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Contents

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The influence of pH on damaged DNA processing by human apurinic/apyrimidinic endonuclease 1

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Key words: human APE1, FRET-substrate, stopped-flow kinetics

Motivation and Aim: Human apurinic/apyrimidinic endonuclease 1 (APE1) is a key participant in the cascade of DNA base excision repair reactions. APE1 initiates the search and repair of apurinic/apyrimidinic (AP) sites in DNA, which are not only cytotoxic, but also can lead to mutagenesis, if left unrepaired. It is known from X-ray diffraction data that enzyme amino acid residues interact preferentially with one of the duplex strands to form usually hydrogen bonds and electrostatic contacts. The enzyme active site is formed by a large number of polar amino acids, which provide extensive contact with the phosphate groups of the DNA substrate binding site. Therefore, the aim of this study was to elucidate the influence of pH on the efficiency of formation of the enzyme-substrate complex and catalytic reaction.
Methods and Algorithms: In the present work, we used stopped-flow fluorescence techniques to conduct a comparative kinetic analysis of the conformational changes of the enzyme and DNA substrate molecules during recognition and cleavage of the abasic site in the pH range from 5.5 to 9. DNA-substrate contained FRET pair FAM-BHQ1 and furan residue F as nonreactive analog of AP-site.
Results: We have observed the changes in FAM fluorescence during the interaction of APE1 with the FAM-F substrate lead to a fast decrease in the fluorescence intensity (within 10 ms) followed by the increase phase in the time range 50 ms to 10 s. It was proposed that the initial decrease in the FRET signal reflects the decrease in the distance between FAM and quenching BHQ1 residues due to DNA bending in the complex with APE1. This step was detected only in the case of pH from 5.5 to 7.0. The increase in FAM fluorescence intensity in the next phase most likely reflects a release of the cleaved DNA product from the complex with the enzyme. The kinetic curves were fitted to sum of exponential equations. The rate constants for the formation of the catalytically competent complex and the observed catalytic constant were calculated.
Conclusion: It was shown that the activity of APE1 increases with increasing pH due to acceleration of the rates of catalytic complex formation as well as catalytic reaction.
Acknowledgements: This work was supported by grant from Russian Science Foundation No. 18-14-00135.

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Topological properties of graph of hydrogen bonds forming in SOD1 protein indicate critical regions in its structure

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Key words: ALS, SOD1, hydrogen bonds, elastic network models, graph theory

Motivation and Aim: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which manifests in two forms: familial and sporadic [1]. The second most prevalent and studied cause of familial ALS is mutations in the SOD1 gene, which codes for the superoxide dismutase-1 enzyme. One of hypotheses on the mechanism for disease progression posits an aggregation of misfolded SOD1 proteins caused by mutations.
Methods and Algorithms: Spatial structures of the 35 mutant SOD1s were obtained by introduction of mutations into the human WT SOD1 protein structure (PDBID: 2V0A) using FoldX software. The WT and 35 mutant structures were modelled with elastic networks modelling (EN) using ElNemo with the default parameters set. A table of hydrogen bonds was constructed for the WT SOD1 and its mutants by the cpptraj utility using AmberTools 13. Rigid fragments in hydrogen bonds graph were detected by starfish [2]. A topology of the graph was constructed using nested stochastic block model from Python's graph-tool package. Graph vertices whose remove decreases connected component size in the graph were found with Python's networkx package.
Results: Mutant SOD1' residues being bottleneck vertices in the graph were found to form hydrogen bonds lacking in the WT. This can indicate that mutations in SOD1 modify hydrogen bonds network to weaker one in terms of the network connectivity. Critical regions are found inside the protein structure which are sensitive for structural adjustments and were affected by ALS-linked mutations. These protein structure regions include amino-acid residues from zinc-binding site, zinc-bindnig and electrostatic loop.
Conclusion: The results of graph analysis are in agreement with our previous work [3] in those SOD1 regions which are critical for its structure if affected by ALS mutations. These SOD1 residues identified can be responsible for the pathogenic conformations in the mutants. Taking them into account can be valuable both for understanding the molecular mechanisms of mutant pathogenicity and drug design. The results garnered in this study can aid the design of drugs that not only can prevent aggregate formation, but also repair pathogenic conformations of mutant proteins before aggregation.
Acknowledgements: Methods development was supported by the RFBR grant No. 17-54-49004 and critical regions study was supported by the Russian Science Foundation grant No. 14-44-00011.

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DNA is a new acceptor of PARP3 protein

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Key words: PARP3, mono(ADP-ribosyl)ation of DNA, DNA repair, NHEJ

Motivation and Aim: Poly(ADP-ribose)polymerases, PARPs, represent a protein family that is involves in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. Through the whole family that includes 17 participants with very different structures and cellular functions, only three proteins – PARP1, PARP2 and PARP3, possess DNA-dependent catalytic activity on attaching of ADP-ribose from NAD+-molecule to the acceptors [1]. Interestingly, that PARP1 and PARP2 catalyzed synthesis of long stretch of poly(ADP-ribose)polymers, whereas the most data are consider that PARP3 is a mono(ADP-ribose)transferase.

For a long time, amino acid residue of the protein substrate such as aspartate, glutamate and lysine, have been exclusive acceptor for PARP-enzymes. However, it was recently discovered by Talhaoui et al. that DNA-dependent PARP1 and PARP2 can also modify DNA [2, 3]. Here, we demonstrate that DNA-dependent PARP3 can modify DNA and form a specific primed structure for further use by the repair proteins.

Methods and Algorithms: In this study, we discovered the capacity of recombinant human PARP3 to modify partial DNA duplexes and investigated in detail the nature of the DNA modification and its processing by the DNA repair proteins.

Results: Present study demonstrates that DNA-dependent PARP3 can modify DNA and that this ADP-ribosylated DNA could be ligated to double-stranded DNA by DNA ligases. Moreover, ADP-ribosylated DNA could serve as a primed DNA substrate for PAR chain elongation by the purified proteins PARP1 and PARP2 as well as by cell-free extracts.

Conclusion: Based on the obtained results we propose that ADP-ribose modification of DNA can be involved in cellular pathways that are important for cell survival in the process of double-strand break formation. Moreover, our results suggest that the different PARPs might act in concert to ensure the efficiency of some cellular processes.

Acknowledgements: Supported by the Russian Science Foundation 14-24-0038.

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In vitro lesion bypass by human PrimPol

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Key words: PrimPol, DNA translesion synthesis (TLS), 8-oxo-G, thymine glycol, 5-formiluracil (5-fU), AP-site, O^6-me-G and 1,N^6-ethenoadenine (εA)

Motivation and Aim: Several human DNA polymerases efficiently incorporate dNTPs opposite DNA lesions during the process called DNA translesion synthesis (TLS). Human PrimPol is a DNA primase with DNA polymerase activity which plays an important role in maintenance of genome integrity. The primary function of PrimPol in vertebrate cells is reinitiation of stalled DNA replication forks at the DNA damage sites and non-B DNA structures. Experiments suggest that PrimPol is capable of initiating *de novo* DNA synthesis downstream of DNA damage using the DNA primase activity. *In vitro* studies also show that human PrimPol is capable of bypassing some DNA lesions in a manner of TLS-polymerase. The TLS activity of PrimPol was demonstrated opposite 8-oxo-G, abasic site (AP-site) and photoproducts. However, the efficiency and accuracy of dNTP incorporation differ among studies and the activity of PrimPol on templates with other common DNA lesions is yet to be characterized.

Methods and Algorithms: In this work, we carried out analysis of the TLS activity of human PrimPol opposite a series of common DNA lesions: 8-oxo-G, thymine glycol, 5-formiluracil (5-fU), AP-site, O^6-me-G and 1,N^6-ethenoadenine (εA).

Results: We demonstrate that PrimPol possesses the properties of TLS polymerase and, in presence of Mg^{2+} ions as metal cofactor, PrimPol carries out very efficient and accurate synthesis past 8-oxo-G and 5-fU lesions caused by oxidative stress. The steady state kinetic analysis has shown that incorporation of complementary dCTP opposite 8-oxo-G is 8-fold higher than incorporation of non-complementary dATP. PrimPol incorporates correct dATP 20-fold more efficient then dGTP opposite 5-fU. In the presence of Mg^{2+} ions, PrimPol also bypasses AP-site and O^6-me-G with moderate efficiency but is blocked opposite thymine glycol and εA. PrimPol bypasses O^6-me-G in error-prone manner preferably incorporating non-complementary dTTP opposite the lesion. Mn^{2+} ions significantly stimulate the TLS activity of PrimPol: PrimPol carries out efficient synthesis opposite all tested DNA lesions including thymine glycol and εA but demonstrates very low accuracy of dNTPs incorporation. We also demonstrate that the accessory protein PolDIP2 stimulates the TLS activity of PrimPol *in vitro*.

Conclusion: These data suggest that the TLS activity of PrimPol have possible relevant functions *in vivo*. In particular, the combined primase and polymerase activities of PrimPol might facilitate replication of DNA with clustered damage.

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The point mutations in the fingers domain increase the fidelity of DNA synthesis on undamaged DNA and abrogate DNA translesion synthesis in Y-family of DNA polymerases

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Key words: Y-family of DNA polymerases, Pol iota, Pol eta, DNA damage, active site

Motivation and Aim: Eukaryotic Y-family of DNA polymerases Pol iota and Pol eta play a key role in efficient and accurate replication of DNA with damage (DNA translesion synthesis, TLS) but demonstrate very low fidelity of DNA synthesis on undamaged DNA templates.
Methods and Algorithms: To better understand the mechanism of translesion and error-prone DNA synthesis by Pol iota and Pol eta we investigated substitutions of evolutionary conserved active site residues in the fingers domain, involved in interactions with the templating and the incoming nucleotides. We analyzed the efficiency and fidelity of DNA synthesis by the mutant human and yeast polymerase variants opposite thymine dimers, abasic site, thymine glycol, 8-oxoguanine, 1,N^6-ethenoadenine and on undamaged DNA.
Results: The point amino acid substitutions Y39A and Q59A in human Pol iota and Q55A and R73A mutations in yeast Pol eta decreased the catalytic activity of DNA polymerases on undamaged templates and significantly affected DNA damage bypass. In the presence of Mg^{2+} ions, the Q59A amino acid substitutions abolished the incorporation of dNTP opposite 1,N^6-ethenoadenine by Pol iota suggesting that the residue Glu59 plays a role in the Hoogsteen base pairs formation during catalysis. The Q55A substitution in Pol eta significantly reduced the efficiency of thymine dimers bypass, R73A had a stronger effect on the activity opposite abasic site, while both substitutions impaired replication opposite thymine glycol. Importantly, Y39A and Q59A mutations in human Pol iota and R73A and, to a lesser extent, Q55A substitutions in yeast Pol eta increased the fidelity of DNA synthesis opposite undamaged DNA.
Conclusions: Altogether, these results reveal a key role of conservative residues of the fingers domain of Y-family DNA polymerases in replication opposite various types of DNA lesions and highlight the evolutionary importance of TLS function at the cost of accuracy of DNA replication.
Acknowledgments: This work was supported by RFBR grant No. 17-00-00264 and the RAS program “Molecular Biology. New groups”.

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Development of Tdp1 inhibitors based on natural biologically active compounds

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Key words: tyrosyl-DNAphosphodiesterase I, inhibitor of tyrosyl-DNA phosphodiesterase I

Motivation and Aim: Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is a promising target for anticancer therapy based on damage of tumor DNA caused by topoisomerase-1 (Top1) inhibitors. Tdp1 plays a key role in the repair of Top1-DNA covalent complexes formed by Top1 inhibitors such as camptothecin and its clinical derivatives.
Presumably, inhibition of Tdp1 can enhance the therapeutic effect of Tor1 inhibitors, sensitizing tumor cells to their action [1].
The development of new inhibitors of DNA repair enzymes based on natural compounds and their derivatives is particularly relevant, since such compounds often have complementarity to targets of biological origin. We used derivatives of coumarin, terpenes and chromen for Tdp1 inhibitors screening.
Methods and Algorithms: Screening of compounds – potential inhibitors is carried out using a real-time fluorescence measurement method, which makes it possible to determine the initial reaction rate with high accuracy [2]. The effect of the selected compounds on the proliferation of transplanted tumor cell lines and the evaluation of cell death are studied using the MTT test.
Results: All studied compounds have a pronounced inhibitory effect. The dependence of the inhibitory activity on the structure of the compounds was revealed. Among the developed inhibitors, there are both moderately toxic and non-toxic compounds.
Conclusion: The compounds studied can be used to develop on their basis more effective inhibitors of Tdp1. The therapeutic effect of such substances can be a selective increase in the activity of Top1 inhibitors in tumors. Non-toxic inhibitors of Tdp1 are of particular interest, since will avoid additional side effects.
Asknowledgments: Supported by Russian Science Foundation (grant No. 16-13-10074).

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Comparative analysis of lactaptin produced in bacterial and eukaryotic cells. Purification and activity

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Key words: lactaptin, casein, anticancer protein, affinity chromatography

Motivation and Aim: Lactaptin is a fragment of human milk κ-casein, it has been previously shown to induce the apoptosis of cancer cells in culture with no cytotoxic activity toward non-malignant cells. Its recombinant analogue (RL2) produced in E.coli demonstrated tumor growth suppression *in vivo*. Given that correct folding of recombinant human proteins occurs in eukaryotic cells we constructed new recombinant plasmid for expression lactaptin analog EL1 (eukaryotic lactaptin 1) in human cells. Besides a leader sequence for secretion, recombinant lactaptin contained hexahistidine-tag. Recombinant lactaptin EL1 production, secretion, harvesting and purification were in the focus of our work.

Methods and Algorithms: EL1production and secretion by HEK293T cells was detected by Western-blot analysis in cell lysates as well as in cultural medium. To purify EL1 we used cation-exchange and immobilized metal ion affinity chromatography (IMAC).

Results: IMAC is a commonly used protein purification procedure. However, enrichment of eukaryotic cell culture medium with histidine and cysteine results in competitive binding of these amino asids with immobilized metal. Moreover, we found that numerous histidine-containing serum proteins contaminated EL1 eluates. To overcome these difficulties we performed cation-exchange chromatography as an initial step. The chromatography protocol (resin, ionic concentrations and pH) was optimized to remove free histidine and proteins with similar retention properties from the EL1-containing solutions. Using IMAC chromatography for subsequent EL1 purification we obtained the protein with up to 95 % purity.

Conclusion: Detailed protocol for recombinant lactaptin EL1 purification was developed.

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Activity of single nucleotide polymorphic variants of human AP-endonuclease 1

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Key words: human APE1, stopped-flow method, pre-steady state kinetics

Motivation and Aim: A single nucleotide replacement in gene can lead to developmental disorders and even to lethal effect if it occurs in reproductive cells or to carcinogenesis if mutation proceeds in somatic cells. At present, five to nine systems of damage correction were distinguished, among which the most active study is conducted with base excision repair (BER) system. This system is polycomponent and functioning due to the coordinated action of more than 10 proteins. One of the major proteins of the BER system is the AP-endonuclease 1 (APE1). The human APE1 is a globular protein of 318 amino acids with a molecular mass 35.4 kDa. The main role of APE1 is the correction of apurinic-apyrimidinic (AP) sites generated spontaneously or as a result of N-glycosylase reaction catalyzed by monofunctional DNA-glycosylases. The goal of our research was to analyze the effects of single nucleotide polymorphism of APE1 on the recognition of AP-sites in DNA and catalytic reaction.
Methods and Algorithms: In the current study, we constructed and purified by the standard methods the APE1 SNP variants: Asn222His, Arg237Ala, Arg237Cys, Gly241Arg, Arg274Gln, Pro311Ser, Met270Ala and Met270Thr. These amino acid residues were chosen because some of them are involved in DNA coordination, while others are involved in the catalytic conversion of AP-sites. Comparative kinetic analysis of catalytic processes for APE1 variants was performed by stopped-flow method. The effects of SNP APE1 on conformational changes in enzyme and specific DNA-substrates were analyzed by detection of fluorescence intensities of tryptophan and 2-aminopurine, respectively.
Results: The data obtained in the current work made it possible to determine the kinetic scheme describing interactions of the mutant forms of the enzyme with the DNA substrates, to calculate the rate constants of the elementary stages and to specify the stages of the process affected by these amino acid substitutions. Stopped-flow kinetic study for all mutant forms revealed that such amino acid substitutions change APE1 activity by influence on the different stages of enzymatic process. However, all mutant forms of the enzyme retain the ability to cleave the AP-site.
Conclusion: The natural SNPs in the enzyme APE1 display a significant effect on the recognition, binding and catalytic conversion of AP-sites in DNA.
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Disaccharide nucleosides as inhibitors of DNA repairing enzymes

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Key words: disaccharide nucleosides, poly(ADP-ribose)polymerase 1, tyrosyl-DNA phosphodiesterase 1, DNA repair

Motivation and Aim: Numerous drugs have been developed, which have or resemble nucleosidic structure. Disaccharide nucleosides is a group of natural compounds forming poly(ADP-ribose) (PAR) and found in tRNA, antibiotics, and other physiologically active compounds [1]. Earlier we have developed the synthetic methodology for the synthesis of 2′-O-α-D-ribofuranosyladenosine, a monomeric unit of PAR, which is an important biopolymer, participating in DNA repair [2]. Therefore, chemical synthesis of disaccharide analogues may be an advantageous area for the discovery of a novel compounds inhibiting DNA-repairing enzymes to increase the effectivity of anticancer therapy.

Methods and Algorithms: We have synthesized a series of disaccharide purine and pyrimidine nucleosides by the formation of an *O*-glycosidic bond between a nucleoside carrying one free hydroxyl group and an activated monosaccharide. All the compounds were characterized by NMR and UV spectroscopy and by LC-APCI and LC-HRMS. Inhibitory effect of disaccharide nucleosides was studied on two DNA-repairing enzymes – poly(ADP-ribose)polymerase 1 (PARP-1) and tyrosyl-DNA phosphodiesterase 1 (Tdp-1). PARP-1 inhibitory assay was performed using [^{3}H]-NAD$^+$. Tdp-1 activity was measured using real-time fluorescence assay in the presence of fluorophore-quencher containing oligonucleotides.

Results: In the series of disaccharide nucleosides pyrimidine derivatives were found to be effective Tdp-1 inhibitors, with IC_{50} being in low micro molar range, and weak PARP-1 inhibitors, with IC_{50} being $\sim 10^{-5}$ M. Disaccharide nucleosides did not demonstrate cytotoxic effects at concentration up to 1 mM against human cell lines.

Conclusion: Thus, the obtained results have revealed disaccharide nucleosides as a promising class of compounds, inhibiting key DNA-repairing enzymes: PARP and Tdp-1.

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Processivity of DNA repair enzymes

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Key words: target search, DNA-glycosylase, DNA repair

Motivation and Aim: The problem of rapid target search in DNA is faced by transcription factors, restriction endonucleases, DNA repair enzymes and other sequence- or structure-specific DNA-binding proteins. Theoretically, the fastest target search in DNA can be achieved by combining one-dimensional diffusion along the DNA contour (processive search) and three-dimensional diffusion (distributive search). The balance between these search modes depends on many factors affecting DNA-protein interactions, such as the presence of mono- and divalent cations, competing proteins, crowding effect, etc. Presently, the mechanisms of target search are understood only for a handful of enzymes.
Methods and Algorithms: We have recently developed an assay to study target search by DNA repair enzymes, based on cleavage of oligonucleotide substrate containing two targets. Thus, the distance between the targets can be precisely controlled, and any modification can be introduced into DNA. Subsequently, the probability of correlated cleavage (P_{cc}) is estimated, reflecting the efficiency of enzyme transfer between the specific sites. In this work, we have investigated five repair enzymes: E. coli endonuclease VIII (Nei), its human homologs NEIL1 and NEIL2, and uracil-DNA-glycosylases (UNG) from E. coli and vaccinia virus.
Results and conclusion: As expected, P_{cc} of all enzymes depended on the ionic strength of the solution and the presence of Mg^{2+}. UNG from vaccinia virus was the most sensitive to these factors, raising questions about its proficiency as a suggested processivity factor of viral DNA polymerase. Nei, NEIL1 and NEIL2 showed a peak of P_{cc} at low but non-zero ionic strength indicating that nonpolar interactions contribute to binding of these proteins to nonspecific DNA. This conclusion was also supported by analyzing amino acid conservation in the catalytic core of Nei. Introduction of bulky fluorescent group between two specific sites greatly reduced the ability of glycosylases to slide along DNA.
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Role of miR-126a in regulation of expression of anti-apoptotic protein BCL2

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Key words: BCL2 expression, miR-126, tumor cells, target *in vitro* assay

Motivation and Aim: Anti-apoptotic B cell lymphoma 2 (BCL2) family members play a key role in the regulation of intrinsic apoptosis. In many types of cancer apoptosis is suppressed due to upregulation of these proteins through multiple mechanisms. MicroRNAs (miRs) may also control *BCL2* gene expression at post-transcriptional level. Thus far 12 miRNAs have been validated to target *BCL2* [1]. Using *in silico* approach we found that miR-126 host gene *Egfl7* has ESRs and DRE binding sites in promoter [2]. Then we performed experiments to measure miR-126 expression under xenobiotic exposure and validated its interaction with 3′UTR of *BCL2* target gene.
Methods and Algorithms: We analyzed ChIP-seq data obtained from the encode project (T47D and Ishikawa cells treated with estradiol, respectively GSM803539 and GSM803422) to identify miR-coding genes with ESRs binding sites in their promoter regions [3]. To study of gene expression we used human cell cultures MCF-7, breast and endometrial cancer tissues. For detection of miR and *BCL2*, *Egfl7* expression the stem-loop RT-PCR and RT-PCR respectively were used. For luciferase reporter experiments, 293FT and HepG2 cells were transfected with the pmirGLO vector with the corresponding inserts (bcl2 3′UTR/bcl2 3′UTR mutated) and the corresponding mimic/miR-126-3p inhibitors (+126mim/+126inh).
Results: In breast cancer, BCL2 protein expression was associated with low tumor grade, ER positivity, and favorable prognosis. We have shown that BCL2 is more frequently overexpressed in endometrial cancer tissues than in normal adjoined tissues. To understand the mechanism of elevated expression of this protein in tumour cells we determined the miR126a expression, which may potentially interact with BCL2 mRNA. Since host gene *Egfl7* of this miR has ESRs and DRE binding sites we incubated MCF7 cells with various doses of benzo(a)pyrene (BP). It was shown 2 fold increase of miR126a and *Egfl7* expression under the BP exposure. At the same time, the expression level of *BCL2* target gene decreased in 5 times. Moreover, the expression level of miR-126a in luminal B type of breast cancer is reduced by 3-4 times. These results confirmed the tumor suppressor feature of this miR which may regulate *BCL2* expression. To proof this suggestion we preformed luciferase reporter experiments and have shown that luciferase activity in 293FT cells transfected with the vector containing insertion of bcl2 3′UTR and the corresponding mimic was reliably reduced by 20 %, whereas an increase in the signal by 55 % was observed with the inhibitor. Similar results were obtained on HepG cells.
Conclusion: The results showed that miR-126a may regulate BCL2 gene expression in breast and endometrial cancer. Besides, ligands of AhR capable activate the transcription of Egfl7 and miR-126 in MCF-7 cells, followed by inactivation of the BCL-2 gene expression, and may be considered as potential therapeutic agents in the treatment of these cancers.
Acknowledgements: Supported by the RSF (project No. 15-15-30012).

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Rational design of small-molecule compounds targeting CD95 programmed cell death pathway

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Key words: CD95, apoptosis, virtual screening

Motivation and Aim: There are two types of apoptosis induction: intrinsic-mediated via mitochondria and extrinsic-mediated via death receptor (DR) activation. Currently six DRs are characterized: CD95/Fas, TNF-R1, TRAILR1/2, DR3 and DR6, while CD95/Fas is one of the most studied members of the DR family. The induction of apoptosis via CD95 is largely controlled by the Death-Inducing Signaling Complex (DISC), which is formed upon CD95 stimulation. CD95 DISC comprises oligomerized, CD95, the adaptor protein FADD, procaspases-8/10 and cellular FLICE inhibitory proteins (c-FLIP). Deregulation of the CD95 pathway accompanies a variety of tumors and neurodegenerative diseases. Currently a limited number of small-molecule agents targeting this pathway is available. Development of DISC targeting compounds is of great interest and the goal of the current work.
Methods and Algorithms: Structure-based molecular modeling techniques including molecular docking, virtual screening, homology modeling and molecular dynamics simulations combined with cellular CD95 activation assays were applied.
Results: Computer-aided design of small-molecule compounds targeting DISC proteins allowed to reveal first agents with propensity to target DISC oligomerization. Novel strategies for inhibition and activation of DISC platform by allosteric and protein-protein interaction mechanisms were proposed.
Conclusion: Combination of structure-based *in silico* and cellular experimental approaches allow to reveal lead compounds for targeting CD95 pathway and development of new anticancer and new anti-neurodegenerative drugs.
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Growth of interest to research in the field of medical genetics according to the analysis of scientific publications

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Key words: interest to scientific research, apoptosis, ANDSystem, associative gene networks

Motivation and Aim: The number of publications in the areas of biology, medicine, and biotechnology grows dramatically, which makes important the computer-based analysis. To date, over 28 million of abstracts highly relevant to biology and medicine can obtained from the PubMed database, and this number keeps growing. A study of such data allows estimating the growth of interest to the researches in the field of medical genetics. The identification of genes in scientific studies for which there is a growing interest can be useful in finding promising candidates for genotyping and drug targets. Such identification can be performed using the text-mining tools.
Methods and Algorithms: The analysis of literature was performed using the ANDSystem package that incorporates utilities for automated extraction of knowledge from Pubmed published scientific texts and databases [1].
Results: On the example of analysis of publications related to apoptosis, a set of genes with a growing interest involved in apoptosis, was formed. Reconstruction of the gene network using this initial set of genes allowed to identify new genes that are functionally closely related to the initial list. The interest to these new genes may appear in the nearest future.
Conclusion: Thus, the identified gene-candidates can be promising for planning experiments on genotyping and search for drug targets.
Acknowledgements: The development of methods for analyzing interest to researches in the field of medical genetics by scientific publications was supported by the Federal target program "Research and development in priority areas of development of Russia's scientific and technological complex for 2014-2020"
Agreement on granting a subsidy as of October 23, 2017 No. 14.601.21.0015 between the Ministry of Education and Science of the Russian Federation and Tomsk NRMC on conducting research project titled: Development of a forecast for the implementation of the priority of scientific and technological development defined in paragraph 20c "personalized medicine, high-tech health care and health saving technologies, including the rational use of medicines (primarily antibacterial)" Scientific and technological development strategy of the Russian Federation. The unique identifier of the project is RFMEF160117X0015. The state agreement identifier is 0000000007417PE10002.
The reconstruction and analysis of gene networks, involved in apoptosis, was supported by the Russian Science Foundation grant "Programmed cell death induced via death receptors: Delineating molecular mechanisms of apoptosis initiation via molecular modeling" 14–44-00011.

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Biological activity of the new photoactive ruthenium nitrosyl complexes: cytotoxicity and effects on DNA repair

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Key words: photopharmacology, MTT assay, ruthenium nitrosyl complex, nitric oxide, DNA repair, BER

Motivation and Aim: Nitric oxide plays an important role in different biological processes in healthy cells, such as neurotransmission, blood pressure control, immunity, and also in the process of protecting the body against the spread of cancer cells during metastasis. The biological activity of ruthenium nitrosyl complexes is mainly related to the possibility of NO release in biological solutions due to photochemical activation or reduction by biological reductants such as ascorbic acid or NADH. On the other hand, it is known that a solution saturated with NO gas is capable of inhibiting the activity of the prokaryotic DNA glycosylase Nth, the main player of the base excision DNA repair (BER). Thus, the study of both the antiproliferative potential of nitrosyl ruthenium complexes on human cell lines, such as the study of the activity of the eukaryotic DNA repair enzymes under conditions of NO saturation *in vitro*, is necessary for understanding the mechanisms of inhibiting the proliferation of cancer cells.
Methods and Algorithms: We measured the cytotoxicity potential in human cell lines of the recently synthesized ruthenium nitrosyl complexes lines by MMT analysis. To analyzed *in vitro* activity of the eukaryotic DNA repair enzymes we used NO saturation conditions controlled by photochemical activation.
Results: In recent decades, a new direction in medicine has developed – photopharmacology, in particular, ruthenium nitrosyl complexes provide the possibility for the photodynamic therapy as potential anticancer drug. Measuring of the cytotoxicity the ruthenium nitrosyl complexes in human mammary adenocarcinoma (MCF-7) and immortalized human embryonic kidney (HEK-293) cell lines revealed that the $[RuNO(betta\text{ - }Pic)_2(NO_2)_2OH]$ complex exhibited inhibition of the growth of cancer cells at remarkably low micromolar concentrations. This ruthenium nitrosyl complex inhibited the activity some eukaryotic BER enzymes containing [4Fe-4S] cluster after controlled photoinduction *in vitro*. Thus, the ruthenium nitrosyl complexes seem are very promising for photocontrolled NO-dependent toxicity conditions for *in vitro* applications and research.
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Molecular model of DNA glycosylase stimulation by human apurinic/apyrimidinic endonuclease 1

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Key words: oxidative DNA damage, apurinic/apyrimidinic site, base excision repair, DNA glycosylase, AP-endonuclease

Motivation and Aim: The base excision repair (BER) pathway consists of sequential action of DNA glycosylase and apurinic/apyrimidinic (AP) endonuclease necessary to remove a damaged base and generate a single-strand break in duplex DNA. Human multifunctional AP endonuclease 1 (APE1) plays essential roles in BER by acting downstream of DNA glycosylases to incise a DNA duplex at AP sites and remove 3′-blocking sugar moieties at DNA strand breaks. Human 8-oxoguanine-DNA glycosylase (OGG1), methyl-CpG-binding domain 4 (MBD4), and alkyl-N-purine-DNA glycosylase (ANPG) excise a variety of damaged bases from DNA. The major human apurinic/apyrimidinic (AP) endonuclease, APE1, stimulates DNA glycosylases by increasing their turnover rate on duplex DNA substrates. At present, the mechanism of the stimulation remains unclear.
Methods and Algorithms: Electron microscopy (EM) indicated that APE1 can oligomerize onto a DNA fragment. Atomic force microscopy (AFM) revealed that APE1 oligomerization induces a kink in the DNA backbone to detect an abasic site. To elucidate the molecular mechanism of the APE1-catalyzed stimulation, we employed the stopped-flow fluorescence analyses of the interaction of APE1 with human DNA glycosylases OGG1, MBD4^{cat}, and ANPG^{cat} bound to their DNA-substrates.
Results: TEM and AFM imaging of APE1-DNA complexes showed that polymerization of APE1 on DNA proceeds in an apparently sequence-independent manner. Full-length APE1 oligomerization along DNA induces helix distortions, which in turn enable stimulation of DNA glycosylases. It was shown that truncated APE1 protein lacking the first N-terminal 61 amino acid residues (APE1-NΔ61) cannot stimulate DNA glycosylase activities of OGG1, MBD4, and ANPG^{cat} on duplex DNA substrates. Altogether, these results suggest that the first 61 N-terminal residues of APE1 participate in protein polymerization along DNA and in formation of oligomerlike complexes by binding to undamaged DNA.
Conclusion: We propose that APE1 oligomers on DNA induce helix distortions thereby enhancing molecular recognition of DNA lesions by DNA glycosylases *via* a conformational proofreading/selection mechanism. APE1-mediated structural deformations of the DNA helix stabilize the enzyme–substrate catalytic complex and promote dissociation of human DNA glycosylases from the product AP-site with a subsequent increase in their turnover rate.
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Lesion recognition by bifunctional DNA-glycosylase Endo III and its catalytic mutants

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Key words: base excision repair, structural transitions, thermodynamic analysis, pre-steady state kinetics

Motivation and Aim: Endonuclease III is a bifunctional DNA-glycosylase from *E. coli* that exhibits both N-glycosylase and AP-lyase activity. It is known that interaction of enzyme with DNA substrate proceeds through several conformational rearrangements in its structure. Here we used stopped-flow technique to register conformational dynamics of DNA substrates arising from the Endo III action.
Methods and Algorithms: Structural changes in DNA substrates were registered using fluorescent analog of DNA base incorporated opposite the specific base. The DNA duplexes contained 5, 6-dihydrouracile base, non-cleavable analog of abasic site and native guanosine as non-damaged DNA-duplex. Changes in fluorescence intensity were registered in 5–37 °C temperature range. To calculate the rate constants of DNA conformational transitions, a number of kinetic curves for each DNA substrate were obtained. Additionally, several mutant forms (K120A, D138A) were constructed to determine the functional role of each amino acid residue in the catalytic process.
Results: It was shown that mutant forms Endo III K120A and D138A lack of both N-glycosylase and AP-lyase activities. Understanding the mechanism of the catalytic complex formation was improved by thermodynamic analysis of the binding and cleavage steps. The dependences of equilibrium constants on temperature were analyzed according to the van't Hoff equation allowing calculating Gibbs free energy, enthalpy and entropy of individual interaction stages. The analysis of the temperature dependence of the reaction rate constant k_{cat} using the Eyring equation provided the values of standard activation enthalpy and standard activation entropy of the transition state.
Conclusion: The thermodynamic analyses of catalytic processes performed by Endo III from *E. coli* reveal that this enzyme employs common energetic features at the main steps of base lesion recognition as other DNA-glycosylases from human and bacteria. Mutational analysis of Endo III reveals that Lys120 takes part both in the processes of nonspecific binding and subsequent recognition of the damage because substitution K120A significantly decelerates conformational changes of the duplex during the complex formation. Moreover, substitution D138A causes a complete loss of the ability of Endo III to distort a DNA double chain.
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Novel therapeutic approaches based on lactaptin action

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Key words: apoptosis, lactaptin, oncolytic viruses, immunotherapy

Motivation and Aim: Immunotherapeutic approaches become a new hope for cancer treatment. Clinical success was demonstrated for adoptive cell transfer of T cells expressing chimeric antigen receptors (CARs) as well as for oncolytic viruses. However, both technologies are needed to be improved. Lactaptin was discovered as a molecule specifically inducing death of various cancer cells *in vitro* and *in vivo*. So we propose to use lactaptin transgene for armoring CAR NK-cells and vaccinia virus.
Methods and Algorithms: We have engineered double recombinant vaccinia virus (VV) coding human granulocyte-macrophage colony-stimulating factor (GM-CSF) and apoptosis-inducing protein lactaptin (VV-GMCSF-Lact). To engineere "armored" CAR NK-cells secreting an anticancer peptide lactaptin at the first stage we designed lentiviral constructs allowing stable transduction of human cell lines with cassettes encoding two secreted forms of lactaptin.
Results: VV-GMCSF-Lact activated a set of critical apoptosis markers in infected cells: phosphatidylserine externalisation, caspase -3, -7 activation, DNA fragmentation, up-regulation of pro-apoptotic protein BAX and efficiently decreased mitochondrial membrane potential of infected cancer cell. Investigating immunogenic cell death (ICD) markers in cancer cell infected with VV-GMCSF-Lact we demonstrated VV was efficient in calreticulin and HSP70 protein externalisation, cellular high-mobility group box-1 (HMGB1) decreasing and ATP secretion. The analysis of antitumor activity against advanced MDA-MB-231 tumor in mice revealed that VV-GMCSF-Lact delay tumor growth up to 94 %.
Lactaptin was successfully produced in HEK293T and YT cell lines. Its in vitro activity in the conditioned media was measured against a panel of sensitive cancer cells: MDA-MB-231 breast adenocarcinoma, PC3 prostate cancer and T98G glioblastoma. We evaluated that lactaptin from conditioned media showed greater than 50-fold increase in cytotoxicity compared to the recombinant lactaptin produced in *E. coli.*
Conclusion: We demonstrated that lactaptin has a great potential for improving immunotherapeutic approaches against cancer.
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Towards understanding of apoptosis regulation using computational biology

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Key words: apoptosis, system biology, CD95, death receptor

Apoptosis is a programme of cell death, which is essential to all multicellular organisms. There are two signaling pathways of apoptosis: intrinsic that is mediated by mitochondria and extrinsic, that is mediated by a family of death receptors. CD95 (APO-1/Fas) is a member of the death receptor family. The CD95 death-inducing signaling complex (DISC), comprising CD95, FADD, procaspase-8, procaspase-10, and c-FLIP; serves as a key platform for initiator procaspase-8 activation leading to induction of apoptotic and non-apoptotic pathways. The enormous advantage for studies of the death receptor networks is provided by the state of the art computational technologies, in particular by systems biology. Systems biology is an emerging field of science that combines mathematical modeling with quantitative experimental methods, providing a quantitative assessment of the signaling pathways. Despite the fact that death receptor-mediated signaling has been studied to a high level of detail, its quantitative regulation until recently has been poorly understood. This situation has dramatically changed in the last years. Creation of mathematical models of death receptor signaling, in particular in immune cells, led to an enormous progress in the quantitative understanding of the network regulation and provided fascinating insights into the mechanisms of death receptor control. It will be discussed how our systems biology studies provide new understanding of the death receptor signaling in cancer cells and create a platform for the drug development in the context of diseases associated to defects in death receptor signaling pathways.

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

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Key words: PARP1, PARP2, poly(ADP-ribose), BER, NER, role of PARP1 in longevity

Motivation and Aim: The phenomenon of nicotinamide adenine dinucleotide (NAD+)-dependent poly(ADP-ribosyl)ation catalyzed with PARP1 was discovered long time ago, but it is still unclear how this post-translational modification governs a multitude of cellular processes including DNA repair. When interacting with the damaged DNA, PARP1 catalyzes the synthesis of a long branched poly(ADP-ribose) polymer (PAR) by using NAD^+ as a substrate. PAR can be attached to the acceptor amino acid residues of nuclear proteins or to PARP1 itself. This process leads to reorganization of the functional protein complexes involved in base excision repair (BER) and in nucleotide excision repair (NER). The aim of the present research was to investigate the role of poly(ADP-ribosyl)ation in regulation of BER and NER and to search new targets of PARylation catalyzed with PARP1 and PARP2. The activities of BER and NER systems were investigated in cells of long-living animal Heterocephalus glaber (H. glaber) in comparison with cells of Mus musculus (mouse).
Methods: Biochemical and immunochemical approaches, RT-PCR, fluorescence titration methods, atomic force microscopy (AFM), light-scattering technique.
Results: PARP1 interacts with BER and NER proteins as well as with DNA intermediates of BER containing breaks or apurinic/apyrimidinic (AP-sites). PARP1 and PARP2 activities regulate BER and NER processes. The new mechanism of stimulation of PARP1 activity by multifunctional protein YB-1 which involved in BER was found. The level of poly(ADP-ribose) synthesis was higher in the cells of H. glaber in comparison with mouse cells as well as H. glaber cell extracts displayed higher level of PARP1 covalent binding to DNA probes containing chemically reactive groups.
Conclusion: The results obtained show the key role of PARP1 in regulation of BER and NER processes and the new mechanisms of stimulation of PARP1 activity by oncoprotein YB-1. The data show the elevated activity of PARP-1 in cells of long-living mammals that can speak in favor of the various roles of PARP1 in longevity and aging.
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DNA polymerases beta and lambda: gap-filling synthesis on the intact and damaged DNA templates in base excision repair

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Key words: base excision repair, cluster lesions, DNA polymerases, PARP1

Motivation and Aim: DNA polymerases beta and lambda (Polβ and Polλ) belong to the X-family and possess similar activities. Both enzymes have dRP lyase activity and the ability to effectively foster gap-filling reactions. We showed earlier that under certain conditions these DNA polymerases can catalyze lesion bypass across benzo[*a*]pyrene-derived (BP-dG) DNA adduct during repair of apurinic/apyrimidinic (AP) sites [1]. Cluster-type DNA damages that include both a modified nucleotide and a bulky lesion can be generated under combined action of oxidative stress and genotoxic polycyclic aromatic hydrocarbons derivatives. In the present study we analyzed the ability of DNA polymerases β and λ to fil gaps generated in DNA after cleavage of AP sites located in different positions to the BP-dG adduct and their interaction with AP-site cleaving enzyme – AP endonuclease 1 (APE1). Effect of DNA repair regulating protein – poly(ADP-ribose)polymerase 1 (PARP1) on Polβ and Polλ activities was also studied.
Methods: Biochemical and immunochemical approaches, fluorescence titration methods, molecular dynamic simulation.
Results: The activity of Polβ and Polλ depends on the location of the gap relative to the modified nucleotide as well as on the conformation of the bulky adduct and on the reaction conditions. DNA duplex with the AP site in 3′ adjacent (+1) position to nucleotide paired with the modified dG was the most difficult to be processed by APE1 and DNA polymerases. The repair process was more effective when both APE1 and Polβ were added simultaneously to this DNA. No functional cooperation between APE1 and Polλ was observed. Polβ was also able to insert the correct nucleotide in the gap arising after AP site hydrolysis in DNA with the AP site in position +2 and +3. Polλ was more sensitive to stereoisomeric form of BP-dG adducts and performed DNA synthesis with less fidelity than Polβ. Although PARP1 moderately inhibited both DNA polymerases, in the presence of NAD+ only Polβ activity was completely restored.
Conclusion: Polβ tolerates BP-dG adducts in DNA and in cooperation with APE1 can correctly process AP site clustered with bulky lesion. Polλ is sensitive to stereoisomeric form of BP-dG adducts and to PARP1 catalyzed modification and can play a specific role in the repair of cluster lesions.
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Tyrosyl-DNA phosphodiesterase 1 (Tdp1) and its natural mutant SCAN1 inhibitors as prototypes of drugs

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Key words: Tyrosyl-DNA phosphodiesterase 1, Tdp1 inhibitors, SCAN1

Motivation and Aim: The ability of cancer cell to recognize DNA damage and initiate repair is the key mechanism of their resistance to chemotherapy. Therefore, the search of DNA repair enzymes inhibitors can serve as a strategy for the development of anticancer drugs. One of the most promising target enzymes for drug development is tyrosyl-DNA phosphodiesterase 1 (Tdp1) [1]. It plays an important role in the removal of DNA damage caused by topoisomerase 1 (Top1), its inhibitor camptothecin and anticancer drugs (camptothecin derivatives – topotecan and irinotecan) [2]. Thus, a Tdp1 inhibitors application can potentiate tumor cells to chemotherapy.

Furthermore, there is a natural mutant of Tdp1 (SCAN1), which is responsible for the development of neurodegenerative disease – a spinocerebellar ataxia syndrome with axonal neuropathy (SCAN1). SCAN1 phenotype does not appear until the second decade of life and is not associated with an increased risk of cancer or immunodeficiency states. Probably, pathology is caused by the accumulation of covalent intermediates SCAN1-DNA formed during the reaction [3]. Presumably, suppression of SCAN1 activity will prevent the progression of the disease.

Materials and methods: The Tdp1 and SCAN1 real-time activity measurements were carried out with fluorophore-quencher-coupled DNA-biosensor, previously designed in our laboratory [4]. Cell cytotoxicity was determined using standard MTT-test [5].

Results: We performed the screening of 34 compounds – derivatives of coumarin, β-carboline and leelamine. The most active derivatives have IC_{50} values of 0.1–1 mkM against Tdp1 and 3.5–10 mkM against SCAN1. SCAN1 inhibitors were found the first time. The compound showed low cytotoxicity (> 100 mkM) on A-549 and WI-38 cell lines.

Conclusion: Tdp1 and SCAN1 inhibitors can likely underlie the development of anticancer drugs and/or drugs preventing or slowing the progressive cerebellar ataxia to improve quality of life of SCAN1 patients.

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Mechanisms of poly(ADP-ribosyl)ation and its role in genotoxic stress response

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Key words: Poly(ADP-ribosyl)ation, PARP1, DNA repair, p53, WRN, genotoxicity

Motivation and Aim: PARP1 catalyzes the posttranslational protein modification with poly(ADP-ribose) (PAR) of variable chain length and branching frequency. Poly(ADP-ribosyl)ation (PARylation) is involved in diverse cellular functions, such as DNA repair, transcription, and regulation of cell death, and contributes to various pathophysiological conditions. Importantly, PARP inhibitors are being employed and further developed in clinical cancer therapy. The overall aim of our research interests is to unravel the biochemical and cellular functions of PARP1-mediated PARylation during genotoxic stress response.
Methods and Algorithms: We employ a broad spectrum of state-of the art bioanalytic, biochemical, molecular biological, bio-imaging, and genetic techniques, including quantitative isotope dilution mass spectrometry, gene expression and proteomics analyses, high-end microscopy and automated image analysis, and genetic engineering via CRISPR/Cas9.
Results: This talk will give a brief overview of our latest published results dealing with biochemical and cellular mechanisms of PARylation, including its role in the regulation of the tumor suppressor protein p53 [1–3]. In addition the relevance of PARylation in patho-physiological mechanisms in *in vivo* mouse models will be highlighted [4, 5]. Furthermore, unpublished results will be presented.
Conclusion: PARylation represents a complex and versatile post-translational modification with key roles in genotoxic stress response on a cellular level and significant contributions to patho-physiological states on the organismic level. Thereby pharmacological interventions hold a high potential in terms of cancer treatment and other pathophysiological conditions.
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Modulatory effect of PAR on PARP1-YB-1 interactions

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Key words: Y-box binding protein 1 (YB-1), base excision repair (BER), poly(ADPribose)polymerase 1 (PARP1), poly(ADP-ribose) (PAR)

Motivation and Aim: Y-box-binding protein 1 (YB-1) is a multifunctional protein involved in a lot of cellular processes. YB-1 is a DNA- and RNA-binding protein. By binding to nucleic acids, YB-1 is engaged in many DNA- and mRNA-dependent processes such as DNA replication and repair, transcription and mRNA translation. Currently, YB-1 is considered as one of noncanonical proteins of base excision repair (BER). YB-1 has an increased affinity to damaged DNA and interacts in vivo and in vitro with several BER proteins, regulating their activities. Recently, it was shown that YB-1 also interacts with poly(ADP-ribose)polymerase 1 (PARP1), the key regulator of BER. PARP1 binding to damaged DNA results in its activation followed by synthesis of nucleic acid-like polymer of ADP-ribose (PAR) originating from NAD^+. As a protein post-translational modification, PAR performs numerous functions in the regulation of BER. The main target of PARylation is PARP1 itself. It was found that PARP1 modify YB-1 with PAR, and YB-1 is able to stimulate the activity of PARP1. Interestingly, YB-1 also interacts non-covalently with PAR. The aim of present research was to investigate potential role of PAR on PARP1-YB-1 interactions.
Methods: Gel-mobility shift analysis, gel electrophoresis
Results and Conclusion: In the present work we explored the influence of purified PAR on YB-1-PARP1 interplay during PARylation. Our data demonstrate that PAR in low concentration may promote interactions of YB-1 and PARP1, resulting in increased modification of both proteins.
Conversely, YB-1 modification level and PARP1 activity are decreased by excess PAR due to disconnection of functional coupling of YB-1 and PARP1.To conclude, we found that the interaction of PARP1 and regulatory protein YB1 may be modulated by PAR during PARylation process.
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Correlation of markers of autophagy andregional metastasis in colorectal cancer

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Key words: colorectal cancer, lymphatic metastasis, autophagy, Beclin-1, mTOR

Motivation and Aim: Colorectal cancer (CRC) is the third most frequently diagnosed cancer in the World, more than one million people are diagnosed colon cancer annually. Despite the fact that colorectal cancer is histological homogeneous, in some cases it is impossible to determine the further prognosis and treatment taking into account the tumor stage and it's differentiation. This problem is due to the fact that each tumor has a unique molecular profile, which is characterized by various genetic and epigenetic changes. Over this issue the approach to the treatment of CRC also based onevaluation of tumor molecular and genetic profile. Such technique allows choosing a more specific treatment and predicting the tumor response to therapy. Considering the fact that colorectal cancer is a genetically heterogeneous tumor, the prognosis and treatmentprimarily depends on molecular pathogenesis that is why researching of autophagy and low molecular weight markers in tumor is very promisingly.To study the correlation of autophagy between lymphatic metastasis in CRC and to evaluate the expression characteristics of the studied proteins in the tissue of the primary tumor.
Materials and Methods: We investigated the surgical material obtained from 100 patients with colorectal cancer (stage T1-4N0-2M0), the mean age of patients being 57.4±9.1 years. Treatment before the operation was not carried out. By a standard technique, the tissue of the primary tumor node, as well as all the removed and lymph nodes, was histological examined.Primary tumor node and all removed lymphatic nodes were evaluated by standard histological methods. Tumor stage was established by WHO (2010) classification. All cases which wereordinary adenocarcinoma. We also evaluated lymphatic nodes for presents or absence metastatic lesions. The tissue of the primary tumor was subjected to immunohistochemical examination.We used antibodies to Beclin-1 (Abcam, Anti-beclin 1 antibody, rabbit, polyclonal) and mTOR (SpringBioscience, RabbitAnti-HumanmTORPolyclonalbAntibody, rabbit polyclonal) Expression of the above proteins was assessed using the Histo-score method.
Results: The received data indicate that expression of autophagy proteins Beclin-1 and m-TOR was same positive as presence of lymphatic metastasis as their absence. The results also show that the level of expression Beclin-1 and mTOR is significantly higher in those cases when lymph nodes are affected by metastases (respectively: for Beclin-1 – $p = 0.004$, for mTOR – $p = 0.004$.) We also established positive correlation between level of expression beclin-1 in cells of adenocarcinoma and amount of effected lymphatic nodes.
Conclusion: In this research we established correlation between the expression of Beclin-1 and mTOR proteins in adenocarcinoma cells with lymphatic metastasis.

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Analysis of programmed cell death in associative gene Network of glaucoma reconstructed using ANDSystem

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Key words: primary open-angle glaucoma (POAG), apoptosis, ANDSystem, gene networks

Motivation and Aim: Nowadays one of the leading causes of irreversible blindness is primary open angle glaucoma (POAG) affecting more than 44 million people worldwide. Excess apoptosis is one of the factors involved in degradation of retinal ganglion cells during POAG [1]. In this work analysis of the role of apoptosis in associative gene network of POAG was performed.

Methods and Algorithms: The list of genes associated with POAG was created based on the information from databases (OMIM, ClinVar, GWAS catalog and SNPedia) and ANDSystem [2]. Genes involved in apoptosis were taken from KEGG database. Gene network reconstruction and analysis was performed using ANDSystem.

Results: Automatic analysis of information from databases and ANDSystem revealed 156 genes associated with POAG. Gene Ontology enrichment analysis showed that these genes are involved in apoptosis, extracellular matrix organization, angiogenesis and other biological processes. According to the value of betweenness centrality TP53 is one of the most central participants of both POAG and apoptosis gene networks. It was shown, for example, that SNPs in TP53 are associated with POAG and protein p53 can decrease the level of MMP1 (its level is increased in POAG). The elevated MMP1 can participate in activation of collagen degradation. Collagen breakdown, in turn, is involved in trabecular meshwork disruption, which is a known pathogenesis factor of POAG.

Conclusion: It was shown that the apoptosis pathway is tightly related to POAG gene network. Apoptosis genes play the central role in the gene network of POAG. Gene TP53 was found to be one of the most central players in both POAG gene network and apoptosis pathway.

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Translesion DNA synthesis by DNA polymerase iota and it's variants

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Key words: DNA polymerase iota, DNA translesion synthesis, polymorphisms, 8-oxo-G, thymine glycol, AP-site, O^6-me-G and 1,N^6-ethenoadenine (εA)

Motivation and Aim: Genomic DNA is continuously attacked by exogenous and endogenous agents in cells. DNA lesions, if unrepaired, block replication fork progression causing stress. Moreover, DNA damage lead to genomic mutations and related diseases such as cancer. Specialized DNA polymerases are able to bypass lesions in the process called DNA translesion synthesis to overcome replication stress. Human polymerase iota (pol i) is a Y-family DNA-polymerase which as able to bypass a variety of endogenous DNA lesions (O^6-me-G, TG, 8-oxo-G, AP). The structure of pol i active site also stabilizes Hoogsten base pairing between the templating and incoming nucleotides and promotes efficient and accurate DNA synthesis opposite some bulky lesions such as ethenoadenine (eA). Several amino acid polymorphisms of human pol i were recently described (including clinically relevant variants) but their effect on the TLS activity of enzyme is yet to be determined.
Methods and Algorithms: In this work, we analyzed the efficiency and fidelity of DNA synthesis opposite AP-site, thymine glycol, 8-oxo-G, O^6-me-G and e-A by several pol i variants: R71G, ?236?, ?251?, ?532?, ?706?. Amino acid substitutions were introduced by site-directed mutagenesis and corresponding proteins were purified from *S. cerevisiae*.
Results and Conclusions: We showed that ?236?, ?251?, ?532?, ?706? variants did not significantly affect the TLS activity pf pol i. However, R71G pol i variant dramatically impaired the catalytic activity and changed the specificity of dNTP incorporation opposite DNA lesions. The R71G polymorphism significantly reduced the ability to bypass all tested lesions but eA. In particular, R71G variant incorporated dATP, dTTP and dCTP opposite AP-site and 8-oxo-G with lower efficiency compared to the wild type enzyme. We also observed decrease in incorporation of dCTP opposite O^6-me-G, TG and eA lesions. Nevertheless R71G variant showed the DNA polymerase activity similar to the wild type enzyme when replicating through eA suggesting that this polymorphic variant supports Hoogsteen interactions during base paring in the active site of pol i.
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PARPs and PARGs orchestrate the assembly/disassembly of FUS/Poly(ADP-ribose) compartments at DNA damage sites and FUS translocation to cytoplasmic stress granules

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Key words: poly(ADP-ribosyl)ation, mRNA-binding proteins

Motivation and Aim: The stability of the genome is controlled by a complex machinery of repair that counteracts DNA damage, the major guilty in cancer and ageing related diseases [1]. After DNA damage, mammalian cells trigger a cascade of events that starts from poly(ADP-ribose)polymerases (PARPs) recruitment to site of DNA damage. Nuclear PARPs, PARP1 [2], recognize damaged DNA and synthetize long and branched poly(ADP-ribose) polymers (PAR) resulting in covalent modification of itself and other DNA binding proteins [2]. The protein poly(ADP-ribosyl)ation (PARylation) is a reversible post-translational modification. PAR polymers covalently attached to acceptor proteins is hydrolyzed by poly(ADP-ribose) glycohydrolase (PARG), which makes protein PARylation a reversible post-translational modification [2]. Recently, PARylation has been shown for RNA binding proteins, which accumulate in DNA regions damaged by short laser beam exposures raising issues about their putative role in DNA repair [3]. Here we focus our attention on the RNA binding protein FUS (fused in sarcoma) during genotoxic stress. FUS is one of the most abundant RNA-binding proteins that can be PARylated after exposure to genotoxic stress and interact with PAR. However, the role of the PAR dependent FUS accumulation at damaged DNA in the cellular response to genotoxic stress remains unclear.
Methods and Algorithms: To address the above-mentioned issue, we developed an original approach based on a single molecule analyzes by atomic force microscopy (AFM). Such approach enables to visualize the molecular assemblies formed by FUS at DNA damage sites after PARP1 activation *in vitro*. In cells, we used immunofluorescence, immunoblotting and RNA interference to detect intracellular compartmentalization of FUS after exposure to genotoxic stress.
Results: In vitro, we found that FUS is recruited to DNA damage sites by binding to PAR and subsequently forms large molecular assemblies thus concentrating damaged DNA into compartments and demonstrated their reversibility through the hydrolysis of PAR by PARG.
Conclusion: We suggest that PAR-dependent relocation of FUS may participate to an adapted translational response to DNA damages.
Acknowledgements: Supported by Vernadsky program of the French Embassy in the Russian Federation and RFBR 18-04-00882.

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Non canonical roles of BER enzymes in RNA processing: novel perspectives in cancer biology through the study of APE1 RNA- and protein-interactomes

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Key words: BER, RNA, apurinic/apyrimidinic endonuclease 1 (APE1), interactome

The Base Excision Repair (BER) pathway, initially studied as a mere DNA repair pathway, has been later found to be implicated in the expression of cancer related genes in human. For several years, this intricate involvement in apparently different processes represented a mystery, which we now are starting to unveil. The BER handles simple alkylation and oxidative lesions arising from both endogenous and exogenous sources, including cancer therapy agents. Surprisingly, BER pathway involvement in transcriptional regulation, immunoglobulin variability and switch recombination, RNA metabolism and nucleolar function is astonishingly consolidating. An emerging evidence in tumor biology is that RNA processing pathways participate in DNA Damage Response (DDR) and that defects in these regulatory connections are associated with genomic instability of cancers. In fact, many BER proteins are associated with those involved in RNA metabolism, ncRNA processing and transcriptional regulation, including within the nucleolus, proving a substantial role of the interactome network in determining their non-canonical functions in tumor cells. Mammalian apurinic/apyrimidinic endonuclease 1 (APE1) is a key DNA repair enzyme in canonical BER involved in genome stability but also in the non-canonical expression of genes involved in oxidative stress responses, tumor progression and chemoresistance. However, the molecular mechanisms underlying APE1's role in these processes are still unclear. Recent findings point to a novel role of APE1 in RNA metabolism. Through the characterization of the interactomes of APE1 with RNA and other proteins, we demonstrate here a role for APE1 in pri-miRNA processing and stability via association with the DROSHA processing complex during genotoxic stress. We also show that endonuclease activity of APE1 is required for the processing of miR-221/222 in regulation expression of the tumor suppressor PTEN. Analysis of a cohort of different cancers supports the relevance of our findings for tumor biology. We also show that APE1 participates in RNA- and protein-interactomes involved in cancer development, thus indicating an unsuspected post-transcriptional effect on cancer genes. Maybe these new insights of BER enzymes, along with their emerging function in RNA-decay, may explain BER essential role in tumor development and chemoresistance and may explain the long-time mystery. Although recent works have provided tremendous amount of data in this field, there are still lot of open questions.

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Autophagy modulation by antitumor protein lactaptin

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Key words: lactaptin, cancer, inducers and inhibitors of authophagy, cytotoxic and antitumor effect

Motivation and Aim: Lactaptin, the proteolitic fragment of human milk kappa-casein, induces apoptosis of various cultured cancer cells. Earlier we have demonstrated lactaptin treatment led to processing LC3-I to LC3-II form in treated cells that was defined as autophagy. Combination of lactaptin with autophagy inhibitor chloroquine (CQ) strongly increased their cytotoxicity against various cancer cells. We supposed that lactaptin besides apoptosis induced also pro-survival autophagy in treated cancer cells. However, lactaptin-dependent autophagosome formation and autophagic flux have not been demonstrated yet.
Methods and Algorithms: Lactaptin analog (RL2) produced in *E. coli* was used for experiments. Autophagosome formation has been detected by transmission electron microscopy. Changes in autophagy-related protein were detected by Western Blot analysis. Lysosome dynamic was monitored by fluorescent microscopy. Autophagy inhibitors and inducers were used to increase or decrease lactaptin-dependent autophagy.
Results: Effective suppression of RL2-induced autophagy by inhibitor CQ was confirmed by electron microscopy: in the presence of RL2 and CQ the number of autophagosomes two-times increased without the implementation of catabolic processes (autophagic flux). It was found that spermidine and 3-methyladenine (3MA) did not affect the cytotoxicity RL2 *in vitro*, but CQ, Ku55933 and rapamycin increased cytotoxic effect of RL2 *in vitro*. Thus, RL2 stimulates pro-survival autophagy of cancer cells, and its inhibition potentiates the cytotoxic effect of RL2 *in vitro*. It was shown that RL2 treatment decreased p62 while in combination with inhibitors of autophagy (3MA, CQ, Ku55933) we detected up-regulation of p62.
Tumor growth inhibition and survival outcomes after RL2 and CQ treatment were estimated using mice, bearing cyclophosphamide-resistant lymphosarcoma RLS. We demonstrated that intravenous injections of RL2 (12 mg/kg) in combination with CQ (50 mg/kg) enhanced the antitumor effect of monotherapy. Tumor growth inhibition was 24.9 % for RL2, 78.4 % for CQ and 86.5 % for RL2 in combination with CQ. It was shown that survival rate in the group receiving RL2 and CQ was 100 % compared to 22.2 % in the control.
Conclusion: Autophagy inhibitors potentiate the cytotoxic effect of RL2 *in vitro*. Combination of lactaptin analog with CQ enhanced antitumor effect in mice.
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CRISPR/Cas9-mediated cleavage of protospacer with DNA damage

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Key words: DNA damage, CRISPR/Cas9 system, Cas9/sgRNA cleavage

Motivation and Aim: A CRISPR/Cas9 system widely uses in the genome editing. The main principle of genome editing is the formation of double-strand breaks in DNA and following DNA reparation. A Cas9/sgRNA complex is targeted to the protospacer in DNA by PAM recognition and R-loop formation. It was shown that mismatches in the sequence of a protospacer influence negatively on the DNA binding and cleavage by the Cas9/sgRNA complex [1]. However, an influence of the DNA damage on cleavage efficiency by the Cas9/sgRNA previously wasn't studied. Thereby we decided to investigate the Cas9/sgRNA cleavage of duplexes and plasmids with point DNA lesions in PAM and the protospacer sequence.
Methods: To detect the DNA cleavage product *in vitro*, we've obtained radiolabeled duplexes with substitutions such as 8-oxo-2′-deoxyguanosine (oxoG), uridine (U) and tetrahydrofuran abasic site (F). These substitutions contained both in PAM (5′-TGG-3′) and the protospacer sequence. In addition, plasmids with the same substitutions as in duplexes have been used as substrates.
Results: Surprisingly, any substitutions in PAM sequence (5′-TGG-3′) of guanosine on the tetrahydrofuran abasic site or 8-oxo-2′-deoxyguanosine led to cleavage resistance for duplex substrates. The opposite situation was observed when substrates were plasmids. 8-Oxo-2′-deoxyguanosine in the second and third positions of PAM partially reduced of cleavage efficiency while tetrahydrofuran abasic sites in the same position didn't effect on activity of the Cas9/sgRNA complex. The cleavage efficiency of duplexes with substitutions at the protospacer positions upstream of PAM of non-target strand decreases in the row (8G → F) – (8G → oxoG) – (16C → U) – (14C → U).
Conclusion: Our results have shown that the DNA cleavage by the Cas9/sgRNA complex depends not only on the type of DNA damage, but also on the type of substrate.

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Structural and biochemical insights of new atypical FPG/NEI DNA – glycosylases

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Key words: DNA damage, DNA repair, formamidopyrimidine–DNA glycosylase, endonuclease VIII, protein crystalology, Bacteroides thetaiotaomicron

Motivation and Aim: DNA glycosylases are enzymes that maintain genome integrity and they are a key element of base excision DNA repair. Due to their ability to recognize base lesions created by ionizing radiation, alkylating and oxidizing agents, these enzymes are still a subject of particular interest and can find use in different areas. Recently, by whole-genome sequencing of some bacteria reveal new Fpg/Nei-like DNA – glycosylases. However, these new Fpg/Nei-like enzymes show very low percentage of sequence identity to existing representatives of Fpg/Nei superfamily. In this work we aim to investigate structural and biochemical aspects of new Fpg/Nei-like DNA – glycosylases of different bacteria.
Methods and Algorithms: We have used molecular cloning and optimized protocol of protein purification to get new Fpg/Nei-like proteins. Using biochemical approaches, we have got first understanding of affinity and substrate specificity of these enzymes. By protein crystallography method we have crystallize and resolve the structures of new Fpg/Nei-like proteins from *Bacteroides thetaiotaomicron*.
Results: In the molecule of Fpg/Nei superfamily belonging proteins there are some structural features that provide the mechanism of catalysis. Most Fpg/Nei glycosylases have high-conservative PELPEVET *N*-terminal motif and use their *N*-terminal proline residue as a key catalytic nucleophile. Additionally, Fpg/Nei proteins have helix-two-turn-helix domain and zinc-finger motif to contribute residue to the active site. However, new Fpg/Nei-like proteins have atypical N-terminal residues such as isoleucine and lysine and significant diversity of *N*-terminal motif. We have crystallized Fpg/Nei-like protein from *Bacteroides thetaiotaomicron* with 2.0 A resolution and reveal the pretense of Zn-finger domain in C-terminal of protein, a-helix rich region in N-terminal and some another Fpg-like features. However, substrate preferences make these proteins similar to Nei. *Conclusion*: We have revealed structural and biochemical aspects of new atypical Fpg/Nei-like proteins to determine relationships these proteins to known glycosylases families by protein crystallographic approach.
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TDP 1 inhibitors as potential antitumor drugs

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Key words: tyrosyl-DNA polymerase 1 inhibitors, usnic acid, bile acids

Motivation and Aim: Tyrosyl-DNA-phosphodiesterase 1 (Tdp1) is promising target for antitumor therapy based on damage of tumor DNA, induced by topoisomerase 1 (Top1) inhibitors such as camptothecin and its clinical derivatives [1]. Thus, inhibition of Tdp1 may enhance therapeutic effect of Top1 inhibitors, sensitizing tumor cells to their action [2]. The objectives of the study were: search of effective Tdp1 inhibitors among derivatives of the natural biologically active compounds usnic and bile acids; definition for the most effective inhibitors their own cytotoxicity against tumor cell lines and their influence on the cytotoxic effect of camptothecins; study of the influence of the most promising compounds on the tumor growth and metastasis of Lewis carcinoma *in vivo*.
Methods and Algorithms: The inhibitory properties of compounds were determined using real-time detection of Tdp1 activity [3]. Cytotoxicity of compounds along and in combination with camptothecin derivative topotecan was determined using a standard MTT test for tumor cell lines. For *in vivo* experiments, mice C57BL/6 were used with Lewis carcinoma metastasizing into the lungs.
Results: *In vitro* studies have established high inhibitory activity of usnic acid derivatives against Tdp1 (half inhibitory concentrations IC_{50} were in the range of 20 to 150 nM). Also we have found a new class of Tdp1 inhibitors based on the bile acid scaffold with IC_{50} up to 0.29 μM. In combination with topotecan, some of usnic acid derivatives increase the cytotoxic effect of the former from 2 to 12 times, being used in the non-toxic concentrations. *In vivo* studies have shown that under the influence of the usnic acid derivatives, a 30 % decrease in the volume of the primary tumor and at least two-fold enhancement of the antimetastatic effect of topotecan on Lewis carcinoma take place.
Conclusion: The use of the Tdp1 inhibitors made it possible to unequivocally demonstrate increasing influence on the antitumor effect of topotecan in relation to the transplanted tumor of mice, both on its primary grafts and on distant metastases.
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Poly- and mono(ADP-ribosyl)ation of DNA strand breaks by PARP2 and PARP3 enzymes

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Key words: PARP2, PARP3, DNA PARylation, BER

DNA dependent poly(ADP-ribose) polymerases (PARP1-3) play important roles in the repair of DNA strand breaks and are known to be activated in the presence of DNA strand breaks to catalyze poly- or mono(ADP-ribosyl)ation (PARylation or MARylation, respectively) of themselves or other nuclear acceptor proteins. Each PARP recognizes distinct sets of DNA structures with breaks, suggesting that PARP1, 2 and 3 have non-overlapping functions in DNA repair. At present, the detailed molecular mechanisms of PARP-dependent DNA breaks repair remain unknown. Previously, we showed that mammalian PARP1 and PARP2 can catalyze covalent addition of ADP-ribose units not only to proteins but also to DNA strand breaks containing terminal phosphates or a 2′-OH group, thus producing a covalent PAR–DNA adduct in vitro. The PARP-catalyzed DNA PARylation is a reversible process because PAR can be entirely degraded by poly(ADP-ribose) glycohydrolase (PARG). Here, we examined DNA ADP-ribosylation activity and the DNA substrate preference of PARP3 as compared with structurally similar PARP2. PARP3 can effectively produce MAR–DNA adducts covalently linked to terminal phosphates at DSB and SSB termini of short and long DNA molecules, exhibiting similar substrate specificity with PARP2. Notably, ADP-ribosylation of 5′-terminal thiophosphates at DSB termini by PARPs generates MAR–DNA adducts resistant to PARG hydrolysis. We found that depending on configuration of DNA strand breaks, the DNA termini can become preferred acceptor sites for ADP-ribosylation as compared to proteins. According to the data obtained, we propose a putative mechanistic model of DNA strand break-oriented DNA ADP-ribosylation by PARP3 or PARP2. Our findings reveal effective PARP3- or PARP2-catalyzed ADP-ribosylation of ~3-kb DNA plasmid-based substrates and DNA PARylation activity in nuclear extracts from HeLa cells. Finally, immunoblotting of purified genomic DNA from PARG-depleted HeLa cells after genotoxic treatment provides indirect evidence of the presence of PAR–DNA adducts in live cells. These results suggest that certain types of complex DNA breaks can be effectively ADP-ribosylated by PARPs in cellular response to DNA damage.

Acknowledgements: This work was supported by grants from La Ligue Nationale Contre le Cancer "Equipe LNCC 2016", EDF RB 2016-17, CNRS PRC-Russie 1074 "REDOBER", Russian Science Foundation 14-24-0038 and 17-74-20075.

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Targeted DNA damage and repair: the cell's multitool for genome regulation

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Key words: DNA damage, DNA repair, epigenetics, targeted mutagenesis

Motivation and Aim: Cytosine methylation is a generally acknowledged mechanism of gene activity regulation. Disregulation of epigenetic DNA methylation accompanies almost all cancers. While the mechanism of methylation has been known for a while, until recently it was not clear how the 5-methylcytosine (mC) marks could be erased from DNA. In the past few years, it was discovered that mC is removed by controlled damage by TET1–TET3 dioxygenases and/or AID and APOBEC deaminases followed by excision by DNA glycosylases, the enzymes that so far have been implicated in genome maintenance through DNA repair.
Methods and Algorithms: We have used molecular dynamics computer simulation to explain the specificity of the demethylation enzymes and Monte Carlo simulation to analyze the flows through this pathway. In addition, we have biochemically characterized plant enzymes that directly remove mC from DNA, and processing of the 5′-adjacent nucleotides during base excision repair.
Results: TET2 dioxygenase and TDG and MBD4 DNA glycosylases, engaged in the active demethylation pathway, seem to derive their specificity for DNA bases in the active demethylation pathway from being able to correctly position them in the active site pocket, unlike the non-substrate bases. The repair steps are limiting the flow through the whole active demethylation pathway. In addition to the canonical pathway direct mC damage, methylation in human cells can be erased through off-target DNA repair when the initiating lesion resides nearby mC, and artificially erased by foreign mC–DNA glycosylases. Overexpression of plant demethylating DNA glycosylase ROS1 in human cells also caused a global decrease in DNA methylation.
Conclusion: Targeted DNA damage and repair emerge as a new paradigm of genome dynamics, in which a number of non-canonical DNA bases are not strictly unwanted lesions that need to be removed as soon as possible but play an important role as regulatory marks.
Acknowledgements: Supported by RSF (No. 17-14-01190), FASO (No. 0309-2018-0021) and NSU (No. 5-100 Program).

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Cross-talk between apoptosis and autophagy: the role of suppressed translation

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Key words: macroautophagy/autophagy inhibition, suppressed translation, caspase-8-dependent apoptosis

Macroautophagy/autophagy inhibition under stress conditions is often associated with increased cell death. Recent data demonstrate a transcriptional regulation of several autophagy genes as a mechanism that controls autophagy in response to starvation. We found that despite the significant upregulation of mRNA of the essential autophagy initiation gene *ULK1*, its protein level is rapidly reduced under starvation. Although both autophagic and proteasomal systems contribute to the degradation of ULK1, under prolonged nitrogen deprivation, its level was still reduced in *ATG*7 knockout cells, and only initially stabilized in cells treated with the lysosomal or proteasomal inhibitors. Under starvation, protein translation is rapidly diminished and, similar to treatments with the proteosynthesis inhibitors, is associated with a significant reduction of ULK1. Inhibiting the mitochondrial respiratory complexes or the mitochondrial ATP synthase leads to upregulation of the ULK1 mRNA and protein expression in an AMPK-dependent manner. These inhibitors could also drastically increase the ULK1 protein in lung adenocarcinoma cells (LACC) with knockout of the *ATG13*, where the ULK1 expression is significantly diminished. We also found that under nutrient limitation, activation of caspase-8 was significantly increased in autophagy-deficient lung cancer cells, which precedes mitochondria outer membrane permeabilization, cytochrome *c* release, and activation of caspase-9, indicating that under such conditions the activation of caspase-8 is a primary event in the initiation of apoptosis as well as essential to reduce clonogenic survival of autophagy-deficient cells. As expected overexpression of inhibitor of FLICE reduces caspase-8 activation and apoptosis during starvation, while its silencing promotes efficient activation of caspase-8 and apoptosis in autophagy-deficient LACC even under nutrient-rich conditions. Similar to starvation, inhibition of protein translation leads to efficient activation of caspase-8 and cell death in autophagy-deficient LACC. Thus, here for the first time we report that suppressed translation leads to activation of caspase-8-dependent apoptosis in autophagy-deficient LACC under conditions of nutrient limitation. Our data suggest that targeting translational machinery can be beneficial for elimination of autophagy-deficient cells via the caspase-8-dependent apoptotic pathway.
Acknowledgements: This research was supported by grant from RSF (14-56-00056).

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Компания Huawei является ведущим мировым поставщиком ИКТ-решений. Благодаря установлению взаимовыгодных отношений с нашими партнерами и заказчиками компании Huawei удалось добиться существенных преимуществ в сфере операторских сетей, корпоративного и потребительского бизнеса, а также в сфере облачных технологий. Мы стремимся создавать максимальные преимущества для операторов связи, предприятий и потребителей путем разработки конкурентных ИКТ-решений и услуг. Оборудование и решения Huawei используются в более чем 170 странах мира. Компания обслуживает более трети населения земного шара.

Имея богатый опыт и технические знания в области НИОКР, Huawei придерживается стратегии тесного сотрудничества и интеграции с корпоративными заказчиками и предоставляет им широкий спектр высокоэффективных клиентоориентированных ИКТ-решений и услуг, на базе глубокого понимания их потребностей. Согласно этой стратегии Huawei предлагает широкий выбор передовых ИКТ-решений в сфере государственного управления, общественного сектора, финансов, транспорта, электроэнергетики, крупных предприятий, а также малых и средних предприятий (SME). Эти решения охватывают корпоративные сети, универсальные системы связи и взаимодействия (UC&C), системы облачных вычислений и центры данных, системы корпоративной беспроводной связи, сетевого электропитания, а также инфраструктурные услуги.

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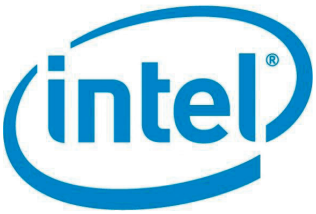

Корпорация Intel

Корпорация Intel была основана в 1968 году Робертом Нойсом и Гордоном Муром. На протяжении 50 лет Intel создает инновационные технологии, открывающие новые возможности для людей.

Корпорация Intel является мировым лидером в области микроэлектроники и информационных технологий. Intel создает технологии для умного мира эпохи больших данных. Основное внимание корпорация уделяет созданию интеллектуальных решений для умного мира, от устройств Интернета вещей и пользовательских ПК до коммуникационной инфраструктуры, технологий для центров обработки данных и суперкомпьютеров.

Штаб-квартира корпорации расположена в г. Санта-Клара, шт. Калифорния. Общий штат Intel насчитывает более 100 тыс. сотрудников в более, чем 60 странах по всему миру. Главным исполнительным директором корпорации является Роберт Свон (Robert Swan).

Intel в России

Первое представительство Intel в России было открыто в 1991 году в Москве. Сегодня в российских офисах Intel в Москве и Нижнем Новгороде работают более 800 человек.

В московском офисе компании представлены отделы маркетинга и развития бизнеса, группы по разработке программного обеспечения, юридический отдел.

В НИОКР центре Intel в Нижнем Новгороде создаются новые и инновационные продукты для разработки ПО. Сегодня он является одним из крупнейших центров исследований и разработок Intel в Европе. Более **700 специалистов и инженеров** разрабатывают программные инструменты и приложения для архитектур Intel. В Нижнем Новгороде также размещаются различные группы поддержки бизнеса (например, административно-хозяйственная часть, финансовый отдел, отдел ИТ, отдел кадров).

Центр исследований и разработок Intel в Нижнем Новгороде

Нижегородский офис Intel был является центром экспертизы корпорации в области высокопроизводительных вычислений, разработки программного обеспечения в области численных методов и беспроводной связи.

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(ООО «МПБА диагностика»)
Адрес: 109147, г. Москва, ул. Марксистская, д. 3,
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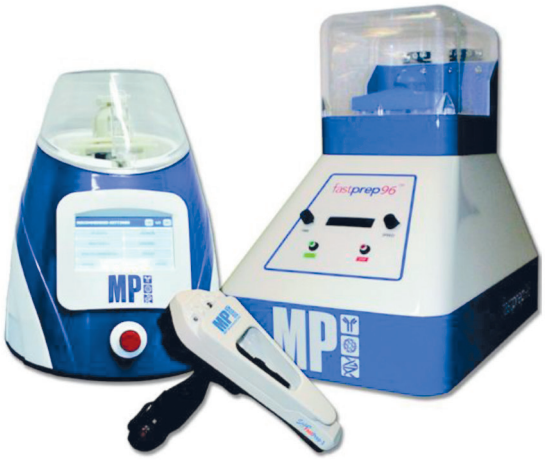
☎ Тел./факс:
+7(495)604-13-44
E-mail rus@mpbio.com

WEB mpbio.com; mpbio.ru

Компания ООО «МПБА диагностика» является дочерней компанией MP Biomedicals, ранее известной как ICN Biomedicals, основанной в 1959 году, признанного лидера в области производства широкого спектра химических реактивов, оборудования для пробоподготовки (система для гомогенизации FastPrep) и наборов реагентов. Каталог продукции компании MP Biomedicals включает более 55000 наименований высококачественных продуктов для проведения биохимических исследований, фармацевтического и биотехнологического производства, для различных отраслей иммунологии и генетики.



ООО «Рош Диагностика Рус» – официальный импортер продукции Roche в России и лицензиат компании F.Hoffmann–La Roche Ltd.

Roche Sequencing Solutions, подразделение Roche, ориентированное на решения для NGS, а в частности на пробоподготовку к NGS, предлагает:

-**Наборы KAPA Biosystems для приготовления библиотек ДНК** (включают баркодированные адаптеры, частицы для очистки, наборы для оценки концентраций ДНК и библиотек методом ПЦР в реальном времени).

-**Наборы для направленного отбора генов перед NGS:**

NimbleGen SeqCap EZ –гибридизационное обогащение панелей генов, экзомов, транскриптомов и метиломов;

HEAT-seq - амплификационное обогащение панелей генов, в том числе и панелей онкогенов;

AVENIO –гибридизационное обогащение панелей онкогенов из внеклеточной опухолевой ДНК и анализ данных.

ООО «Рош Диагностика Рус» предлагает комплексные решения, включающие в себя не только оборудование и реагенты, но и технический сервис, обучение персонала и постоянную методическую поддержку.

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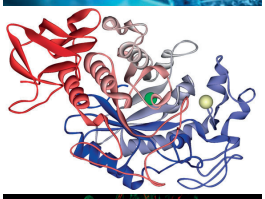
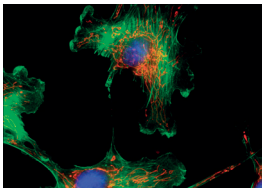
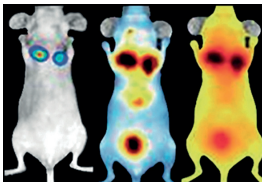
Благодаря уникальному портфолио продукции и опыту наших специалистов мы выполняем поставки и внедрение комплексных решений для разнообразных задач в области молекулярной и клеточной биологии.







Молекулярно-генетические исследования

- Системы для выделения и молекулярного анализа одиночных клеток Becton Dickinson
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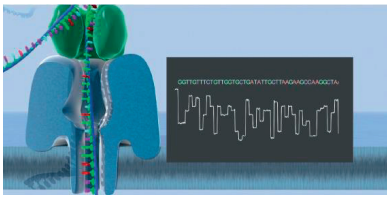
- Все для ПЦР, реагенты,наборы, пластик, амплификаторы.
- Нанопоровые секвенаторы Oxford Nanopore Technologies, наборы для секвенирования ДНК и РНК.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Компания АЛЬБИОГЕН — официальный дистрибьютор illumina и Lucigen

Компания ООО «АЛЬБИОГЕН» с 2015 года является эксклюзивным (единственным) официальным торговым представителем и дистрибьютором компании illumina на территории Российской Федерации, Республики Беларусь, Республики Казахстан и Республики Узбекистан.

Нашей задачей является обеспечение полного доступа клиентов к передовым технологиям и сервисам illumina, включая современные системы NGS и анализа ДНК-биочипов, программное обеспечение для биоинформатики и весь спектр реактивов.

ООО «АЛЬБИОГЕН» предоставляет полный комплекс услуг, связанных с продажей, технической поддержкой и сервисным (гарантийным и постгарантийным) обслуживанием продукции компании Illumina, а также обучением пользователей работе на данном оборудовании.

Инновационная и стремительно развивающаяся компания illumina Inc., являющаяся мировым лидером в области геномных технологий, заключила соглашение с компанией АЛЬБИОГЕН, специализирующейся на поставках оборудования и расходных материалов для секвенирования нового поколения (NGS) и анализа на ДНК-биочипах.

Новейшие продукты компании illumina, создаваемые совместно с ведущими мировыми учеными, позволяют изучать геном на очень глубоком уровне и дают возможность для новаторских достижений в науке, медицине, сельском хозяйстве и потребительской геномике. Более 90% научных статей, связанных с технологиями секвенирования нового поколения, сделаны при помощи оборудования Illumina.

Сотрудничество с компанией АЛЬБИОГЕН направлено на то, чтобы сделать технологии NGS и анализа ДНК-биочипов более доступными на территории Российской Федерации и в странах СНГ.

Компания АЛЬБИОГЕН использует свой обширный опыт в области продаж и продвижения продукции, знания передовых технологий и сеть региональных представителей для обеспечения быстрой, эффективной и бесперебойной работы лабораторий клиентов illumina.

Компания АЛЬБИОГЕН также является официальным дистрибьютором компании Lucigen, основными продуктами которой являются ферменты и реагенты для секвенирования нового пколения и молекулярной диагностики.

Компания СкайДжин предлагает к поставке со склада в Москве и под заказ наборы реагентов, оборудование, расходные материалы, реактивы, а также специализируется на сервисном обслуживании и поверке дозаторов, лабораторных весов различных производителей. Мы предлагаем гибкие условия работы и очень большой ассортимент продукции.

Поставляемая нашей компанией продукция широко используется в научно-исследовательских лабораториях и R&D центрах, лабораториях секвенирования, при решении практически любых молекулярно-биологических задач.

Большая часть производителей в нашем портфолио - это прямые, эксклюзивные поставки. Мы являемся первым звеном в поставках для таких компаний как New England Biolabs, Agilent Technologies, Oxford Nanopore Technologies, QIAGEN, 10x Genomics, NIMAGEN, Integrated DNA Technologies, Thermo Fisher Scientific, SIGMA-ALDRICH, BioSan, Gilson.

К флагманским продуктам наших линеек относятся:

- Набор для пробоподготовки образцов от New England Biolabs ULTRA II FS с интегрированной системой фрагментации и другие наборы серии ULTRA для образцов ДНК, РНК и микроРНК;
- Digital NGS: готовые панели и наборы для обогащения на основе ПЦР от QIAGEN с мономолекулярным баркодированием;
- Специализированные наборы для работы с микроРНК и анализа экспрессии от QIAGEN-Exiqon;
- Нанопоровые секвенаторы третьего поколения: портативный секвенатор MinION, высокопроизводительный секвенатор GridION;
- Уникальная система Chromium производства 10x Genomics для автоматической пробоподготовки геномов и транскриптомов единичных клеток.

За дополнительной информацией о производителях, товарах, ценах и условиях поставки обращайтесь к нашим квалифицированным специалистам.

Будем рады ответить на Ваши вопросы и помочь выбрать качественное и недорогое решение для Ваших задач!

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ХИМЭКСПЕРТ

Агентство Химэксперт

Информация о компании:

Компания Химэксперт существует 16 лет и давно зарекомендовала себя, как надежный поставщик приборов, реактивов и расходных материалов для молекулярной биологии. Мы собрали для своих клиентов самые интересные и перспективные бренды, большинство из которых в России можно приобрести только у нас.

Химэксперт предлагает оборудование для анализа ДНК и РНК, в том числе и методами NGS, фундаментальных протеомных и цитологических исследований, фармацевтики и биотехнологий, прикладного тестирования, включая идентификацию личности и установление родства в криминалистике и судебно-медицинской экспертизе.

Наши клиенты выбирают Химэксперт потому что:

- Химэксперт всегда находит самые прогрессивные решения в области Life Sciences. Наша компания постоянно расширяет свое портфолио и в курсе последних веяний в области молекулярной биологии
- Химэксперт осуществляет полную техническую и методическую поддержку наших клиентов: обратившись к нам, вы получаете помощь квалифицированных сотрудников в подборе оборудования и реагентов под поставленные задачи и их последующем использовании
- Химэксперт стремится идти навстречу заказчикам и осуществлять быстрые поставки, так как скорость и четкость исполнения заказов очень важна.

Обратившись к нам, вы можете быть уверены в будущем своего эксперимента.
Начните сотрудничество с компанией Химэксперт и убедитесь в этом на своем опыте!

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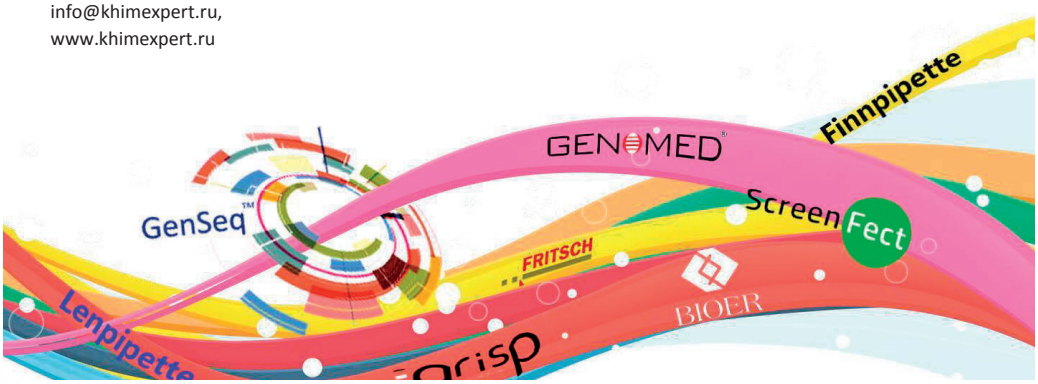

www.genexplain.com

The geneXplain GmbH is glad to welcome you at the BGRS/SB'2018 conference and is proud to introduce you the following software and database solutions for the needs of bioinformatics, systems biology and systems medicine:

geneXplain platform – is a high-performance tool for multi-omics data analysis, which allows identification of new therapeutic targets and biomarkers. A unique feature of the geneXplain platform is its Upstream Analysis. You can register and immediately receive access to a free account.

TRANSFAC database – is a unique collection of transcription factors, their experimentally validated binding sites (TFBS) and a widely known library of positional weight matrices (PWMs). The database has its own integrated methods for TFBS search. It can also be used as an integral part of the geneXplain platform. TRANSFAC is available online or can be downloaded as a set of flat files.

TRANSPATH database – is one of the biggest and most famous collections of signaling and metabolic pathways, which counts over 489000 reactions. The database can be applied for master-regulators search within the geneXplain platform. TRANSPATH is also available online in one package with HumanPSD database or can be downloaded as a set of flat files.

HumanPSD database – is a collection of genes, proteins and micro-RNAs, which includes information about disease biomarkers and clinical trials for various diseases. Besides the detailed biomarkers data, the database contains information about drugs.

BRENDA database – is a comprehensive enzyme and enzyme-ligand information system. Its manually derived core contains over 3 million data points about 77,000 enzymes annotated from 135,000 literature references.

PASS – is a software tool for evaluating the general biological potential of organic compounds based on their structural formula. This program predicts main and side pharmacological effects, molecular mechanisms of action, specific toxicities, and antitargets, actions associated with the metabolism and transport of pharmaceutical substances and their influence on gene expression.

PharmaExpert – is a software tool for analysis of the biological activity spectra of substances predicted by PASS and selecting compounds with the desirable set of biological activity, for analyzing the relationships between biological activities, drug-drug interactions and for multiple targeting of chemical compounds.

GUSAR – is a software tool for analysis of quantitative structure-activity/structure-property relationships (QSAR/QSPR) based on the structural formulas of the compounds and data on their activity/property, and for prediction of activity/property for new compounds. GUSAR can be easily applied to different routine QSAR/QSPR tasks, for building multiple models, and for prediction of the different quantitative values simultaneously.

If you got interested in any of the products, provided by GeneXplain, or you have any questions, please contact us by e-mail info@genexplain.com. We will be glad to help you!

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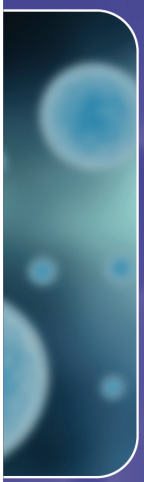

```
function [ t, x ] = firstReactionMethod( ...
    stoich_matrix, propensity_fcn, tspan, x0,...
    rate_params, output_fcn, max_out)

if ~exist('rate_params', 'var')
    rate_params = [];
end

num_rxns = size(stoich_matrix, 1);
num_species = size(stoich_matrix, 2);

%Simulation loop
while t(rxnCount) <= max(span)
    % Step 1: calculate propensities
    a  = propensity_fcn(X(rxnCount,:), rate_params);
    % Step 2: identify the reaction that will occur
    r = rand(1,num_rxns);
    taus = -log(r)./a;
    [tau, mu] = min(taus);
    % Update time and execute reaction mu
    rxnCount = rxnCount + 1;
    T(rxnCount)    = T(rxnCount-1)    + tau;
    X(rxnCount,:) = X(rxnCount-1,:) + stoich_matrix(mu,:);

    if rxnCount > max_out
        warning('SSA:ExceededCapacity','');
        return;
    end
end

% Simulation completed
t = T(1:rxnCount-1);
x = X(1:rxnCount-1,:);
end
```

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Symposium

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