed in lymphocytes obtained from cancer patients, but not in normal controls. We conclude that some of the observed alterations are common characteristics of all types of cancer studied, and can lead to the discovery of novel drug targets and new personalized treatment modes of disease (Tadevosyan Y. V. et al., 2008; Patent AM2256, G01N 33/534; Tadevosyan Y. V. et al., 2009, Patent AM2311, G01N 33/53).

## Novel functions for ubiquitylation in nuclear processes

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Concomitantly to their transcription, nascent transcripts undergo a series of orchestrated modifications resulting in the formation of stable and export-competent mRNPs. Importantly, mRNPs are constantly remodeled during their biogenesis with most processing factors being removed prior to transport to the cytoplasm. Several years ago, we proposed that posttranslational modifications by ubiquitin and recognition by ubiquitin binding domains could play a major role in the dynamics of the supramolecular scaffolds involved in mRNA biogenesis. We originally focused on the UBA of the yeast mRNA nuclear export receptor Mex67. This domain is not only required for mRNA nuclear export, but also contributes to the coordination of transcription and mRNA export via its ability to interact with ubiquitylated target proteins. Our recent data indicate that histone ubiquitylation also influences the appropriate assembly of mRNP. Monoubiquitylation of histone H2B indeed controls integrity and stability of the cleavage and polyadenylation factor, which itself monitors recruitment of nuclear export factors suggesting a direct relationship between histone modifications and posttranscriptional events. Finally, ubiquitylation processes also regulate dissociation of some nuclear factors prior transport to the cytoplasm. Nuclear export of mRNP occur through NPCs, large channels anchored in the nuclear envelope. We determined the ubiquitylation status of all the nucleoporins in yeast and found that ubiquitylation of the NPC might be associated to broad functions of the NPC. In particular, we identified an unexpected role for ubiquitylation of Nup 159, a nucleoporin located at the cytoplasmic face of the NPC, in cell cycle progression.

## SAYP and Brahma are important for «repressive» and «transient» Pol II promoter-proximal pausing

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Drosophila SAYP the homologue of human PHF10/BAF45a is metazoan coactivator that is associated with Brahma complex. SAYP is essential for the recruitment of TFIID and Brahma on promoters of actively transcribed genes. Here we studied the role of SAYP in DHR3 activator driven transcription of *ftz-f1* gene encoding NR, the member of ecdysone cascade. The repressed state of the *ftz-f1* is formed by transcription stalling and is characterized by pre-recruited Pol II complex on promoter and Pol II promoter-proximal pausing around 1.5 kb downstream the start of transcription. SAYP and Brahma form «nucleosome barrier», the region of high nucleosome density ahead of paused Pol II. RNAi knockdown of SAYP leads to Brahma removel, elimination of nucleosome barrier and to the synthesis of the full-length transcript. During active transcription Pol II is also paused at 1.5 kb downstream promoter. In line with general view this promoter-proximal pausing correlates with Pol II CTD Ser2 phosphorylation. Knockdown of SAYP represses the Ser2 CTD phosphorylation, interferes with transcription elongation and increases the time interval in which transcription maximum is reached. Thus SAYP as a part of Brahma complex participates both in repressive and transient Pol II pausing.