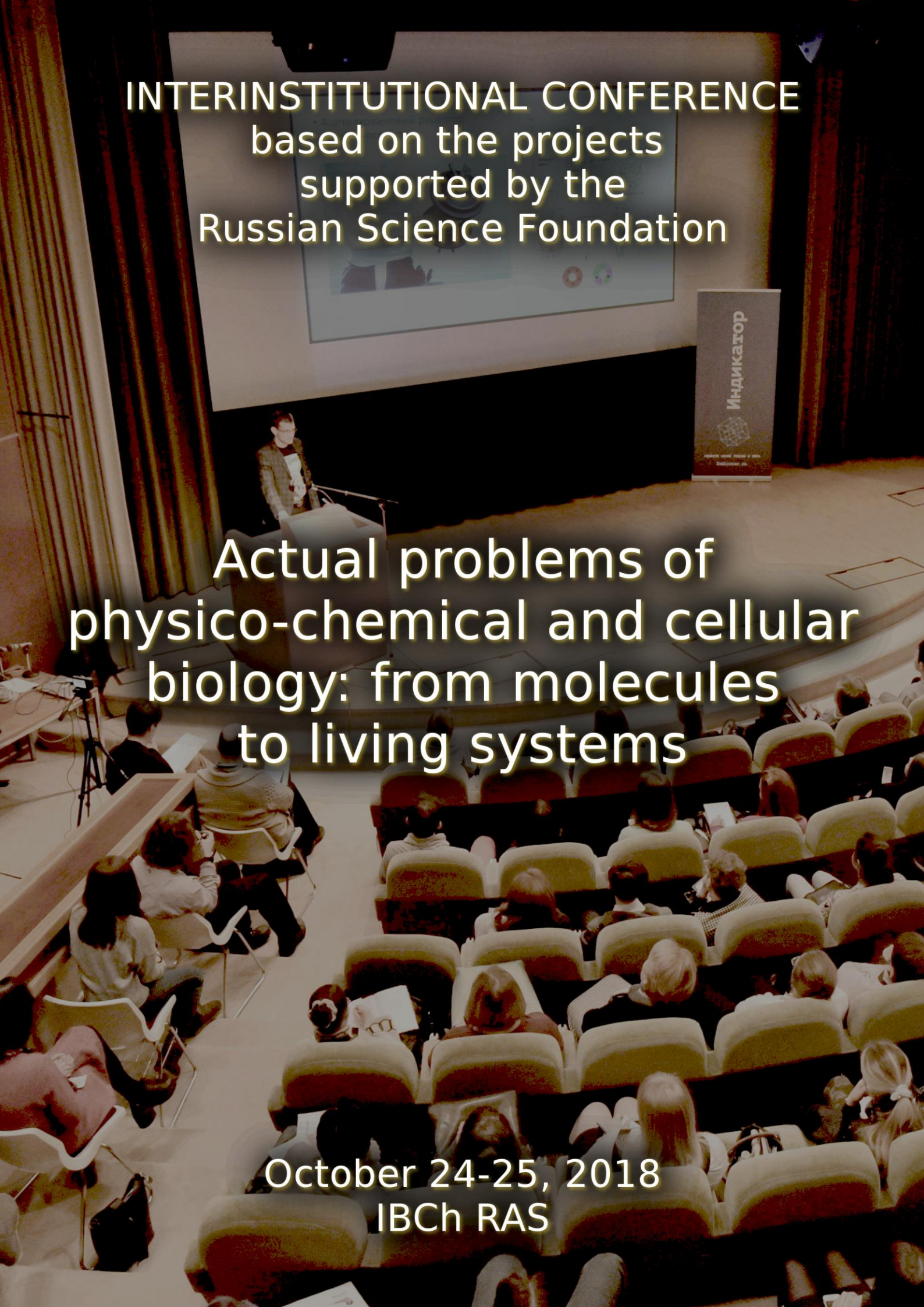

INTERINSTITUTIONAL CONFERENCE
based on the projects
supported by the
Russian Science Foundation

Actual problems of physico-chemical and cellular biology: from molecules to living systems

October 24-25, 2018
IBCh RAS

INTERINSTITUTIONAL CONFERENCE

based on the projects supported by the Russian Science Foundation

RESEARCH CENTER OF BIOTECHNOLOGY

ACTUAL PROBLEMS OF PHYSICO–CHEMICAL AND CELLULAR BIOLOGY: FROM MOLECULES TO LIVING SYSTEMS

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OCTOBER 24-25, 2018, IBCh RAS

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PROGRAM

24 October 2018 (Wednesday)		
8:15	REGISTRATION	
8:45	CONFERENCE OPENING	
Chair: E.D. Sverdlov		
9:00 – 9:35	A.G. Gabibov	Proteins and peptides in the post-genomic era. structural and functional research for solving fundamental problems and directed design of innovative medicines
9:35 – 10:10	V.T. Ivanov	Systemic study of the biodiversity of peptides (peptidomics)
10:10 – 10:40	A.N. Tomilin	Basic and applied aspects of pluripotent stem cells
10:40 - 10:55	*Coffee Break*	
Chair: T.V. Ovchinnikova		
10:55 – 11:15	N.A. Mikhailova	Molecular-cell technologies for treatment of socially significant diseases: perspective portrait of the INC RAS on the results of integrated program's implementation
11:15 – 11:50	V.G. Debabov	Microbes and electricity
11:50 - 12:15	E.S. Kornilova	Comparative analysis of EGF receptor functioning in stem and transformed human cells
12:15 - 12:40	E.V. Bocharov	Structural basis of signal transduction *via* transmembrane domains of bitopic receptors
12:40 – 13:00	I.V. Smirnov	Social important biocatalists: screening, generation and structural-functional analysis
13:00 - 14:00	*Lunch*	
Chair: E.A. Bonch-Osmolovskaya		
14:00 - 14:35	T.V. Ovchinnikova	Peptide factors of innate immunity system
14:35 - 15:00	N.V. Ravin	Metagenomic analysis as the instrument for studying uncultured microorganisms
15:00 - 15:25	I.V. Yampolsky	Chemistry of light: fluorescent proteins, luciferins, luciferases
15:25 - 15:50	O.G. Lyublinskaya	HyPer-based measurements of stem cell redox parameters

15:50 - 16:00	M.G. Khotin	Center of cell technologies - from development of cellular products to their production
16:00 - 16:15	*Coffee Break*	
Chair: I.V. Smirnov		
16:15 - 16:30	Y.A. Lomakin	Exposure to the Epstein-Barr viral antigen induces affinity-maturated anti-myelin antibodies
16:30 - 16:45	I.V. Kublanov	Extremophilic archaea as a source of novel glycosidases
16:45 - 17:00	V.F. Lazarev	Glyceraldehyde-3-phosphate dehydrogenase as a new biological target
17:00 - 17:10	A.N. Kaminskaya	Design of new diagnostic marker for multiple sclerosis based on fluorescence peptide-based sensor of the myelin-specific abzymatic activity
17:10 - 17:20	A.V. Selenina	The functional significance of the components of the ubiquitin-proteasome system in the reprogramming and differentiation of mammalian cells

25 October 2018 (Thursday)

Chair: I.V. Guzhova		
9:00 – 9:35	E.D Sverdlov	Master regulatory proteins and genes responsible for development and carcinogenesis as exemplified by pancreas
9:35 – 10:10	N.N. Nikolsky	Stem cells as a cell technology basis
10:10 – 10:45	B.A. Margulis	Chaperonic drugs for the therapy of oncological and neurodegenerative pathologies: development and aprobation
10:45 - 11:00	*Coffee Break*	
Chair: N.V. Pimenov		
11:00 – 11:35	N.V. Bovin	Dynamics of glycolipids in cell membranes and model systems
11:35 - 12:00	A.P. Sinitsyn	Bioconversion of lignocellulosic feedstocks
12:00 - 12:25	I.I. Suvorova	Role of autophagy in the regulation of pluripotency and self-renewal of embryonic stem cells
12:25 - 12:50	M.B. Tereshina	Genes that disappeared in evolution, as regulators of brain development and regeneration

12:50 - 14:00	*Lunch*	
Chair: E.S. Kornilova		
14:00-14:35	A.G. Petrenko	Structure and mechanisms of activation of receptor tyrosine kinases
14:35 - 15:00	N.M. Yudintceva	Application of the tissue engineering and cell therapy for the reconstruction of urinary organs
15:00-15:25	S.N. Gavrilov	Novel genomic determinants of respiratory metabolism in extremophilic archaea
15:25 - 15:50	A.V. Borodkina	Human endometrial stromal cells senescence: typical features and possible concequences
15:50 - 16:20	A.A. Belogurov	Journal "Acta Naturae" - the prospect of working with the Russian scientific audience
16:05 - 16:20	*Coffee Break*	
Chair: A.A. Belogurov		
16:20 - 16:35	A.V. Stepanov	Selection of ligands for personalized CAR-T therapy of leukemia and lymphoma
16:35 - 16:50	T.Yu. Starkova	Linker proteins H1 and HmgB1 in chromatine of embryonic stem cells of the mouse
16:50 - 17:00	A.E. Mamedov	Presentation of myeline autoantigenes on the major histocompatibility complexes class II catalyzed by HLA-DM
17:00 - 17:10	E.Y. Kochetkova	Targeted elimination of senescent tumor cells through suppression of MEK/ERK pathway
17:10 - 17:20	R.S. Kalinin	Universal CAR-T for therapy of oncological diseases based on the barnase-barstar complex
17:20	CONFERENCE CLOSING	

ORAL PRESENTATIONS

PROTEINS AND PEPTIDES IN THE POST-GENOMIC ERA. STRUCTURAL AND FUNCTIONAL RESEARCH FOR SOLVING FUNDAMENTAL PROBLEMS AND DIRECTED DESIGN OF INNOVATIVE MEDICINES

A.G. Gabibov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Combinatorial chemistry and biology became a hallmark of life science in XXI century.
We developed microfluidicapproaches for screening microbiota, biocatalytic clones, antibody diversity and specific chimeric antigen receptors (CARs). We report the development of a novel platform to significantly enhance the efficacy and safety of Follicular lymphoma treatment. Combinatorial autocrine-based selection is used to rapidly identify specific ligands for these B cell receptors on the surface of FL tumor cells. The selected ligands are used in a CAR-T protocol. Ultrahigh¬throughput screening techniques can identify unique functionality from millions of variants. To mimic the natural selection mechanisms that occur by compartmentalization in vivo, we developed a technique based on single-cell encapsulation in droplets of a monodisperse microfluidic double water-in-oil-in-water emulsion .The combination of droplet generating machinery with FACS followed by next-generation sequencing and liquid chromatography-mass-spectrometry analysis of the secretomes of encapsulated organisms yielded detailed genotype/phenotype descriptions. This platform was probed with uHTS for biocatalysts anchored to yeast with enrichment close to the theoretically calculated limit and cell-to-cell interactions. The versatility of the platform allowed the identification of bacteria, including slow-growing oral microbiota species that suppress the growth of a common pathogen. We developed a novel platform for maturation of antibody molecule insilica. In vitro selection of antibodies from large repertoires of immunoglobulin (Ig) combining sites using combinatorial libraries is a powerful tool, with great potential for generating in vivo scavengers for toxins. We approached this goal using quantum mechanics/molecular mechanics (QM/MM) calculations to achieve maturation in silico.

Supported by the Russian Science Foundation grant № 14-50-00131.

SYSTEMIC STUDY OF THE BIODIVERSITY OF PEPTIDES (PEPTIDOMICS)

V.T. Ivanov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Peptides comprise an extremely diverse, ubiquitous class of low molecular natural products participating in a large number biochemical processes that take part in living organisms. In the present communication peptidome is considered as a derivative of proteome formed at the final stage of transformation of primary genetic information:

genome → transcriptome → proteome → peptidome

In this work analysis has been carried out of a series of biological objects by means of high resolution time-of-flight mass spectrometry coupled with high performance liquid chromatography. Interpretation of experimental spectral data was done by comparison with the spectra predicted for amino acid sequences following from genomic data banks.

The first plant peptidome was documented on the example of *Physcomitrella patens* moss. Over 20000 individual peptides were identified and assigned to respective protein precursors. Over 140 were shown to originate from short open reading frames of moss genome. Functional activity has been demonstrated for a number of peptides in specific plant test systems.

The second part of the talk will be related to biomedical applications of peptidomic research. Peptide composition of cerebrospinal fluid of patients with infectious meningitis and Guillain-Barre syndrome provided valuable information on pathphysiological mechanisms of those diseases. Peptidomic analysis of human blood plasma and serum revealed reproducible differences between samples taken from patients with oncological disorders (ovarian cancer, colorectal cancer and leukemia). These data might open new horizons for oncodiagnostics. For the first time it was shown that our blood contains essential number (5-10% of the total over 5000) of peptides generated by human microbiota. The possible biological role of such peptides will be discussed.

The work is supported by RSF, project №14-50-00131 and by RFBR, project №17-00-00461.

BASIC AND APPLIED ASPECTS OF PLURIPOTENT STEM CELLS

A.N. Tomilin

Institute of Cytology of the Russian Academy of Sciences, Saint-Petersburg

Pluripotent stem cells have advanced to the cutting edge of cell biology and, likely, the whole biomedicine because of the tremendous potential of these cells in disease modelling and cell-tissue-replacement therapies, inferred from unique features of these cells, which are unlimited self-renewal capacity and the ability to differentiate into all cell types of adult organism. During my talk I will present our recent results which concern both fundamental and applied aspects of pluripotent stem cell.

The studies presented in the talk have been supported by the RSF, grant № 14-50-00068.

MOLECULAR-CELL TECHNOLOGIES FOR TREATMENT OF SOCIALLY SIGNIFICANT DISEASES: PERSPECTIVE PORTRAIT OF THE INC RAS ON THE RESULTS OF INTEGRATED PROGRAM'S IMPLEMENTATION

N.A. Mikhailova

Institute of Cytology, Russian Academy of Sciences, Saint-Petersburg

The integrated development program of the Institute of Cytology RAS, implemented within the framework of the RSF grant № 14-50-00068, consists of three interrelated directions aimed at solving scientific problems that are promising for the treatment of socially significant diseases: "Stem cells as the basis of cellular technologies", "Effective programs of cell aging" and "Transformed and cancer stem cells as targets for antitumor agents".

The goals and objectives of the program are aimed at solving the scientific priorities of cell biology, developing new methods for the therapy of diseases based on fundamental research of normal, stem cells and tumor cells.

The results demonstrate the significant impact of the integrated program on the development of the INC RAS, namely: raising the level of fundamental and applied research, strengthening human resources, improving the material and technical base, developing international cooperation, creating knowledge-intensive products, demanded by the economy and society.

The maximum number of project participants is 136, including 19 post-graduate students of the INC RAS. The proportion of young scientists is 66.7%. During the project implementation more than 160 of WoS and Scopus articles (September 2018) have been published, 10 PhD dissertations have been defended. The results of the works are presented at prestigious scientific events (more than 60 oral presentations). The INC RAS initiated and organized a regular international conference "Cell technologies at the edge: research and practice" (2016, 2018), an annual mini-symposium "Molecular oncology", a conference "Biomedical cell products - development, implementation, production".

Significant success is the increase of applied research level, on the creation of new cell products for the treatment of diseases, as well as the GLP standard preclinical studies of drugs, based on cell test models *in vitro*.

The key achievement of the project is the organization of the Cell Technology Center, the creation of which eliminates the main long-term imbalance of the INC RAS – this makes it possible to transfer biomedical technologies to practical health care. The Center is designed according to the international GMP standard, equipped with world-class equipment, including a robotic complex for the BMCP production and their preclinical and clinical research, registration and market entry. This is the first production site in Russia among the institutes of the Russian Academy of Sciences that corresponding to the requirements of the 180-Federal Law and GMP and GTP standards.

All the works were supported by the Russian Science Foundation grant № 14-50-00068.

MICROBES AND ELECTRICITY

V.G. Debabov

State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center" Kurchatov Institute", Moscow

Bacteria can transfer electrons into environment with the help of carrier molecules or directly onto the electrode solid surface. A limited group of bacteria with this transfer ability is called electrogene. This phenomenon of electron transfer to the anode is the basis of microbial Fuel Cell (MFC) functioning. This presentation will describe composition and optimization of MFC and molecular mechanisms of electron transfer to electrode. An opposite process – transfer of electrons from cathode to bacterial cell – results in electro-biosynthesis. In the process that occurs in the device called Microbial Electrolysis Cell, microorganisms on the cathode fix CO2 while synthesizing organic compounds (most often acetate). This presentation will consider electro-biosynthesis parameters and conditions and the mechanism of CO2 fixation by electro-active bacteria. Electricity generation with the help of microorganisms and CO2 fixation by electro-biosynthesis are new technologies that are ecologically friendly and are well compatible with water recycling and with the use of renewable energy sources (solar and wind). This presentation will discuss the ways of perfecting these processes with special attention to optimizing the microorganism properties.

COMPARATIVE ANALYSIS OF EGF RECEPTOR FUNCTIONING IN STEM AND TRANSFORMED HUMAN CELLS

M. Kharchenko[1], R. Kamentseva[1], B. Kosheverova[1], M. Istomina[1,2], O. Semyonov[1,3], I. Litvinov[1], A. Salova[1], T. Belyaeva[1], E. Kornilova[1,2,3]

[1]Institute of Cytology, Russian Academy of Sciences, Saint-Petersburg;
[2]Saint-Petersburg Polytechnikal University, Saint-Petersburg;
[3]Saint-Petersburg State University, Saint-Petersburg

The system of Epidermal Growth Factor and its Receptor is a popular model in studies of signal transduction, proliferative signal in particular, and regulated endocytosis along lysosomal degradative pathway. One of the reasons for this is the existence of immortalized cell lines with a high level of receptor protein, which provides reliable detection of ligand-receptor complexes. However, these cell lines were isolated, as a rule, from tumors, and their growth does not depend on EGF (HeLa cells, etc). Furthermore, overexpression of EGF receptor has been shown to correlate with malignant transformation, and drugs based on antibodies to EGF receptor or inhibitors of receptor tyrosine kinase are already used in the clinic.

Obviously, search for not-transformed EGF-dependent cell lines is extremely important for understanding physiological significance of EGF-stimulated processes. Endometrial MSCs are primary culture of normal cells, actively proliferating and capable of differentiation. It is believed that a set of factors other than EGF is responsible for high proliferative activity of MSC. However, we found that the EMSC lines obtained at the Institute of Cytology RAS express a high level of EGF receptor, comparable to that in the cell lines originated from tumors. Moreover, presence of EGF in the medium significantly accelerated proliferation of self-renewing EMSC. Short-term effect of EGF on EMSC leads to a long-lasting stable activation of MAP kinases and Akt, whereas in HeLa cells it is transient.

Analysis of intracellular behavior of EGF receptor complexes in EMSC has shown that they are endocytosed and undergo the same pathway to lysosomes as in HeLa cells, although in EMSC organization of vesicular apparatus and tubulin cytoskeleton has some peculiarities. Thus, in HeLa cells, EGF receptor complexes in endosomes first co-localize with APPL1 / 2 signal protein involved in activation of early response genes, and are then associated with early endosomes carrying EEA1 protein responsible for fusion and endosome maturation on the way to lysosomes . On the contrary, in EMSC endosomes may carry all three proteins simultaneously, but in segregated domains, up to translocation to perinuclear region. Heterogeneity of responses of EMSC lines is discussed.

This work was supported by the Russian Science Foundation, grant № 14-50-00068.

STRUCTURAL BASIS OF SIGNAL TRANSDUCTION *VIA* TRANSMEMBRANE DOMAINS OF BITOPIC RECEPTORS

E.V. Bocharov, D.M. Lesovoy, O.V. Bocharova, A.S. Urban, S.A. Goncharuk, K.S. Mineev, K.D. Nadezhdin, P.E. Volynsky, R.G. Efremov, A.S. Arseniev

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Membrane bitopic receptors of type I (e.g. receptor tyrosine kinases and receptors associated with JAK protein kinases) are involved in development regulation and homeostasis of human organism, playing central role in cell proliferation, differentiation and adhesion. A necessary condition of biochemical signal transduction through a plasmatic membrane is lateral dimerization (and/or an oligomerization) of bitopic receptors, either ligand-dependent or ligand-independent, accompanied by conformational rearrangements of all the domains of the receptor, including the α-helical transmembrane domains (TMD). During the Project, the main aspects of structure-function relationship for bitopic receptors of epidermal growth factors, fibroblast growth factors and growth hormone are considered. We determined the number of dimeric conformations of the TMDs in different membrane-mimicking environments (detergent micelles and lipid bicelles) using high-resolution NMR spectroscopy combined with MD-relaxation in explicit lipid bilayer. Fine adaptation of intermolecular polar and hydrophobic contacts that we found to accompany the TMD dimer formation suggests that certain membrane properties can govern the TMD helix-helix packing and, thus, their alteration can trigger the receptor state. Pathogenic transmembrane mutations found for the bitopic receptors relatives are located in narrow regions (so-called ‘hot spots’) within the specific TMD helix-helix interfaces assuming that the intermolecular interactions inside membrane are important for receptor dysfunction in human organism. In the light of the obtained biophysical and biochemical data it is shown that functioning of bitopic receptors is mediated not only by protein-protein interactions, but also by the state of the surrounding lipid environment as one of the main components of a self-consistent signal transduction system. The novel principles of intercellular signal transduction mediated by lipid membrane supplement the molecular mechanisms of bitopic receptors functioning proposed earlier and explain a number of the paradoxes observed upon activation of wild-type receptor and the receptor with pathogenic transmembrane mutations.

The work is supported by the Russian Science Foundation grant № 14-50-00131.

SOCIAL IMPORTANT BIOCATALISTS: SCREENING, GENERATION AND STRUCTURAL-FUNCTIONAL ANALYSIS

S.S. Terekhov, Yu.A. Mokrushina, A.M. Kudzhaev, A.G. Andrianova, M.Yu. Zakharova, S.O. Pipiya, A.S. Nazarov, D.V. Danilov, A.A. Zinchenko, E.N. Kaliberda, TV Rotanova, L.D. Rumsh, I.V. Smirnov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Biocatalytic function is one of the most advanced functions inherent in living systems. This function is realized in a limited set of biological objects, the bulk of which are protein molecules - enzymes and antibodies. Biocatalytic activity is widely used in various fields of modern industrial and pharmaceutical biotechnology. An important role is played by enzymatic processes in the development of various pathological processes. In our work, we developed a platform for high-throughput screening of functionally active biocatalysts (enzymes and antibodies) and their inhibitors using a yeast display system. This technology is universal and allows you to search for compounds that have any functional activity, including antibiotic.

During the project implementation, studies were carried out to investigation of the catalytic mechanism of the socially significant and dangerous enzymes. In particular, we studied the properties of the deletion form of Lon-proteinase, one of the key components of a protein quality control system that maintains the integrity of the cellular proteome. The studies of IgA1 protease, one of the pathogenicity factors of microorganisms, were carried out, which results in weakening the immunity of mucous membranes. This manifests itself in increasing the adhesion of bacteria to the epithelium and colonizing them with mucous membranes. The mechanism of resistance of HIV-1 protease to the majority of inhibitors of the first generation was investigated. Thus, the results obtained during the implementation of the project have prospects for applied application for protecting the organism from pathogenic high-molecular compounds.

The work is supported by Russian Science Foundation grant № 14-50-00131.

PEPTIDE FACTORS OF INNATE IMMUNITY SYSTEM

T.V. Ovchinnikova, S.V. Balandin, I.V. Bogdanov, I.A. Bolosov, A.A. Emelianova, S.K. Zavriev, A.A. Kalashnikov, D.V. Kuzmin, M.B. Marggraf, D.N. Melnikova, P.V. Panteleev, E.A. Rogozhin, S.V. Sukhanov, S.V. Sychev, A.A. Tagaev, E.I. Finkina, Z.O. Shenkarev, V.T. Ivanov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Endogenous antimicrobial peptides (AMPs) are evolutionally ancient factors of the innate immunity of multicellular organisms. AMPs play a key role in the defense against infections. Every biological species develops its own set of AMPs that provide an effective protection against pathogenic microflora. Peptides of various structures and similar protective functions were isolated from tissues of invertebrates, vertebrates, and plants. AMPs are active against bacteria, yeast and filamentous fungi, protozoa, and enveloped viruses. AMPs differ in their spectra of antimicrobial activities and are able to complement and reinforce each other. AMPs are also characterized by a wide variety of mechanisms of their action that involve not only disruption of target cell membranes, but effect specific inhibition of metabolism processes via interactions with specific molecules on the surface or within the cell. Endogenous AMPs can also play a role of mediators of the immune system (immunomodulators) by activation of phagocytosis and chemotaxis, and by stimulation of production of cytokines. Studies of structures, biological functions and mechanisms of action of various animal and plant AMPs were fulfilled under the project. Biological significance of AMPs antimicrobial properties *in vitro* was examined. Main mechanisms of AMPs action toward microbial membranes were investigated, and principles of selectivity of interactions were analyzed. Search and investigation of animal and plant AMPs as host defense factors provide a better understanding of general mechanisms of innate immunity and open the way to development of novel medicines.

This work was supported by the Russian Science Foundation, project No. 14-50-00131.

METAGENOMIC ANALYSIS AS THE INSTRUMENT FOR STUDYING UNCULTURED MICROORGANISMS

N.V. Ravin

Institute of Bioenginieering, FRC Biotechnology Russian Academy of Sciences, Moscow

In XX century microbiology was based on the studies of pure microbial cultures, and that made possible the characterization of their metabolic pathways and biochemical processes, as well as the estimation of their environmental impact. Development and application of molecular methods made it clear that more than 99% of microorganisms present in natural ecosystems could not be cultivated in laboratories. Of 100 known prokaryotic phyla about a half has no cultivated representatives. At present, genomic analysis makes the main instrument for studying "uncultured" microorganisms, with the metagenomic analysis and single-cell genomics being the major approaches used. The first approach is based on the analysis of integrated microbial community genome (metagenome). The metagenomic data analysed by means of bioinformatic tools help to characterize the community composition and genetic potential, as well as to reveal single microbial genomes including those of uncultured ones.

Using metagenomic approach, we investigated microbial communities of extreme ecosystems. Deep-subsurface water horizons located at the depth of 2 to 3 km in Western Siberia became our main research object. These ecological niches are characterized by anaerobic conditions, high pressure and elevated temperature. Analysis of these metagenomes showed that in many case less than a half of organisms represented known species, while the rest belonged to uncultured groups some of which were not known even by their 16S rRNA sequences. Analysis of metagenomes allowed us to obtain about 50 bacterial genomes including those of "uncultured" phyla *Aminicenantes, Armatimonadetes, Riflebacteria* and BRC1. Sequencing of single-cell genomes from microbial communities of deep-subsurface and Lake Baikal sediment samples allowed us to obtain the genomes of bacteria representing phyla *Aerophobetes, Aminicenantes, Atribacteria, Caldiserica, Parcubacteria, Microgenometes* and NC10.

The work of our group was supported by the Russian Science Foundation grant № 14-14-01016.

CHEMISTRY OF LIGHT: FLUORESCENT PROTEINS, LUCIFERINS, LUCIFERASES

I. Yampolsky, Y. Bolt, A. Gorokhovatsky, A. Kotlobay, T. Mityushkina, K. Palkina, V. Petushkov, T. Chepurnykh, A. Bubyrev, V. Solovyeva, I. Myasnyanko, E. Shakhova

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Many living organisms emit light, a phenomenon named bioluminescence. There are estimated to exist ~ 40 different chemical mechanisms underlying the generation of "cold light". The energy required for light production is generated by the oxidation of a small organic molecule, luciferin, catalyzed by a specific enzyme, luciferase. More than 100 species of bioluminescent higher fungi are known. During the last few years, the international research group led by the speaker reported elucidation of two novel luciferins and their mechanisms of action. In 2016 the same group identified and cloned fungal luciferase and the enzymes of luciferin biosynthesis. Discussed are structure elucidation of earthworm and fungal luciferin, cloning of fungal bioluminescnce enzymes, light emission mechanism and perspectives of practical applications of fungal bioluminescence.

This work was supported by the Russian Science Foundation grant № 14-50-00131.

HYPER-BASED MEASUREMENTS OF STEM CELL REDOX PARAMETERS

O.G. Lyublinskaya

Institute of Cytology Russian Academy of Sciences, Saint-Petersburg

Reactive oxygen species (ROS) are normal products of aerobic metabolism. ROS mediates signaling processes in cells and, simultaneously, are harmful intracellular pro-oxidants. Dual function of ROS in cell, as a signaling molecules or toxins, is realized due to the difference in the concentration and duration of ROS pulses, as well as in the localization of ROS sources. However, the task of numerical determination of intracellular ROS concentration is still a challenge. For example, existing estimations of intracellular H_2O_2 concentrations, an effective cellular pro-oxidant, are scarce (1 – 700 nM, Stone and Yang, 2006) and based exclusively on the analysis of extracellular H_2O_2 generation. The data on rate constants of H_2O_2 elimination by normal human cells are also quite limited yet. The present work is aimed at characterization of basic redox parameters of cultivated stem and differentiated human cells with the use of HyPer, genetically encoded H_2O_2 biosensor. To perform numerical evaluations, we analyzed the kinetics of the biosensor oxidation during incubation of cells with hydrogen peroxide. By fitting these data with theoretical equations describing the kinetics of oxidation/reduction of intracellular proteins (Brito and Antunes, 2014) we derived the key redox parameters of cells, including the basal intracellular H_2O_2 concentration and rate of H_2O_2 elimination by cells.

Research is supported by the Russian Science Foundation, grant № 14-50-00068.

CENTER OF CELL TECHNOLOGIES - FROM DEVELOPMENT OF CELLULAR PRODUCTS TO THEIR PRODUCTION

M. Khotin

Institute of Cytology, Russian Academy of Sciences, Saint-Petersburg

New technologies based on human living cells are gradually entering practical health care all over the world and allow successfully combating previously untreatable pathologies. The Institute of Cytology Russian Academy of Sciences is a pioneer and one of the leaders in the Russian Federation in the research and transfer of cell products. Such studies have been conducted at the Institute for more than 25 years. The most successful of them since 2006 are used to restore extensive damaged skin and treat trophic ulcers. However, previously there were obstacles for the development of such studies. The condition for successful application development is, among other things, the possibility of their implementation. An essential requirement for this in the case of cell technologies is the availability of an appropriate infrastructure - not only research laboratories, but production facilities, biobanks, the introduction of the necessary quality management standards.In implementation of the complex program for the development of the organization, implemented at the INC RAS with the support of the Russian Science Foundation (RSF) in the period 2015-2018, it was possible to overcome this restriction and update the infrastructure necessary for performing research, to create an experimental production of biomedical cellular products. The new production is unique for Russia and the first in the structure of the Academy of Sciences. Thus, by 2017 a new structure has been created - the Center of cell technologies (CCT).As part of the CCT the stages of the development of new cell products, tissue engineering structures, their components (extracellular matrix proteins and biopolymer membranes), preclinical research and production were structurally separated and introduced. Use of robotic technologies of cultivation, biobanking and cell analysis, which allow to ensure standartization and high productivity of all stages of work. Implemented GxP system of good practices: GLP (good laboratory practice) for preclinical research and GMP (good manufacture practice) for production. The Center of Cell Technologies is a modern structure, corresponding to the most international standards, which is capable of providing the entire cycle of innovative development in the field of cellular technologies from R&D to production.

Work is supported by the Russian Science Foundation, grant № 14-50-00068.

EXPOSURE TO THE EPSTEIN-BARR VIRAL ANTIGEN INDUCES AFFINITY-MATURATED ANTI-MYELIN ANTIBODIES

Y.A. Lomakin[1,2], A.A. Belogurov[1,2,3], A.G. Gabibov[1,3]

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[2]Kazan Federal University, Kazan;
[3]Lomonosov Moscow State University, Moscow

Multiple Sclerosis (MS) is an autoimmune chronic inflammatory disease of central nervous system (CNS). Cross-reactivity of neuronal proteins with exogenous antigens is considered as one of the possible mechanisms of MS triggering. Previously we showed that monoclonal myelin basic protein (MBP)-specific IgGs are cross-reactive with Epstein-Barr virus (EBV) protein LMP1. Here we report evidence that exposure of mice to LMP1 may result in induction of myelin-reactive autoantibodies *in vivo*. Only part of such anti-MBP antibodies are cross-reactive towards LMP1 but majority is occurred as a result of epitope spreading. We suggest that chronic contact with viral antigen rather than multiple rapid exposures to it is more sufficient in inducing switch of B cells from viral to myelin antigen. Moreover, even in inbred animals being almost identical in terms of genome, such switch was observed only in 20% of animals, indicating that this evidence is occurred by a chance rather than systematically. Our findings provide novel insights into still enigmatic link between EBV infection and MS development, determining several criteria that are beneficial for induction of self-reacting antibodies on the background of viral infection.

Current study was supported by Russian Science Foundation grant № 17-74-30019.

EXTREMOPHILIC ARCHAEA AS A SOURCE OF NOVEL GLYCOSIDASES

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Archaea, representing a third domain of life, are being a significant component of modern Earth's microbiota. While detected in all natural environments, in many of them they are being in minority in comparison with bacteria. On the contrary in extremal habitats (hydrothermal springs, hypersaline brines etc.) archaea outnumber bacteria and played the major role in biological processes, occurred there. Their deep rooting on the tree of life as well as extremal environments they are inhabited determine their unique metabolic pathways, enzymes, transporters, regulatory mechanisms etc. Besides that, the extremal life style reflects high stability of their molecules and enzymes in particular, which often named as extremozymes. All these specify high biotechnological potential of extremophilic Archaea, first of all in the fields of metabolic engineering, synthetic biology, and so-called white biotechnologies and 2nd generation biofuels (biofules from lignocelluloses, xylan and their derivatives) in particular.

The aim of this work is to search the novel extremophilic (first of all thermophilic and haliphilic) archaea, capable of hydrolyzing raw material polysaccharides for 2nd generation biofulels production, to determine the mechanisms of polysaccharide degradation and utilization of the monomers in central carbohydrate metabolism. A special emphasis was put on detection and characterization of novel highly stable glycosidases (cellulases, xylanases etc.), involved in this process. In the course of the work we have isolated more than ten strains of hyperthermophilic and halophilic archaea, growing on polysaccharides, reconstructed *in silico* the central carbohydrate metabolism of few of them, discovered a unique multi-domain cellulase from hyerthermophilic archaeon *Themococcus* sp., obtained several cellulases, active at 4M NaCl from novel halophilic archaea and a lot more.

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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AS A NEW BIOLOGICAL TARGET

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the key enzyme of the glycolytic cycle. Nevertheless, in recent years, many other functions of this protein have been demonstrated by scientists. GAPDH is involved in the DNA repairing, the response of cells to oxidative stress, the enzyme is involved in apoptotic signaling. GAPDH is sensitive to changes in the "redox status" of the cell, which can lead to denaturation of the enzyme. Similar events are characteristic for both neurodegenerative and oncological diseases.

Thus, the appearance of beta-amyloid in neurons or in the intercellular space or the development of neuroinflammatory processes inevitably leads to oxidative stress. Oxidation of the enzyme in neurodegenerative diseases leads to the formation of cytotoxic aggregates including GAPDH. Such aggregates are able to enter the intercellular space and cause the death of surrounding cells. On the other hand, tumor cells in cancer diseases are forced to exist in conditions of hypoxia. The change in the homeostasis of reactive oxygen species in the cell affects many physiological processes, including influencing the ability of cancer cells to proliferate and migrate. Our recent studies have demonstrated the role of GAPDH in the response of tumor cells to hypoxia. We found that the enzyme in response to hypoxia denatures, and denaturation of the enzyme, in turn, leads to a decrease in cell viability and increase sensitivity to antitumor drugs. Such data led us to the idea that for the therapy of oncological diseases it is necessary to reduce the resistance of GAPD to oxidation and denaturation.

Thus, the studies that we have conducted over the past few years prove that the GAPDH protein is an extremely promising target for therapeutic intervention in both neurodegenerative and oncological diseases.

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DESIGN OF NEW DIAGNOSTIC MARKER FOR MULTIPLE SCLEROSIS BASED ON FLUORESCENCE PEPTIDE-BASED SENSOR OF THE MYELIN-SPECIFIC ABZYMATIC ACTIVITY

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Multiple sclerosis (MS) is one of the most socially significant autoimmune diseases spread all over the world. This neurological dysfunction is characterized by chronic inflammation, demyelination, axonal and oligodendrocyte loss, caused by activation and migration of immune cells into the central nervous system. Early diagnostics is one of the necessary requirements for the successful MS treatment. The phenomena of natural antibodies with catalytic activity is widely studied during last few decades. These antibody-enzymes or abzymes may either protect organism or contribute to the development of the autoimmune abnormalities. Previously we showed that myelin-reactive autoantibodies from patients with multiple sclerosis had an ability to recognize and hydrolyze encephalitogenic MBP peptide 81–103 flanked by two fluorescent proteins, designated EPeFRET (*Encephalitogenic Peptide Fluorescence Resonance Energy Transfer*). Here we report next generation of this biomarker for MS based on the antibody-mediated degradation of the novel chemically synthesized FRET substrate representing fluorophore Cy5 and quencher QXL680 interconnected by the MBP peptide 81-99 – Cy5-MBP_{81-99}-QXL680. This substrate is degraded being incubated with purified antibodies from mice with experimental autoimmune encephalomyelitis and patients with MS in contrast to those from non-immunized mice and healthy donors. Analysis of fluorescence increase rate revealed statistically significant difference between rate of hydrolysis of Cy5-MBP_{81-99}-QXL680 by IgG isolated from active progressive MS patients compared to active non-progressive MS patients and healthy donors. Cy5-MBP81–99-QXL680 fluorescent biosensor may be targetedly used as a predicative biomarker of MS conversion into the active progressive phenotype, differentiating newly developed active progressive from long-termed active progressive phenotype.

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THE FUNCTIONAL SIGNIFICANCE OF THE COMPONENTS OF THE UBIQUITIN-PROTEASOME SYSTEM IN THE REPROGRAMMING AND DIFFERENTIATION OF MAMMALIAN CELLS

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The ubiquitin-proteasome system plays an important role in maintaining protein homeostasis and in the regulation of many cellular processes. The proteasome is a multi-subunit protease complex, consisting of a core 20S particle and 19S regulatory particles. Under certain conditions, the constitutive catalytic subunits of the 20S particle beta1, beta2 and beta5 can be replaced by special subunits – β 1i/LMP2, β 2i/Mecl-1 and β 5i/LMP7. In this case the proteasomes, designated as immunoproteasomes (IPs), are involved in antigen presentation. There is also an additional regulator of the proteasome and IP - PA28α/β. It has been shown that, there is an increased expression of IP subunits in human ES (embryonic stem) cells, while the expression of these subunits drops during their differentiation. In addition, an inhibition of these subunits reduces the expression of genes associated with pluripotency and increases the expression of genes associated with differentiation. These observations imply that IPs are involved in regulation of pluripotency and differentiation of pluripotent cells. Another intriguing issue regards a possible role of IPs in the induction of cellular pluripotency. Also, it is not known how IPs participate in maintaining the naive and primed pluripotency of mouse and human ESCs. This work is devoted to the study of the maintenance of protein homeostasis in pluripotent mouse cells using the ubiquitin-proteasome system. We showed that during the whole process of reprogramming under the selective inhibitor of LMP7 PR-957 and the inhibitor of the proteasome MG-132, there is a marked decrease in the formation of clones of induced pluripotent stem cells (iPSCs). The analysis of reprogramming of cells derived from knockout embryos MECL-1, LMP7 and PA28α/β also showed a strong decrease in the formation of iPSCs clones, which indicates the participation of the proteasomes, IPs and regulatory particle PA28 in the process of reprogramming. We also found out that the synthesis of IPs in mouse ESC begins when naive cells are differentiated into primed cells from 5 days, which may indicate the role of these complexes in "late" pluripotent cells.

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MASTER REGULATORY PROTEINS AND GENES RESPONSIBLE FOR DEVELOPMENT AND CARCINOGENESIS AS EXEMPLIFIED BY PANCREAS

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Samples of fetal (FP), normal (NP) and cancer pancreas (CP) were collected, mRNA and cDNA prepared and the expression levels of master regulatory genes of pancreas development *PDX1, PTF1A, SOX9, HNF1b, GATA4, KLF5* and *ZEB1* measured.

In comparison to NP, in FP the expression of *SOX9, GATA4, PDX1, PTF1a* is increased, whereas in CP the expression of *PTF1a* is lowered, *GATA4* and *HNF1b* not changed, *PDX1* is lowered or not changed, *SOX9* increased, lowered or not changed and the expression of *KLF5* and *ZEB1* is increased significantly. A positive correlation of the expression levels of *KLF5* and *ZEB1, SOX9* and *PDX1* in CP was observed.

To single out the role of master genes in a proper cancer cells we studied the transcription and translation levels in five cell lines derived from CP of different grades. These cells differed in epigenetic modifications of the regulators and in expression of master genes.

The comparison of the master genes expression in clinical samples and in cell lines revealed only partial correlation. Our data demonstrate the negative correlation of the *KLF5* and *ZEB1* expression in cell lines indicating their antagonistic role in CP. This contradicts to the mentioned above positive correlation of the expression of these genes in the CP samples. Similar discrepancy was observed for *PDX1* and *SOX9.* Thus, the cells do not reflect correctly the behavior of tumor as a whole, possible due to the influence of tumor cells heterogeneity and stromal component which is rather high in CP.

The cell lines model can be particularly informative for study of the metastasis. The PANC1 cells transduced with *PDX1* were used for xenotransplantation experiments using *Danio rerio* embryos (in collaboration with IMG RAS). It was shown that the elevated expression of *PDX1* leads to the suppression of metastasis. The knockdown of *SOX9* changes *SNAIL, SLUG,* and *HES1* expression revealing its participation in regulation of these genes in CP cells.

Our results with cell lines and clinical samples put forward the KLF5 gene and protein as a prospective target for CP therapy.

The conception of cancer therapy targeted not at cancer cells but to the inhibition of interactions between cancer and stromal tumor components is proposed.

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STEM CELLS AS A CELL TECHNOLOGY BASIS

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3D bioprinting of tissues and organs is currently being widely discussed by scientific community and mass media. Great attention is being given to the development of printing methods, to the creation of biocompatible scaffolds, while cells itself are taken for granted. The cellular material is suggested to be available in quantities that can be provided only by *in vitro* cell cultivation. Moreover, it is tacitly implied, that this material has to be more or less homogeneous and reproducible. On the other hand, a concern about the ability of cultivated stem cells for oncogenic transformation is highly escalated. To resolve the existing controversies, an in-depth study of cultured stem cell behavior is required. In the framework of the project " STEM CELLS AS A CELL TECHNOLOGY BASIS ", the properties of cultured human embryonic stem cells, fibroblasts and mesenchymal stem cells of different tissues, including the human endometrium (eMSCs), were investigated. In comparison with other mesenchymal stem cell cultures, eMSCs are shown to possess an increased differentiation potential and appeared to be one of the most adequate and profitable objects for fundamental and applied research. Using the methods of classic and molecular karyotyping, and also whole transcriptome analysis, we showed that these cells are not subjected to transformation and revealed no evidence of oncogenity during prolonged cultivation or under stress (oxidative, heat shock, genotoxic) conditions. However, it was also established, that stress conditions induce premature senescence of eMSCs. In addition, within the framework of this project, the research on evaluation of the therapeutic use effectiveness of eMSCs in the treatment of infertility was carried out. Experiments on transplantation of cell suspension and cell spheroids to experimental animals have shown that the application of human eMSCs allows to overcome infertility which is caused by Asherman's syndrome. Some other important results related to the project are presented by Dr. A.N. Tomilin, A.V. Selenina, Dr. T.Yu. Starkova, Dr. N.A. Mikhailova, Dr. M.G. Khotin, Dr. N.M. Yudintseva, Dr. E.S. Kornilova, Dr. O.G. Lyublinskaya.

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CHAPERONIC DRUGS FOR THE THERAPY OF ONCOLOGICAL AND NEURODEGENERATIVE PATHOLOGIES: DEVELOPMENT AND APROBATION

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Molecular chaperones particularly Hsp70 are known as key regulators of protein homeostasis of a cell; they maintain polypeptides in active, soluble form and correct the structure of damaged proteins. Chaperonic activity Hsp70 is the base of its protective function that is employed in therapy of heart diseases and that is a real humble in oncological therapy. One of goals of our studies was the search for substances able to inhibit heat shock transcription factor HSF1 or to reduce the chaperonic activity of Hsp70 in cancer cells. Using a few of modified assays we revealed several substances able to reduce the level of Hsp70 synthesis or its chaperonic activity. Since most of chaperone inhibitors are highly toxic, we concentrated on the search of less toxic compounds; the second goal of this study was to reveal substances that can be used in combination with known anti-cancer drugs and by this to reduce side effects of the latter.
Two inhibitors of Hsp70 chaperones were found in combination with doxorubicin to decrease proliferation level of tumor cells of different origin and in experiments with animals to prolong their viability by 20-30%. One of interesting properties of Hsp70 inhibitors was effect of dissociation of regulatory molecules of apoptosis, exemplified by caspase 3 from Hsp70; on our mind the mechanism of such affinity separation may be employed in the design of new drug compositions.

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DYNAMICS OF GLYCOLIPIDS IN CELL MEMBRANES AND MODEL SYSTEMS

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The report covers four sub-directions of this theme.

1. *Modelling of glycocalix by the methods of chemical biology*. Glycolipids capable of anchoring well into biological membranes and forming membrane-like monolayers on surfaces were synthesized. A particular feature of these glycopeptides is conformationally rigid spacer group having the length up to 15 nm, which allows loading of glycans into glycocalix on the predetermined distance from the membrane.

2. *Molecular dynamics and immunochemical properties of Gb3 glycolipids*. Human erythrocytes and endothelial cells bear glycolipid Gb3, where ceramide is directly linked to glycan Galα1-3Galβ1-4Glc. At the same time a high level of natural antibodies to this glycan is observed in human blood. We explained the tolerability of antibodies to natural Gb3 in composition of cell membrane combining molecular dynamics simulation and synthesis of Gb3 analogs.

3. *Physico-chemical and molecular dynamics properties of supramers biot-CMG-DOPE*. This molecule is interesting by the ability of rapid biotinylation of cells or material surfaces for minutes. The study using AFM, TEM, DLS, and molecular dynamics simulation of micelle-like vesicles and flat monolayers demonstrated that only 1% of biotin residues enter the layer periphery, however this is sufficient for the binding with Str.

4. Modeling *of glycopeptide transfer between the cells*. First results on the transfer of synthetic glycolipids between eukaryotic cells, and from eukaryotic to bacterial cells are presented here.

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BIOCONVERSION OF LIGNOCELLULOSIC FEEDSTOCKS

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The process of enzymatic conversion of lignocellulosic biomass (LCB) to fermentable C5 and C6 sugars was developed and scaled up to pilot level. Soft and hard wood sawdust were used as a feedstocks. The technological scheme of pilot plant includes units for mechanical and chemical pretreatment of LCB (reaction ability of LCB was improved x4 times, C5 sugars were mainly formed at this stage, up to 90% of total xylose yield was achieved, which allows to separate C5 and C6 sugars stream); unit for enzymatic saccharification of pretreated LCB to C6 sugars (total enzymatic conversion yield was >55% from total solids, duration of enzymatic saccharification process –16-36 hours, concentration of glucose after the end of saccharification process >60 g/l when initial concentration of LCB was 100 g/l); for concentrating of C5 and C6 sugars by vacuum evaporation; for in house production of enzymes in the form of cultural filtrate of UF-conc.

ROLE OF AUTOPHAGY IN THE REGULATION OF PLURIPOTENCY AND SELF-RENEWAL OF EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs) can indefinitely divide in vitro retaining undifferentiated state, although pluripotency is transient state in vivo. However, culture conditions for self-renewal and pluripotency of ESCs are still not optimal. Autophagy is a key catabolic process that allows self-renewal of intracellular components to degrade by a lysosomal machine in response to various stimuli. At present, the study of autophagy begins to occupy a leading position in the study of the viability of tumor cells and fundamental properties of stem cells, since the involvement of autophagy in maintaining pluripotency and self-renewal of ESCs create a new level of regulation of the stemness. Therefore, there is a need to develop new approaches to address these fundamental issues. Thus, this project is aimed at studying autophagy in tumor and ES cells as a key mechanism maintaining their undifferentiated state.

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GENES THAT DISAPPEARED IN EVOLUTION, AS REGULATORS OF BRAIN DEVELOPMENT AND REGENERATION

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The study of molecular mechanisms of regeneration and early development of the brain is one of the most important problems of modern biology. It is well known that the ability to regenerate limbs is significantly reduced in higher vertebrates (birds and mammals) in comparison with the lower ones (fish, amphibians, reptiles). Moreover, higher vertebrates also differ from the lower ones by a much more developed brain. Earlier we showed that these differences can be explained by the loss in the course of evolution in the ancestors of modern higher vertebrates of a number of genes regulating the regeneration and development of the brain in lower vertebrates. Obviously, the study of the functions of these genes is an extremely important task, the solution of which can contribute to understanding the causes of loss of regenerative abilities and enhanced brain development in higher vertebrates, including humans. In the course of this project, we have studied the mechanisms of the functioning of the genes *Ag1*, *Ras-dva1*, *Ras-dva2* and *Noggin4* by means of various methods of molecular biology and developmental biology during the body appendages regeneration and early development of the brain at two model organisms, representatives of the lower vertebrates - embryos of the clawed frog *Xenopus laevis* and fish *Danio rerio*. As a result, data were obtained on signaling cascades regulated by these genes, as well as data supporting the important role of these genes in regulating limb regeneration and in early brain development in lower vertebrates.

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STRUCTURE AND MECHANISMS OF ACTIVATION OF RECEPTOR TYROSINE KINASES

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Receptor tyrosine kinases (RTKs) play a key role in intercellular signal transmission and regulate the most important processes of cell functions - proliferation, differentiation, migration, apoptosis and malignant transformation.

In the frames of "Receptor and signaling proteins. The analysis of structure and function" grant component, RTKs were studied by a combination of structural and functional approaches. The structure and mechanism of the activation of the insulin receptor-related receptor (IRR), a pH-activated RTK, were studied by mutagenesis, monoclonal antibodies and SAXS analyses. A two-center model of IRR activation was proposed. Membrane fragments of the receptors of neurotrophin, epidermal growth factors and human growth hormone were analyzed by the high resolution heteronuclear NMR spectroscopy in membrane-like environments. Structural determinants of the specific dimerization of transmembrane domains were revealed, and conformational and dynamic models of the RTK function were constructed. The lipid-mediated signal transmission mechanism across the cell membrane was proposed. The opportunity of alternative coordinated protein - protein and protein - lipid interactions of the RTK structural domains in response to ligand binding was shown. For the first time, the use of snake cardiotoxins as natural modifiers of cellular membranes lipid bilayers that can regulate the function of membrane receptors, including RTK, was proposed.

By the use of knockout mice, signaling partners of IRR were revealed, in particular, the pH-sensitive potassium channel TASK2 and phospholipase C gamma 2 . It was shown that in representatives of lower vertebrata – embryos of Xenopus laevis frog, downregulation of IRR expression results in the arrest of embryos development Also, in frogs and Danio rerio fish, the signaling cascades regulated by Ag1, Ras-dva1, Ras-dva2 and Noggin4 genes were described and their important role in regulation of limb regeneration and in the early brain development in the lower vertebrata was discovered.

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APPLICATION OF THE TISSUE ENGINEERING AND CELL THERAPY FOR THE RECONSTRUCTIONOF URINARY ORGANS

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In recent years the interest of urologists to use the methods of tissue engineering and cell therapy in the treatment of pathologies of the urinary tract has increased. This refers to diseases in which organ substitution is required, and the tissues of the gastrointestinal tract and various tissues of the body are used as substitutes. The disadvantages of this approach are postoperative complications, a shortage of tissues for plastics, and an increase in the time of surgery due to the need for a patient's flap.

The aim of the study was to investigate the effectiveness of the tissue engineering graft (TEG) application for the repair of damaged urine bladder (UB) tissue and urethra, as well as the evaluation of the effectiveness of cell therapy in the experimental model of tuberculosis lesion of rabbits of the Chinchilla breed.

TEGs based on bilayer polymer scaffolds seeded with allogeneic mesenchymal stem cells (MSCs) of rabbit bone marrow were prepared for the reconstruction of UB and urethra. To specifically track the used cells in vivo, the latter were labeled with superparamagnetic iron oxide nanoparticles (SPIONs). TEGs were implanted on the model of partial resection of the UB and defect of the dorsal surface of the urethra of rabbits. Cell therapy was used in combination with standard anti-tuberculosis therapy, while the suspension of MSCs labeled with SPIONs was injected into the submucosal layer of the pathologically altered wall of the UB.

Evaluation of the results of the TEGs application and cell therapy was performed following 4, 8 and 12 weeks after the operation. After animal sacrifice, histological and immunohistochemical analyses were performed and tissue cryosections were prepared.

The nanoparticle-labeled cells were detected in various layers of reconstructed tissues that convincingly demonstrate their active participation in the reconstruction process. The developed TEGs with allogenic MSCs facilitated to the effective reparation of damaged tissues of UB and urethra, which is especially important for treatment of pathologies without a possibility of using autologous tissue. The obtained results have demonstrated the efficacy of the combined anti-tuberculosis and cell therapy in the treatment of tuberculosis bladder in rabbits.

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NOVEL GENOMIC DETERMINANTS OF RESPIRATORY METABOLISM IN EXTREMOPHILIC ARCHAEA

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Archaea is one of two domains of life dating back to the last common ancestor (LUCA). Archaea are widely distributed in many ecotopes and often predominate in extreme habitats. Accordingly, extremophilic archaea are likely to possess most ancient, as well as most stable forms of the enzymes, which determine key processes of the cell metabolism.

We are describing novel determinants of anaerobic and aerobic respiration, revealed in the genomes of various extremophilic archaea, namely: multiheme oxidoreductases of hyperthermophilic iron(III) reducers of *Geoglobus* and *Pyrobaculum* genera, molybdopterin polysulfide reductases of an extreme halophile *Halanaeroarchaeum sulfurireducens*, oxygen reductases of a hyperacidophile *Cuniculiplasma divulgatum* and its putative nanoarchaeal symbiont. In particular, we have for the first time revealed the genes of cell surface multiheme cytochromes *c* in archaea. Several novel oxidoreductases of this class have been detected in the genomes of *P. ferrireducens*, *P. arsenaticum* 2319x2 and an obligatory iron(III) reducer *G. acetivorans*. First two archaea contained homologs of iron reductases from mesophilic bacteria, while in the last case, the cytochromes did not have previously characterized homologs but their interaction with insoluble Fe(III) oxide was demonstrated in our biochemical experiments. Thus, we have discovered that extremophilic archaea, and in particular, hyperthermophiles possess the mechanisms for extracellular electron transfer to insoluble acceptors, and consequently, can be utilized for electricity production in microbial fuel cell technologies using a broad spectrum of fermentable and non-fermentable substrates.

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HUMAN ENDOMETRIAL STROMAL CELLS SENESCENCE: TYPICAL FEATURES AND POSSIBLE CONCEQUENCES

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The human endometrium is a dynamic tissue that undergoes about 400 cycles of growth, renewal, differentiation, and shedding during the woman's reproductive life. Cyclical restoration of endometrial tissue is due to the proliferation of resident endometrial stromal cells. The work of our group is focused on the investigation of the molecular basis and consequences of premature senescence of human endometrial stromal cells (eSCs).

First of all, we explored intracellular changes accompanying premature senescence of eSCs. We revealed that senescent eSCs displayed most of the features typical for cellular senescence, e.g. DNA-SCARS formation, activation of the DNA damage response, p53/p21/Rb-mediated cell cycle block, irreversible proliferation arrest, cell hypertrophy and protein synthesis modulation, impairment of the degradation systems and increased SA-β-Gal activity, malfunction of mitochondria and elevated levels of reactive oxygen species. In addition, eCSs premature senescence led to a significant decrease in migration capability and disturbance of adipogenic and osteogenic differentiations. It should be noted that the eSCs senescence adversely affected their tissue-specific decidual differentiation, which determines the success of implantation and the subsequent normal embryo development.

Apart from the intracellular changes described above, using liquid chromatography and high-resolution mass spectrometry, we demonstrated a significant change in the profile of the factors secreted by senescent cells. Factors secreted by senescent eSCs, on the one hand, mediated senescence propagation within cell population, and on the other hand, led to the disruption of the decidual differentiation of the normal cells. Based on the bioinformatics we selected the PAI-1 protein as the key component that might be responsible for the observed adverse effects of senescent cells secretome on the microenvironment. In order to verify the functional role of PAI-1 in the secretome of senescent eSCs, we generated PAI-knockout and PAI-1-overexpressing cells with the use of CRISPR/Cas9 technology.

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SELECTION OF LIGANDS FOR PERSONALIZED CAR-T THERAPY OF LEUKEMIA AND LYMPHOMA

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The introduction of T cells modified with chimeric antigenic receptors (CART) is one of the most developing areas in immuno-oncology. Over the past decade, this area of cell therapy significantly evolves from structure optimization and animal models experiments to successful clinical applications. Initially, a vector of CAR development was directed toward increasing activation, cytotoxicity and persistence of modified T-cells. However, the first clinical trials demonstrated necessary to increase the safety of the existing CART cells. The main restriction of the existing CAR is the unspecific targeting of healthy tissues and organs. We believe that discovering of more selective markers of pathological lymphocytes seems to be an urgent goal for the modern cell therapy. A hallmark of the lymphoproliferative diseases, such as leukemia and lymphoma, is a monoclonal expansion of the tumor lymphocyte. Each malignant clone expresses membrane-tettered antigen-recognizing immunoglobulin, which distinguishes it from all other cells of the body. Identification of ligands specific to BCR and TCR of the lymphoma and leukemia cells will allow us to selective elimination of the pathological lymphocytes without damaging healthy cells. For proof-of-principle, we isolate tumor cells from patients with confirmed leukemia or lymphoma. After the nucleotide sequences encoding the immunoglobulin variable domain genes were identified, phage display or autocrine selection of reporter cells methods were utilized for selection of the specific ligands of malignant T and B cells. The introduction of the selected ligands to the chimeric antigen receptors sequence allows us to obtain safe and tumor-specific CART that effectively suppressed cancer cells in vitro, ex vivo and in vivo. We believe, clinical application of this approach may reduce the risks of complications during CART therapy, as well as increase the safety of cell therapy, which will admit its approval for therapy at earlier stages of cancers.

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LINKER PROTEINS H1 AND HMGB1 IN CHROMATINE OF EMBRYONIC STEM CELLS OF THE MOUSE

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Using by FT (ICR) MS, we detected functionally significant differences in the modification status of the "linker" proteins HmgB1 and H1 in embryonic stem cells (ESCs) and differentiated mouse cells. In particular, overall increase of acetylation of H1.0 and H1.2 in the ESC in the region of C-terminal domains involved in the stabilization of condensed chromatin has been shown. At the same time, the expression level of H1.0 and H1.2 in the ESCs is much lower, which leads to a decrease in the total amount of H1 in these cells. Reducing the amount of H1 in the chromatin of the ESCs and weakening the DNA-H1 interaction due to a decrease in the positive charge of the N- and C-terminal regions of the H1 proteins by acetylation can lead to the formation of a "loose" chromatin structure, which is characteristic of stem cells.

We have shown that the "linker" HmgB1 ESC protein is characterized by the presence of acK2 in the region of the N-terminal domain and deacK81 in the region between the HmgB1 domains, which, according to the published data, leads to a loss of its ability to bend DNA upon interaction and preferential binding of HmgB1 at the ends of macromolecule. This should make a difference in the functioning of the protein in the chromatin of ESCs.

It is shown that the level of expression of HmgB1 in ESC is higher than that of chromatin of differentiated cells, however, CRISPR / Cas9-mediated knockout of HmgB1 is not lethal for cell. In contrast, an increase in the proliferation rate of $HmgB1^{-/-}$ ESCs is observed, as compared to wild-type cells. Combined HmgB1/HmgB2 knockout, also does not lead to a lethal outcome, however, manifests itself as a certain delay of differentiation of the ESCs in vitro into endoderm and neuroectoderm cells. The report also describes differentiation of HmgB1/HmgB2 mutant ESCs in vivo.

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PRESENTATION OF MYELINE AUTOANTIGENES ON THE MAJOR HISTOCOMPATIBILITY COMPLEXES CLASS II CATALYZED BY HLA-DM

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Risk of multiple sclerosis (MS) is known to be increased in individuals bearing distinct class II human leukocyte antigen (HLA) variants, whereas some of them may have a protective effect. Here we analyzed allele distribution of a highly polymorphous HLA-*DRB1* locus for more than one thousand MS patients and healthy individuals of Russian ethnicity. Carriage of HLA-*DRB1**15 and HLA-*DRB1**03 groups of alleles was associated with MS risk, whereas carriage of HLA-*DRB1**01 and HLA-*DRB1**11 was shown to be protective. Analysis of genotypes suggests the compensatory effect of risk and resistant alleles *in trans*. Determination of antigenic specificity of the recombinant HLA-DRB1*01:01 molecule demonstrated the capacity of this HLA to recognize epitopes of myelin basic protein (MBP), a major MS autoantigen. HLA-DRB1*01:01 bound two MBP epitopes, namely MBP_{90-98}, a part of the encephalitogenic fragment, and $MBP_{153-161}$, located in the C-terminal part of the protein, with affinity comparable with its classical antigenic determinant – peptide 306–318 of the hemagglutinin (HA) of the influenza virus. Determination of kinetic parameters of MBP and HA peptides loading on HLA-DRB1*01:01 catalyzed by HLA-DM revealed a significantly lower rate of exchange of CLIP by MBP peptides. Analysis of chimeric MBP-HA antigenic peptides demonstrated that the observed difference is caused by the lack of anchor residues in the C-terminal part of the myelin peptide. Moderate occupation of P6/7 and P9 pockets of HLA-DRB1*01:01 by $MBP_{153-161}$ epitope in contrast to the $HA_{308-316}$ epitope leads to the P1 and P4 docking failure resulting in peptide dissociation and release of the empty HLA-DM–HLA-DR complex. We speculate that observed protective properties of the HLA-*DRB1**01 group of alleles may be directly linked with the ability of HLA-DRB1*01:01 to kinetically discriminate peptides of exogenous and endogenous nature.

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TARGETED ELIMINATION OF SENESCENT TUMOR CELLS THROUGH SUPPRESSION OF MEK/ERK PATHWAY

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Small GTPase Ras is mutated in about 30% of tumors. These tumors are among most aggressive ones, because mutated Ras provides therapy resistance. The current strategy against Ras-expressing cells is linked with kinase inhibitor of Raf/MEK/ERK pathway. However, we've obtained that Ras-expressing cells overcome the pro-apoptotic effect of MEK/ERK kinase inhibitor PD0325901 by activating cytoprotective AMPK-regulated autophagy that degrades damaged mitochondria and restores cellular viability. The aim of the work was to develop a strategy to deprive autophagy of its cytoprotective properties. Autophagy is negatively regulated by mTOR kinase – one of the targets of Ras-ERK pathway. mTOR is also widely known as key regulator of cellular senescence. In senescent cells, mTOR is constitutively active, independently on amino acids and growth factors, and it down-regulates autophagy to maintain hypertrophic, hypersecretory phenotype of senescent cells. Thus, high mTOR activity may be the factor that would attenuate autophagy in Ras-expressing cells exposed to MEK/ERK inhibitor and force cells to apoptosis. We've applied histone deacetylase inhibitor sodium butyrate (NaBut) that induces senescence of Ras-expressing cells. Senescence induction deprives Ras cells of their tolerance to MEK/ERK inhibitor, causing cell death. Senescent cells fail to eliminate damaged mitochondria because they don't complete cytoprotective autophagy. The incapability of senescent cells to complete autophagy in response to MEK/ERK suppression is due to development of senescent phenotype that is linked with anchoring of lysosomes around the nucleus and therefore, uncoupling of autophagosomes and lysosomes. The other factor is relocalization of pro-autophagic, anti-apoptotic oncogenic Ras from plasma membrane to cytosol, where it can't provide autophagy regulation. Senescence induction makes Ras-expressing cells uniquely sensitive to MEK/ERK suppression, thus providing a promising strategy of Ras-transformed cells elimination.

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UNIVERSAL CAR-T FOR THERAPY OF ONCOLOGICAL DISEASES BASED ON THE BARNASE-BARSTAR COMPLEX

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Cancer immunotherapy by T cells modified with chimeric antigen receptors (CART) is one of the most promising directions in oncological diseases treatment. Until recently, the use of CAR T-cell therapy has been restricted to small clinical trials, largely in patients with advanced blood cancers. But these treatments have nevertheless captured the attention of researchers and the public alike because of the remarkable responses they have produced in some patients—both children and adults—for whom all other treatments had stopped working. Progress with CAR T cells has greatly accelerated, with researchers developing a better understanding of how these therapies work in patients and translating that knowledge into improvements in how they are developed and tested. However, a list of complications associated with cytokine storm and tumor lysis syndrome significantly restrict the application and safety of CART therapy.

One of the possible approaches to the controllable CART activity is regulation of the interaction between tumor and CART cells. We modified the basic paradigm of the adoptive cell therapy by the tunable CART, which implies direct contact of the tumor cell with CART cell. A new approach includes a third component, which plays an intermediator function in recognition of cancer cells by CART. The mediator molecule includes a cancer antigen recognition domain and bacterial RNase Barnase, which specifically interacts with its natural inhibitor Barstar exposed on CAR. We generate a set of mediator molecules together with a panel of chimeric antigenic receptors expressing different forms of Barstar with vary linkers connecting Barstar to signal part of CAR. We analyzed the intensity of hybridization of all variants of chimeric receptors with Darpin-Barnase that specific to the tumor antigen HER2neo. We selected the most effective Barstar chimeric receptor configuration. The developed Barstar-CAR construct will be validated for in vitro and in vivo activities. Tunable CART approach will arise a possibility of real-time control under gene-modified T cells during gene therapy.

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