Symposium «Molecular mechanism of human pathologies»

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Foreword

The breakthroughs in modern medicine have led to a significant increase in the lifespan and quality of life. This has also changed the spectrum and frequency of different human diseases. The infectious diseases, the principal cause of mortality a century ago, do not represent a major threat to humanity these days. At the same time, the diseases that have been relatively rare at in the XIX and in the first part of the XX century, have emerged as major challenges for modern medicine. These diseases include cancer, neurodegenerative and autoimmune diseases. They also represent a challenge to modern society, as their treatment is a heavy financial burden on public health systems.

A common point between these diseases is the absence of efficient treatment in most cases and the dependence of outcome on early diagnostics of the disease. Indeed, most cancers develop for several years before appearance of first clinical symptoms. The same is true for neurodegenerative diseases (Parkinson's and Alzheimer's diseases, hyperprolactinemia) that develop during 20–30 years before attaining a clinical stages, and the clinical symptoms appear when 80 % of the specific neurons are attained. Therefore, the understanding of early mechanisms of these diseases is particularly important for the success of treatment and for development of new drugs.

Interestingly, some of the early mechanisms of cancer, autoimmune and neurodegenerative diseases are similar, eg mutations and translocations in immunoglobulin genes may provoke both autoimmune diseases and some cancers. Therefore, cooperation between laboratories working on these subjects may provide new insights into early mechanisms of these diseases.

The meeting «Early mechanisms in human disease» held in Kiev (Ukraine) on May 29 – June 1 has united Ukrainian, Russian, and French scientists working on cancer, neurodegenerative and autoimmune diseases. This is the second meeting in the series, the first one was held in Moscow in October, 2007. The quality of the meeting is reflected in the abstracts published in this issue of «Biopolymers and Cell». I would also thank the local organizing committee and Iryna Pirozhkova (IGR, Villejuif, France), for the wonderful organization of the meeting.

Yegor Vassetzky

Dynamic microtubules are necessary for the assembly of YB-1 containing stress granules

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Following exposure to various stresses (arsenite, UV, hyperthermia, hypoxia), mRNAs assemble into large cytoplasmic bodies known as «stress granules», in which mRNAs and associated proteins may be processed by specific enzymes for different purposes like transient storing, sorting, silencing or other still unknown processes. To limit mRNA damage during stress, the assembly of micrometric size granules has to be rapid and indeed it takes only about 10–15 min in living cells. However, such a rapid assembly is against the rules of hindered diffusion in the cytoplasm, which states that cytoplasmic bodies larger than 100 nm are almost immobile. Consequently, cells must have developed mechanisms to allow the rapid formation of stress granules. In the present work, using HeLa cells and YB-1 as a stress granule marker, we identified and explored experimentally and theoretically three hypotheses to explain how cells overcome the limitation of hindered diffusion: shuttling small mRNP particles from small to large stress granules, sliding mRNP particles along microtubules, microtubule-mediated stirring large stress granules. The data favor the latter hypothesis and underline the necessity of a dynamic microtubules network in the process of YB-1 rich stress granule formation.

Ubiquitin and UBA domains: new players in the coordination between transcription and mRNA nuclear export

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Concomitantly to their transcription, nascent transcripts are loaded with mRNA binding proteins implicated in the processing and packaging of mRNA into stable and export competent mRNPs. These co-transcriptional reactions are tightly coupled and coordinated by the RNA polymerase II (RNAPII) C-terminal domain, which acts as a recruitment platform for the different RNA processing machineries. In addition, more and more evidence show that chromatin remodelling and histone epigenetic marks play a fundamental function in RNAPII dynamic and RNA processing. Fully mature and correctly packaged yeast mRNPs are then released from the transcription site and transported into the cytoplasm by the heterodimeric export receptor Mex67/Mtr2, which promotes their translocation through the nuclear pore complexes. However, Mex67/Mtr2 is not directly recruited to

mRNAs but rather requires specific RNA-binding adaptors that associate with nascent transcripts during the transcription process. On the other hand, ubiquitin conjugation has been recently involved in the regulation of nuclear export of poly(A) + RNA. Interestingly, the yeast mRNA export receptor Mex67 as well as its metazoan counterpart harbours an Ubiquitin-Associated domain (UBA-Mex67) in its C-terminus that participates in the interaction with FG-nucleoporins in nuclear pores. Our results indicate that UBA-Mex67 not only promotes interaction with ubiquitin and ubiquitylated proteins but also the co-transcriptional recruitment of Mex67. Our studies led to the identification of distinct UBA-Mex67 interacting proteins which are implicated in transcription elongation, histone methylation and 3' end processing, and chromatin remodeling respectively. We now propose that the mRNA export receptor Mex67 could play a major role in the coordination of different steps of transcription and mRNA transport through the dynamic interaction of its UBA domain with these various ubiquitylated targets.

Sodium-dependent phosphate transporter NaPi2b as ovarian cancer marker

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Mouse monoclonal antibody MX35 was developed against ovarian cancer. The antibody showed homogeneous reactivity with approximately 90 % of human ovarian epithelial cancers and with a limited number of normal tissues by immunohistochemistry. Although mAb MX35 has been used in a number of clinical trials in ovarian cancer, it has been difficult to define the molecular identity of MX35. We report here that mAb MX35 recognizes the sodium-dependent phosphate transport protein 2b (NaPi2b) in human cancer cells. This conclusion is based on several lines of experimental evidence, including 1) the identification of SLC34A2, the gene coding for NaPi2b, by immunoscreening an ovarian cancer cell line cDNA expression library with mAb MX35; 2) mass spectrometry sequencing of peptides obtained by fragmentation from mAb MX35 affinity-purified antigen, which show complete sequence homology to amino acid sequences in NaPi2b; 3) selective down-regulation of SLC34A2 gene expression by RNA interference and the resulting loss of mAb MX35 binding to MX35-expressing human cancer cells; and 4) demonstration of a specific mAb MX35 reactivity with recombinant fusion proteins and with synthetic peptides of the putative largest extracellular loop of NaPi2b. We has further shown that NaPi2b in cancer cells is expressed on the cell surface as a heavily N-glycosylated protein, with evidence of additional post-translational modifications such as palmitoylation and formation of disulfide bridges in the major extracellular loop. The membrane transporter molecules, such as NaPi2b, represent a new family of potential cell surface targets for the immunotherapy of cancer with monoclonal antibodies.

Catalytic antibodies: from a laboratory concept to physiological entities

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Since the demonstration that catalytic antibodies with catalytic activities may be elicited using transition state analogues, more and more groups have brought evidence that catalytic antibodies are not only a chemical curiosity. Antibodies with various catalytic activities are present in the serum of mammals after a physiological disorder or appear during the evolution of some pathology. We have exploited the idiotypic network of the immune system to generate antibodies that mimic the structure and function of different enzymes with esterase, amidase and protease activities. The appearance of such catalysts in the serum raised the question of their possible beneficial or deleterious physiological role. Different examples of isolation of catalytic antibodies in human serum in relation with autoimmune diseases or after physiological perturbations are now available. In some cases the presence of catalytic antibodies may be indicative for a positive progression of the pathology or, at the contrary, as a deleterious factor. However, due to their early expression, catalytic antibodies may be used in some cases as predictive biomarkers for the development of related diseases.

Catalytic antibodies in pathology

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Discovery of catalytic antibodies (abzymes) was a revolutionary event that created new junctions between chemistry, biochemistry, immunology and pathology. The general concept of complementarity introduced in life sciences by Emil Fisher explains the driving force of various biological processes including genetic machinery, enzyme catalysis, ligand-receptor interaction, antibody-antigen recognition etc. Creation of abzymes as a new class of biocatalysts is based upon the intrinsic properties of immunoglobulin superfamily to produce complementary «molecular imprints» using the hypervariability of CDRs. These «catalytic imprints» could be made from the stable chemical analogs of transition state (TSA) of the enzyme reaction (*Linus Pauling and Bill Jencks*). This approach was successfully developed by Richard Lerner group (*Scripps Research Institute, USA*). The alternative way to create abzymes was proposed by author (*Schuster et al., Science, 1992*). It is generated on the basis of the immunological network hypothesis of Niels Jerne and declares the formation of anti-idiotypic antibody repertoire generated against the active site of corresponding enzyme. This approach allowed us to create abzymes with acetylcholinesterase and protease activities (*Kolesnikov et al. PNAS, 2000, Pillet et al., J. Imm. meth. 2002, Ponomarenko et al., Biochemistry, 2007*). In both cases one try to mimic the highly evolved enzymatic function by selection of antibody catalysts from the vast repertoire of immunoglobulines (*Reshetnyak et al, J. Am. Chem. Soc., 2007*). This may give rise to biocatalysts with new functions, previously unknown for common enzymes,

which may be very profitable for fine organic synthesis. This method stimulated our attempts to make antibody-like acceptors for phosphorus-based poisons. Recombinant antibodies with such functions have been obtained recently in this lab using chemical selection of «naïve» phage-display library. The second advantage of abzyme field is the opportunity to make «catalytic vaccines». Traditional drugs including antibiotics and other small-molecule compounds developed in the pre-biotechnology era showed the limited success in a number of sever bacterial and viral infections. Numerous attempts to combat HIV infection using drug therapy as well as classical vaccination turned out to be ineffective. One of the targets for the novel therapeutic approach may be the main surface antigen, viral envelope protein gp120. The specific cleavage of this protein can lead to the dramatic changes in the immune response toward virus and decrease binding of HIV to CD4 receptor. This task, impossible to be solved by enzyme therapy, may have an effective abzyme alternative (*Durova et al. Mol. Immunol., 2009*). A novel approach for creating catalytic antibodies against pathogens is described (*Ponomarenko et al., J. Imm. meth; Biochemistry, 2002*). This involves utilizing the autoimmune disorder of SJL mice induced by myelin basic protein as a background for raising a protein-specific catalytic response toward gp120. Site-specific abzyme-mediated cleavage of gp120 is demonstrated. This approach developed in this laboratory can be considered as a general strategy to obtain a catalytic vaccine to proteins of interest (*Ponomarenko et al. Biochemistry, 2006*). In our studies we firstly showed that catalytic antibody formation has the strong intrinsic and still enigmatic links with autoimmune diseases. The existence of DNA-specific abzymes in scleroderma, systemic lupus erithematosus (SLE), rheumatoid arthritis and AIDS was described in this laboratory (*Shuster et al. Science 1992, Gololobov et al., PNAS,1995, Gololobov et al. Molecular Immunology, 1997)*. Very recently the input of abzyme activity in neurodegeneration process has been demonstrated (*Ponomarenko et al. Immunology letters, 2006*). Autoantibody-mediated tissue and cell destruction is among the main features of organ-specific autoimmunity. We have described abzyme contribution to neural tissue-specific antigens (Ag) degradation. AutoAb to myelin basic protein (MBP) from humans with multiple sclerosis (MS) and SJL mice with experimental autoimmune encephalomyelitis (EAE) exhibited site-specific antigen degradation (*Ponomarenko et al. PNAS, 2006*). AutoAb from patients with the secondary progressive MS and highest scores on the expanded disability status scale (EDSS) demonstrated augmented catalysis. An established MS therapeutic Copaxone® inhibited reaction in vitro. AutoAb catalysis thus appears to be a specific feature associated with MS pathogenesis and potential marker of disease progression (*Belogurov et al. J. Immunol. 2008*).

Oncogenic redundancy is a significant obstacle to the success of target therapy

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In the 21st century the cancer therapy will be changed from «one size fits all» approach to the personalized one, in which each patient is treated according to the specific genetic defects in his tumor. To enhance glioblastoma (GB) marker discovery, using Serial Analysis of Gene Expression

(SAGE), we found 676 genes with 2-fold change of expression in GB, the most aggressive tumor of human brain. The gene-expression data were used to create a prognostic GB gene-expression signature. Unfortunately, reviewing available results obtained by microarrays we found that they were not always reproducible and the lists of detected genes have quite poor overlapping. Besides possible methodological mistakes, it is more likely that the signatures created by different laboratories use different genes to monitor the same biological processes. Oncogenic redundancy is recognized today as a significant obstacle to for a target therapy. A central role that the insulin-like growth factor system plays in a tumor progression makes it a target for the cancer therapy, but the enhanced IGF-I gene expression in GB was not found, instead, we revealed CHI3L1 among genes with the most pronounced changes in expression. Both, YKL-40 and IGF-I, may activate PI3K and MAPK signaling cascades, which are associated with the control of mitogenesis (Recklies et al., 2002). The expression of homological YKL-39 and CD74, EGFR, CTGF, IGFBP5, IGFBP7, IGF-II, also considerably increased in GB, may activate the PI3K and MAPK cascades similarly to the epidermal growth factor (EGF). Concerning the IGF-II expression in ependimomas and meningiomas, SAGE revealed extraordinary high content of one particular tag belonging to the IGF2-associated mRNA. The open reading frame of this mRNA is unusually localized in the 3'UTR of IGF-II gene and may encode some protein with completely unknown function. Attention of investigators is directed mostly to genes with overexpression in tumors. However, identification of genes which are underexpressed in brain tumors is not less important. 85 genes with 5-fold decrease were found by SAGE, some of them may be considered as potential tumor suppressor genes. The TSC-22 mRNA level is decreased in astrocytic gliomas. The differential expression of TSC-22 was confirmed by Northern, RT-PCR and histochemical analysis. In contrast to YKL-40, TSC-22 may serve as a mediator of TGF- signaling. TGF- binds to the receptor type II that leads to the phosphorylation of the type me receptor, and then activates SMAD2 protein. TSC-22 activates SMAD4 which in combination with SMAD2 is translocated to the nucleus that leads to arrested growth and apoptosis, so the decrease in TSC-22 expression has anti-apoptotic consequences in GB. Therefore, it is possible to see that not only morphological but also molecular heterogeneity of malignant neoplasms and oncogene redundancy cause a necessity of building «passway signatures». It might be very useful to create passway signature for each cancer type so that the optimal treatment can be chosen for each patient.

Detection of key DNA repair proteins responsible for cell radiosensitivity by chemically reactive DNA intermediates

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DNA is continuously damaged by endogenous reactive metabolites and exogenous chemicals or ionizing radiation. A dedicated network of DNA repair mechanisms safeguards DNA integrity to prevent the deleterious consequences of genetic degeneration, notably mutations and cell death leading to cancer and aging. Some key proteins of different DNA repair mechanisms recognizing

DNA damages are known to be responsible for cell radiosensitivity. The most versatile approach to study interaction of such proteins with damaged DNA is affinity labeling by chemically reactive DNA with structural peculiarities mimicking proper DNA damages including clustered ones. We have developed an approach to identify and monitor key DNA repair proteins in cell extracts. Photoreactive branch point DNA intermediate of base excision repair (BER) has been used in cellular extracts of mouse embryonic fibroblasts to identify composition of protein ensembles interacting with damaged DNA and with the BER pathway intermediates. The main target proteins covalently linked to photoreactive BER intermediate formed in cellular extracts were identified by immunoprecipitation assay and by MALDI-MS as poly(ADP-ribose)polymerase 1 (PARP1), flap endonuclease 1 (FEN1), DNA polymerase (Pol), apurinic/apyrimidinic endonuclease 1 (APE1) and the high mobility group box 1 protein (HMGB1). The functions of these proteins in BER were analysed. Natural apurinic/apyrimidinic (AP) sites in DNAs were used as groups reactive to proteins. PARP1, Ku80 subunit of Ku antigen and XRCC1 were shown to efficiently interact with AP sites via Schiff base formation. PARP1 and Ku80 were identified in the cell extract as targets of cross-linking to AP site by immunochemical methods and/or MALDI-TOF-MS. The specificity of Ku antigen interaction with AP sites was proven by more efficient competition of DNA duplexes with an analogue of abasic site than non-AP DNA. Ku80 was cross-linked to AP DNAs with different efficiencies depending on the size and position of strand interruptions opposite to AP sites. Ku antigen was shown to inhibit AP site cleavage by APE 1. We compared the results of dot-ELISA based on anti-Ku80 antibodies and the levels of Ku80 cross-linking to AP DNA in the extracts derived from HeLa and several melanoma cell lines. The efficiency of Ku80 trapping varies considerably depending on the type of cell extract and correlates with the amount of Ku80 in the extracts. Thus, AP site containing DNA can be used as an efficient tool to test the content of Ku antigen in cell extracts. XRCC1 was shown to interact with AP site more efficiently in DNA duplexes containing strand breaks opposite AP site. These proteins participate in repair of single- and double-strand breaks in DNA and control sensitivity of cells to ionizing radiation. A new unknown protein was shown to specifically interact with AP site closely opposed to analog of AP site. The level of these proteins in cancer cells is considered as a relevant factor for the prediction of tumor radiosensitivity and, therefore, AP DNA can be used as a test system for determining the content of these proteins in tumor cells to estimate appropriateness of radiotherapy and treatment outcome. This work was partially supported by RFBR, project N 07-04-00178; Siberian Branch of the RAS, project N 104; FASI, contract N 02.512.11.224 and by RAS, Program «Molecular and Cellular Biology».

Thyroid transcription factor-1 could be a target gene of RET/PTC and canonical Wnt pathways in papillary thyroid carcinoma

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Background and purpose: The RET/PTC1 oncoprotein, exclusively expressed in tumour cells, is an early event in the development of Papillary Thyroid Carcinoma (PTC) but would not be sufficient for the tumour progression. We hypothesize that Wnt/ -catenin signalling pathway may cooperate

with ret/PTC oncogene to promote the disease. Therefore, we assessed the cross-talk between the RET/PTC and the canonical Wnt pathways on the thyroid transcription factor-1 (TTF-1) involved in PTC relapse. Methods: LiCl and SB216763, inhibitors of GSK-3, were used to activate the Wnt signalling. SiRNAs sequences were designed to knockdown RET/PTC1 oncogene. The cross-talk between RET/PTC and Wnt pathways was assessed by combining LiCl or SB216763 with the siRNA RET/PTC1. TTF-1, RET and -catenin expressions were analysed by Q-RTPCR and western blot in the TPC-1 cell line that constitutively express ret/PTC1. Results: Both LiCl, SB216763 and siRNA ret/PTC1 induced the TTF-1 gene expression. The -catenin protein was induced by LiCl and SB216763; the RET gene and protein expression were inhibited by siRNA RET/PTC1. Combined treatments leads to the preservation of ret/PTC1 gene inhibition and minimization of the TTF-1 gene induction. Discussion and conclusion: TTF-1 would be a target gene of the Wnt/ -catenin and RET/PTC pathways. Treatment with siRNA RET/PTC1 inhibits the oncogene expression but induces a major gene involved in tumour aggressiveness. In order to use siRNA RET/PTC1 as a targeted therapeutic against PTC, we need to define them to inhibit the molecular components of Wnt pathway responsible for the TTF-1 activation.

Viral hijacking of mitochondrial lysyl-tRNA synthetase

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Human immunodeficiency virus type 1, HIV-1, is a retrovirus. Its genome is made of two single-stranded RNA molecules. Reverse transcriptase encoded by HIV-1 uses $tRNA_3^{Lys}$ of the host cell to start the reverse transcription of its RNA genome into proviral DNA. During assembly of HIV-1 particles, $tRNA_3^{Lys}$ is taken up along with lysyl-tRNA synthetase (LysRS) from the host cell, the tRNA binding protein that specifically aminoacylates the different $tRNA^{Lys}$ isoacceptors. In human, the cytoplasmic and mitochondrial species of LysRS are encoded by a single gene by means of alternative splicing. We showed that polyclonal antibodies directed to the full-length cytoplasmic enzyme equally recognized the two enzyme species. We raised antibodies against synthetic peptides, that allowed to discriminate between the two enzymes, and found that mitochondrial LysRS is the only cellular source of viral LysRS. Mitochondrial localization of LysRS in HeLa cells is altered after addition of the auxiliary viral protein Vpr into the culture medium. These results open new routes toward the understanding of the molecular mechanisms involved in the specific packaging of $tRNA_3^{Lys}$ into viral particles. Molecular mechanisms involved in viral hijacking of molecules of the host cell will be described.

Two tissue-specific isoforms of translation elongation factor 1A: why one of them is oncogenic?

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eEF1A is the main component of eukaryotic translation elongation machinery. It is responsible for correct binding of aminoacyl-tRNA to A site of ribosome. In tRNA channeling cycle eEF1A forms the complex with GTP and aminoacyl-tRNA and delivers the latter to the 80S ribosome. After the translocation and transpeptidation steps are finished deacylated tRNA stays in the ribosomal E site. Thus, other role of eEF1A in elongation cycle was suggested to pick up deacylated tRNA from E site and to deliver it back to aminoacyl-tRNA synthetase for recharging. Multienzyme complex of aminoacyl-tRNA synthetases associates in cell with multicomponent eEF1H complex containing GDP/GTP exchanging subunits for eEF1A, thus aminoacylation of tRNA and GDP/GTP exchange in eEF1A molecule occur simultaneously. As a result, the ternary complex aminoacyl-tRNA*eEF1A*GTP is formed and ready to go to A site of the ribosome. There are two 97 % similar isoforms of eEF1A (A1 and A2) which are tissue and development-specific. A1 is expressed in all tissues during development but is absent in adult muscles, heart and neurons. A2 is found instead in these tissues. A2 reveals oncogenic properties when appears in non-specific tissue (ovary, pancreas etc.). A2 was found to be highly expressed in ovary, pancreas and breast tumors. Rodent fibroblasts overexpressing A2 demonstrated enhanced focus formation, anchorage-independent growth and decreased doubling time. In addition, A2 expression made NIH3T3 fibroblasts tumorigenic and increased the growth rate of ES-2 ovarian carcinoma cells xenografted in nude mice. Importantly, the copy number at the EEF1A2 locus does not always correlate with expression level of the gene. No functional mutations were found and the EEF1A2 gene is unmethylated in both normal and tumour DNA, showing that overexpression is not always dependent on genetic or epigenetic modifications at the EEF1A2 locus. Thus, we deal with two highly similar proteins both of which are routinely expressed in different tissues and apparently possess the same translation function. However, appearance of A2 in non-specific for that isoform tissue leads to carcinogenesis. There should be an abnormal competition between the isoforms that results in unfavorable outcome. What is the molecular background for such a competition between two very similar proteins? No profound difference between the isoforms was found in different translation tests (GDP/GTP exchange, intrinsic GTPase activity, kinetics of poly(U) translation, tRNA binding). A1 and A2 were also observed to possess similar chaperon-like activity which rules out the involvement of these kinds of activity in providing cancer-related properties of A2. However, we have found more pronounced ability of the A2 isoform to interact with SH2 and SH3 domains of different molecules involved in phosphotyrosine-mediated signaling pathways. Vise versa, A2 isoform completely lost the ability

to interact with calmodulin in the presence of Ca^{2+} while A1 demonstrated a specific complex formation in different tests. These findings indicate a possibility of non-similar involvement of A1 and A2 in cell regulation which could be important for cancer development. To decipher structural features which might contribute to the distinct signaling abilities we have analyzed spatial structures of A1 and A2. Striking conformational dissimilarity of the isoforms was found by X-ray scattering and confirmed by scanning microcalorimetry. A2 isoform behaves like a globular protein in solution while A1 possesses an extended conformation. Moreover, atomic force microscopy revealed the pronounced hydrophobicity of the eEF1A2 surface but hydrophilic nature of eEF1A1 one. Since, apart from translation, eEF1A is apparently involved in cell signaling, actin and tubulin cytoskeleton rearrangement, apoptosis, nuclear transport, ubiquitin-dependent proteolytic system, heat shock, mRNA transport, interaction with viral genomic RNA etc. it is difficult at present to determine exactly which regular cellular process is influenced by unusual appearance of A2 un some tissue. We have shown the background for the competition between A1 and A2 isoforms might not be limited by the minor dissimilarity of primary structures but apparently includes the difference in their spatial organization and surface hydrophobicity. All those factors could have an impact on the oncogenic properties of A2 isoform facilitating interactions which are not present in the tissues containing generally A1 protein only, or excluding contacts which are regular for A1 protein.

Development of surface plasmon resonance biosensor for diagnostics of Ph+ leukemia

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This presentation describes important steps for the development of optical biosensor based on Surface Plasmon Resonance (SPR) for detection of the bcr-abl gene related to Ph+ leukemia. A bioselective element (a specific recognition layer on the sensor surface) of SPR spectrometer for label-free detection of DNA sequence of the hybrid bcr-abl gene is firstly developed using thiol-modified oligonucleotides. Preparation of the bioselective element and subsequent hybridization events were monitored in real time by SPR spectrometer «Plasmon SPR-4m». The thiol-modified single-stranded oligonucleotide mod-Ph is shown to be immobilized efficiently on a gold sensor surface of the SPR spectrometer. Specific hybridization between immobilized mod-Ph and complementary oligonucleotide P1 was detected, whereas no sensor response to non-complementary target was observed. The experimental results on interactions between the oligonucleotides under study are in good agreement with theoretical calculations of thermodynamic parameters of these interactions.

BMP7 is involved in Mantle cell lymphoma secondary drug resistance

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The identification of genes involved in the mechanisms of secondary drug resistance is a challenge. In order to select such genes in mantle cell lymphomas (MCL), we designed a gene profiling experiment based on the use of paired tissue samples obtained before and after treatment of 5 patients. For each gene of each patient, the variation in gene expression was estimated as a ratio between the expression after treatment and the expression before. For each gene, the mean variation was estimated for patients initially resistant and for responders who developed a secondary drug resistance. Genes were considered relevant when the ratio between the variation in expression measured in initially resistant patients and the variation in secondary resistant patients was greater than 2, with an associated P value less than 0.01. Nine relevant genes were selected, BMP7 was the only one with fold change > 5 and the only one with a significantly increased expression at relapse in patients who developed a secondary resistance. The validation of BMP7 as a key gene involved in secondary resistance was performed using cell line cultures. In the MCL cell line expressing BMP7, BMP7 RNA interference markedly increased apoptosis ($P < 0.01$) after exposure to Bortezomib and Cytarabine.

Alternative splicing regulation of intersectin functional interactions in humans

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Neurodegenerative diseases are linked to abnormalities in endocytosis and vesicle-trafficking disorders. The enlarged early endosomes were shown in mouse model of Down Syndrom (DS).

Neurons of individuals with DS demonstrate similar defects as in Alzheimer Disease (AD) patients. Intersectin1 (Itsn1) gene is located on human chromosome 21 in so-called critical region for DS and is over expressed in DS individuals. Moreover, over expression of ITSN1 enhances huntingtin aggregation and neurodegeneration at Huntington's disease. ITSN1 is a membrane-associated adaptor protein involved in clathrin-mediated endocytosis, mitogenic signalling, and actin cytoskeleton rearrangements. Structural and functional diversity of multidomain intersectin in vertebrates is generated by alternative splicing, by usage of alternative promoters and presence of paralogous gene Itsn2. We identified an alternative transcription initiation site in the fifth intron of Itsn1. We revealed 17 alternative splicing events for pre-mRNA of Itsn1 gene. Eleven of them presumably are subjected to degradation whereas six affect different functional domains of ITSN1. Among novel isoforms the shortest one, ITSN1-22a, with C-terminus presented by mobile element LINE2C has been localized in endomembrane compartments. It forms a complex with ubiquitously expressed ITSN1-s isoform. Comparing to the essential inhibition by ITSN1-s and ITSN1-22a, C-terminus of ITSN1-22a has only slight effect on endocytosis. Nevertheless, this C-terminus is turned out to bind the SH3 domain of amphiphysin and the SH3A domain of intersectin suggesting participation of C-terminus in regulation of dynamin-amphiphysin and dynamin-ITSN interactions. An expression analysis of alternatively spliced transcripts revealed brain- and development-specific ITSN1 isoform with exon 20 inclusion affecting the SH3A domain. This exon encodes five additional amino acids in the n-Src loop of the SH3A domain and altered its binding properties. The protein partners were divided into three groups: with higher affinity to the brain-specific isoform, with lower and unchanged ones. Changes in n-Src loop due to insertion of additional amino acids are assumed to evoke rearrangement of charged groups engaged in interaction interface affecting the interaction with PRD containing charged side chains. Set of pull-down experiments with mutants of the SH3A domain confirmed it. Transcripts with exon 20 are detectable at the early stages of brain development. We investigated in details and compared interaction interfaces of ITSN1 and ITSN2 for their binding partners. Some differences were observed in binding for c-Cbl and Sema6a, no differences were found for Sos1 and dynamin 1, Ruk/CIN85 is a specific partner for ITSN1. Furthermore, ITSN1-s, ITSN2 and ITSN1-22a were co-localized significantly. Thus, ITSN proteins provide complex interfaces for interaction between basic endocytic machinery and the members of different cell processes such as signaling, sorting and cytoskeleton rearrangements which are largely regulated by alternative splicing.

Poly(ADP-ribosyl)ation reactions in the control of the balance between life and death

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins mediated by poly(ADP-ribose) polymerases (PARPs), involved in DNA repair, transcription, mitotic segregation, and telomere homeostasis and cell death. PARP-1 is a molecular sensor of DNA breaks, which uses NAD^+ to synthesize poly(ADP-ribose), a signal molecule involved in the recruitment of base

excision/single-strand break repair factors at the damaged site. We have identified another DNA damage dependent PARP, PARP-2. Cellular and animal models deficient in PARP-1 and PARP-2 developed in the laboratory revealed the redundant but also complementary functions of these two proteins in the surveillance of the genome integrity. More recently, we identified specific and essential roles of PARP-2 in cell differentiation. Our laboratory has established in silico the sequence of novel PARPs, bringing to 17 the number of PARP family members. The diversity of functional domains associated to the PARP domain, the variety of subcellular localizations and the novel biological functions discovered, extend considerably the field of poly(ADP-ribosyl)ation reactions to various aspects of the cell biology. Many studies revealed the importance of PARPs and PARG, the poly(ADP-ribose) degrading enzyme poly(ADP-ribose) glycohydrolase, to control poly(ADP-ribose) levels regulating the balance between life-and-death in response to DNA damage. The expression of PARG was knocked down in HeLa cells, leading to poly(ADP-ribose) accumulation. PARG deficient cells showed increased radiosensitivity caused by single and double strand breaks repair defect and alteration of mitosis progression due to mitotic spindle checkpoint defect. Irradiated PARG deficient cells displayed centrosome amplification leading to mitotic supernumerary spindle poles, and accumulated aberrant mitotic figures, which induced either polyploidy or cell death by mitotic catastrophe. Our results suggest that PARG could be a novel potential therapeutic target for radiotherapy.

Mechanisms of the brain plasticity in Parkinson's disease and hyperprolactinemia

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In addition to the monoaminergic (MA-ergic) neurons possessing the whole set of enzymes of MA synthesis from the precursor amino acid and the MA membrane transporter, the neurons partly expressing the monoaminergic phenotype have been first discovered almost twenty years ago. Most of the neurons express individual enzymes of MA synthesis having no MA transporter. These so-called monoenzymatic neurons are widely distributed throughout the brain in adult mammals being even more numerous than MA-ergic neurons. Individual enzymes of MA synthesis are expressed continuously or transiently over certain periods of ontogenesis and in adulthood under functional insufficiency of the MA-ergic neurons, e. g. under their chronic stimulation or in certain neurodegenerative diseases. The above data suggest an important functional role of monoenzymatic neurons. Some monoenzymatic neurons appear to co-express the monoamine transporters. Most numerous monoenzymatic neurons possess enzymes of dopamine (DA) synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC). TH and AADC are enzymatically active in most monoenzymatic neurons being capable to convert L-tyrosine to L-DOPA and L-DOPA to DA or serotonin, respectively. L-DOPA produced in monoenzymatic TH-neurons is supposed to play a role of a neurotransmitter or a neuromodulator providing its action

on the target neurons via catecholamine receptors. Moreover, L-DOPA released from the monoenzymatic TH-neurons can be captured by monoenzymatic AADC-neurons or dopaminergic and serotoninergic neurons for DA synthesis (Kannari et al., 2006). Such cooperative synthesis of MAs is considered as a compensatory reaction under the failure of MA-ergic neurons, e. g. in neurodegenerative diseases like hyperprolactinemia and Parkinson's disease which are developed primarily because of degeneration of DA-ergic neurons of the tuberoinfundibular system and the nigrostriatal system, respectively. Noteworthy, hyperprolactinemia is followed with time by the normalization of prolactin secretion due to stimulation of DA synthesis by the neurons of the tuberoinfundibular system, most probably because of the turning on cooperative synthesis of DA by monoenzymatic neurons. The same compensatory mechanism is supposed to be used under the failure of the nigrostriatal DA-ergic system that is manifested by the increased number of monoenzymatic neurons in the striatum of animals with pharmacologically-induced parkinsonism and in humans with Parkinson's disease. Expression of the enzymes of MA synthesis in non-monoaminergic neurons is controlled by intercellular signals such as classical neurotransmitters (catecholamines), neurotrophic factors (brain-derived neurotrophic factor, glia-derived neurotrophic factor), and perhaps hormones (prolactin, estrogens, progesterone). Thus, a substantial number of the brain neurons express partly the monoaminergic phenotype, mostly individual complementary enzymes of MA synthesis, serving to produce MAs in cooperation that is considered as a compensatory reaction under the failure of MA-ergic neurons.

Intranuclear relocalization of translocated regions: a common mechanism of oncogene activation in lymphomas?

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Lymphoma is a cancer involving cells of the immune system; it represents over 35 different subtypes. We are particularly interested in two lymphoma types, Burkitt's lymphoma (BL) and mantle cell lymphoma (MCL). Most BLs carry a translocation of the c-myc oncogene from chromosome 8 to either the immunoglobulin (Ig) heavy-chain region on chromosome 14 [t(8;14)] or one of the light-chain loci on chromosome 2 (kappa light chain) [t(8;2)], or chromosome 22 (lambda light chain) [t(8;22)]. This translocation juxtaposes Ig heavy chain gene (/IGH/, 14q32) sequences with the c-myc locus, leading to an overexpression of a number of genes, including the c-myc gene. The translocation site may be as far as 500 kb from the translocation point, which makes direct regulation unlikely. MCL is closely associated with the translocation (11;14)(q13;q32). This translocation juxtaposes Ig heavy chain gene (/IGH/, 14q32) sequences with the /BCL-1/locus, leading to an overexpression of a number of genes, including the cyclin D1 gene (CCND1). However, CCND1 overexpression alone is

not sufficient for hematopoietic transformation since mice transgenic for cyclin D1 do not develop MCL. Recent transcriptome studies have revealed that several genes located in the vicinity of the breakpoint on chromosome 11 are overexpressed in MCL cells. This general transcription upregulation might be due to epigenetic processes as in the BL. Chromosomes 8 and 11 are located in a largely heterochromatic region of the nucleus, while chromosome 14 is found in a more euchromatic context. We propose that the t(11; 14) and t(8; 14) translocations induce the transposition of the 11q13 or 8q22 loci from an heterochromatic to an euchromatic region of the nucleus. This movement could then cause the overexpression of the genes located on 11q13 and 8q22. We have studied the localization of the rearranged (11;14)(q13;q32) locus in MCL and (8;14) locus in BL using 3D FISH and have found that the translocated loci are relocalized within the nucleus towards the nuclear center form the peripheral regions. The biochemical and epigenetic mechanisms which may be activated by such translocations are currently under study and will be discussed.

Activation of p53 by MDM2 antagonists has differential apoptotic effects on EBV-positive and EBV-negative Burkitt's lymphoma cells

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P53 inactivation is often observed in Burkitt's lymphoma (BL) cells, due either to mutations in p53 gene or to overexpression of the p53-negative regulator, MDM2. BL is closely associated with Epstein-Barr virus (EBV) infection although not all cases are EBV(+). In EBV-infected cells, viral DNA persists in cells, leading to the production of a limited number of viral proteins. Three patterns of EBV latency have been defined, based on the differential production of these proteins. Most cases of BL express the latency I programme (only EBNA1 protein produced), but some express the latency III (all viral proteins produced). We have shown previously that in EBV(–) BL cells, reactivation of p53, induced by reducing MDM2 protein level, led to apoptosis. We show here that nutlin-3, a potent antagonist of MDM2, activates the p53 pathway in all 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. Furthermore, we also show that prior treatment with sublethal doses of nutlin-3 sensitizes EBV(–) or latency I EBV(+) cells to apoptosis induced by etoposide or melphalan, but protects latency III EBV(+) cells. $P21^{WAF1}$ which is overexpressed in the latter, is involved in this protective effect, as siRNA-mediated inhibition of $p21^{WAF1}$ restored sensitivity to etoposide. Nutlin-3 protects latency III BL cells by inducing a $p21^{WAF1}$-mediated G1 arrest. Most BL patients with wild-type p53 tumors could therefore benefit from treatment with nutlin-3 following a careful determination of the latency pattern of EBV in infected patients.

Gene repositioning upon genotoxic stress

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The translocation t(8;21)(q22;q22) affecting AML1 and ETO genes is known to be one of the frequent chromosome translocations in childhood and treatment related acute myeloid leukemia. Up to now, no data are available concerning mutual positioning of these particular genes in the nucleus of a living cell as well as the mechanism of their realignment. Here we show that there is no marked proximity between these two genes in the nuclei of normal human male Wbroblasts. Moreover, these genes are located in different nuclear layers. Treatment of cells with VP-16 (etoposide), an inhibitor of DNA topoisomerase II (Topo II) widely used in anticancer chemotherapy, causes the ETO gene repositioning which allows AML1 and ETO genes to be localized in the same nuclear layer close to the nucleoli. Inhibitor studies demonstrate that such an effect is likely to be connected with the formation of stalled cleavable complexes Topo II–DNA. Finally, inhibition of ETO gene repositioning by 2,3-butanedionemonoxime (BDM) suggests that this process depends on nuclear myosin. Together, our data corroborate the so called «breakage Wrst» model of the origins of recurrent reciprocal translocation. CHIP assay demonstrated that etoposide treatment and consequent ETO gene relocalization to the perinucleolar space stimulated enrichment of nucleolar form of DNA Topo II beta and nucleolin at Topo II cleavage site. A possible role of nucleoli in the DNA rearrangement and maintaining genome stability is discussed.