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ITSN-1 forms complexes with proteins of the SUMOylation system

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Aim. An important step in elucidating the reasons for the nuclear localization of the neuronal intersectin 1 (ITSN-1) isoform is the study of post-translational modifications capable of affecting the nuclear-cytoplasmic transport of proteins. A distinctive feature of the neuronal splice isoform of ITSN-1 is the presence of a short insertion of five amino acid residues (+VKGEW) located in exon 20 of its SH3A domain. This insertion structurally corresponds to the consensus site of SUMOylation, a post-translational modification representing the process of covalent attachment of small SUMO-protein modifiers to substrates. Since SUMOylation is also one of the most common modifications affecting the cellular localization of a protein, we investigated the ability to form complexes between ITSN-1 and several SUMO family proteins, such as SUMO-1, SUMO-2/3 and UBC9. **Methods.** To study the protein complexes formation between intersectin 1 and SUMO modifiers in the cell, the coimmunoprecipitation from lysates of line 293 cells with transient coexpression of Omni-ITSN1S(+VKGEW) and GFP-SUMO1, Omni-ITSN1S(+VKGEW) and GFP-SUMO2 or Omni- ITSN1S(+VKGEW) and GFP-SUMO3 was used. Precipitation of GST fusion proteins was used to examine the binding of ITSN1 to UBC9 *in vitro*. To do this, the cDNA sequence of full-length UBC9 was cloned into a GST-tagged bacterial expression vector and the recombinant GST-UBC9 was expressed in *E. coli* cells. GST-UBC9 was immobilized on glutathione-sepharose and incubated with lysate of 293 cells containing Omni-ITSN1-S (+VKGEW). **Results.** The results of coimmunoprecipitation indicate the ability of the neuronal isoform of ITSN-1 to precipitate SUMO-proteins *in vivo*. ITSN1-S (+VKGEW) was found to have a higher affinity for SUMO-2/3 than for SUMO-1, and of the two homologues SUMO-2 and SUMO-3, the highest affinity interaction was shown for the ITSN1-S complex (+VKGEW) with SUMO-3. This suggests that SUMO-3 may be the key SUMO-modifier for ITSN-1 *in vivo*. The results of Western blot analysis also demonstrated the ability of the neuronal ITSN-1 isoform to precipitate with the enzyme UBC9. **Conclusion.** The obtained results indicate that the ability to undergo SUMOylation may be unique to ITSN-1 and is likely mediated by the presence of consensus SUMOylation motifs in ITSN-1 absent both in a ubiquitous isoform of the protein and in ITSN-2. Further *in vivo* studies may clarify the role of the interaction of intersectin-1 with SUMO-2/3. Site-directed mutagenesis can also be performed to clarify the exact points of interaction between the modifier and the substrate. Additionally, confirmation of the ability of SUMO-2/3 to form a poly-chain in this particular case along with the screening of interactions between ITSN-1 and E3 ligases will provide a broader functional characterization of these particular molecular processes.

Investigation of the potential antitumor properties of ORN-D-M on the B16 murine melanoma model. Study of gene expression by qPCR using a new intercalating dye

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Aim. Oligoribonucleotides in complex with D-mannitol (ORN-D-M) inhibit cancerous cell lines, in particular the B16 murine melanoma cell line. However, the mechanism of this effect, as well as the influence on the tumors *in vivo* is unstudied. Additionally, in our research, we encountered the problem of insufficient efficiency and high cost of exported PCR dyes, which makes the development of new dyes critical. **Methods.** To develop a new dye for the qPCR technique, three criteria were used: low threshold cycle, low reaction inhibiting level (defined as the value of the trend line on the graph of the dependency of the threshold cycle, and dye concentration), and high relative fluorescence. Optimized PCR mix with new intercalating dye was used for the gene expression analysis in further research. Investigation of the mechanisms of ORN-D-M inhibition effect on the B16 murine melanoma cells was performed by comparison of the relative gene expression level between the group with 48-hours treatment with 5 mg/mL ORN-D-M and the control group without treatment. An influence of ORN-D-M on the solid tumors in the B16 animal model (20-25 g mice of the C57BL6/J line) was studied using different routes of drug administration (peritumoral, oral, and intraperitoneal), which allowed administering the wide range of drug concentrations (0.7-12 mg/animal). We have checked the tumor volume and vitality of mice with the following relative gene expression analysis by qPCR on their spleen and blood samples. **Results.** We selected the dye (produced by «SSI Institute for single crystals of NAS of Ukraine), which differed by the low reaction inhibiting level and high relative fluorescence in comparison with some popular commercial PCR dyes. Based on this dye, the mixture for PCR was optimized and used for further investigations of gene expression. It was found, that ORN-D-M treatment of the B16 murine melanoma cells caused the gene upregulation of some RNA receptors: Tlrs, Eif2ak, and some components of dependent pathways: Nfkb1, Ifns type I. Activation of these pathways can lead to the apoptotic death of cells, so it can be a mechanism of their growth inhibition. However, ORN-D-M did not show an effect on the solid B16 tumor in the monotherapy of tumor-bearing mice. Gene expression analysis of the different immune cell markers in the spleen and blood samples of these experimental mice revealed the difference only between healthy and tumor-bearing mice. **Conclusions.** ORN-D-M can inhibit the B16 cell line growth by upregulation of some components of Tlr- and Eif2ak-dependent pathways. However, ORN-D-M did not show an effect on the solid B16 tumor in murine model. Furthermore, we proposed a new effective intercalating dye, which is produced in Ukraine, for use in the qPCR analysis.

Structure and molecular dynamics modeling of the complex of EMAP II protein with dextran-70

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Aim. The ability of endothelial and *monocyte-activating* polypeptide II (EMAP II) to induce apoptotic processes in tumor cells proves the prospects of using it as an antitumor agent. However, significant structural lability makes the use of proteins for such purpose challenging. In the case of EMAP II, its propensity to form aggregates in solution may result in significant side effects. Using ligands to stabilize the protein structure proved to be a promising method of preventing protein aggregation. The aim of this work is to reveal the mechanism of formation of such complexes and protein stabilization within them, which is crucial for their improvement. **Methods.** The spatial structure of EMAP II complex with dextran 70(hereinafter "Complex") was modeled using the AutoDock4 software. Molecular dynamics (MD) simulation of the complex was carried out at 37 °C in the environment of a 150 mM NaCl solution. All MD calculations and trajectory file conversion were performed with GROMACS 2020.4 software using CHARMM36 force field. VMD and Chimera software were used for trajectory visualization and analysis. **Results.** The results of dextran-70 docking against EMAP II together with previously carried out simulations of the EMAP II aggregates formation suggest that the binding of the polysaccharide to the hydrophobic tryptophan "pocket" and ^{34}DVGEIAPR^{41} unstructured loop in the protein structure is likely to be the mechanism of EMAP II aggregation inhibition and Arg12, Gly36, Glu37, Ile38, Arg41, Lys68, Lys71, Met72, Arg73, Leu76, Lys116, Asn119, Lys121, Lys123, Trp125, and Lys166 residues make up the binding interface. Analysis of the data obtained by MD simulation of the complex molecule at a temperature of 37 ˚C showed a significant decrease in the RMSD values of EMAP II atoms compared to those for free state EMAP II. In case of free state EMAP II, the RMSD values increase sharply during the first 20 ns of the simulation and fluctuate significantly in a wide range, reaching 0.35 nm during 100ns. In the complex with dextran-70, the RMSD values reach a plateau during the first 10 ns of the dynamics and do not exceed 0.25 nm^2 throughout the simulation time, which indicates a higher stability of the protein in the complex comparing to free EMAP II. While the RMSF value of individual areas of free EMAP II exceeded 0.4 nm^2, this value for protein residues in the complex does not exceed 0.25 nm^2. **Conclusions.** The data obtained using molecular docking allow us to reasonably assume the binding interface of EMAP II with the dextran molecule. The analysis of the MD trajectories shows the general stabilization of the EMAP II protein in the complex compared to the free state, which is consistent with the experimental data.

Defining the interactions of G3BP1 with cytoplasmic RNA-binding proteins on cellular and biochemical levels

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Stress granules are non-membrane ribonucleoprotein cell compartments formed by liquid-liquid phase separation that occurs due to local high concentration of key proteins and RNAs in response to stress. The core function of stress granules remains poorly understood, however, it is known that the aberrant assembly or disassembly of these granules has pathological implications in cancer, viral infection and neurodegeneration. The cores of SGs are composed of proteins with RNA-binding activity. One of the key SG proteins is RNA-binding Ras GTPase-activating protein SH3-domain binding protein G3BP1. It is known that G3BP1 has a protein-protein interaction with Caprin1, G3BP2 and several other proteins, but overall the range of this protein interactions and the detail of its involvement in the breast cancer progression remain unclear.

Aim. Discover new interactions of G3BP1 with cytoplasmic RNA-binding proteins that are associated with stress granules and are involved in cancer progression. **Methods.** Molecular cloning, Microtubule bench assay coupled with HCS analysis, immunoprecipitation, Western-blot. **Results.** Using MT bench technology we discovered that RBPs such as PABPC1L, FXR1, FXR2, EIF4B, STAU1, STAU2 and CSDE1 have an affinity for G3BP1 similar to that of its canonical partner, G3BP2 in normal conditions as well as in sodium arsenite induced stress conditions. By far the strongest affinity occurred between G3BP1 and its other canonical partner — Caprin 1. Next, we generated several constructs of G3BP1 protein fragments, including G3BP1ΔC, ΔN, ΔPRM and tested these constructs ability to bind RNA. G3BP1ΔC and ΔPRM have shown 50 percent decreased affinity for RNA. We conducted a series of MT bench assays with G3BP1ΔC, ΔN, ΔPRM constructs, resulting in EIF4B, STAU1, STAU2, FXR1, FXR2 and CSDE1 affinity patterns being similar to G3BP1 ability to bind RNA, notable exception being STAU2 interaction with G3BP1ΔPRM. On the other hand, the G3BP1ΔPRM interaction with Caprin1 decreased drastically compared to WT, which is at odds with to this well-documented interaction. Next up, we performed immunoprecipitations followed by Western-blot analysis. PABPC1L, G3BP2 and Caprin1 showed increased affinity for G3BP1 in RNA-free conditions, while KIF5C, STAU1, STAU2, CSDE1, EIF4B were able to interact with G3BP1 only in normal conditions. **Conclusions.** All proposed RBPs were shown to interact with G3BP1, although Caprin1, G3BP2 and PABPC1L do not rely on RNA for the interaction, but KIF5C, STAU1, STAU2, CSDE1, EIF4B, FXR1 and FXR2 require RNA for the interaction.

Editing D-amino acids by *Thermus thermophilus* leucyl-tRNA synthetase

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Introduction and Aim. Norvaline and homocysteine can be activated and can be edited by leucyl-tRNA synthetase. However, it is unknown about the D-stereospecific editing of this enzyme. Early, it was reported about the D-amino acid editing by alanyl-tRNA synthetase of *Thermus thermophiles* (Rybak *et al.*, 2017), so the purpose of this work is to research the D-amino acid activation and editing by LeuRS of *T. thermophilus*. **Methods.** *Purification of Thermus thermophilus leucyl-tRNA synthetase*. After expression of recombinant protein in the *Escherichia coli* cells, the harvested cell pellet was resuspended in lysis buffer (50 mM Tris-HCl (pH 8.0), 15 mM DTT, 0.1 mM EDTA, 1 mM PMSF, 1 tablet of EDTA-free protease inhibitor cocktail), incubated during 30 min on ice and then centrifuged at 14 000 rpm for 30 min. Then the lysate was heated at 72ºC for 35 min and again centrifuged at the same conditions. The obtained supernatant was dialyzed against the buffer A (20 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 2 mM DTT, 0.1 mM PMSF) during night and then loaded onto DEAE sepharose. It was washed by buffer A and eluted by gradient of NaCl (0-0.4 M). The fractions, containing protein, were detected by the activity test and SDS-PAGE and then combined and dialyzed against buffer A. Hereafter, the protein solution was loaded onto Heparin sepharose and eluted by KCl gradient (0-0.3 M). The Fraction, containing protein, was used in further experiments. *Editing assay.* Overall editing by LeuRS was tested in the reaction of AMP accumulation. The Reaction mixture contained 100 mM Na-HEPES (pH 7.4), 10 mM KCl, 12 mM $MgCl_2$, 5 mM DTT, 30 uM *E. coli* $tRNA^{Leu}$, 200 uM ATP, trace amounts of ^{32}P-labelled ATP, 1 uM TT LeuRS and 30 mM norvaline or homocysteine. The mixture with 2 mM L-leucine was used for a control reaction. It was incubated at 37ºC and stopped at five time point by loaded onto PEI-cellulose. The reaction products were separated by TLC in 0.1 M ammonium acetate, 5 % acetic acid and quantified by phosphorimaging. **Results and Conclusions.** After 30 min incubation, 20 % of AMP were accumulated in the presence of D-Nva and tRNA and 50 % — in the presence of L-Nva and tRNA. Without tRNA, the level of AMP was much lower in both cases thus suggesting the tRNA-dependent editing mechanism. TT LeuRS edits D-Hcys, but is seems to be the tRNA-independent mechanism.

Generation and characterization of novel antibodies specific to Coenzyme A

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Aim. The goal of this work was to obtain monoclonal antibodies against Coenzyme A with specificity other than that of the existent, previously generated 1F10 antibody. The alternative approach of antigen preparation was used for immunization in the hope that it will affect the antigen presentation. **Methods.** To generate the antigen for mice immunization, CoA was cross-linked to Keyhole Limpet Hemocyanin (KLH) and to bovine serum albumin (BSA) using reversible chemical crosslinker SPDP. In order to produce monoclonal antibodies, the hybridoma technique was used. The positive clones were later selected using ELISA method. For further testing of antibody's specificity, the western blot analysis, immunoprecipitation and immunofluorescent analysis were performed. **Results.** We obtained the monoclonal antibody (A11) that recognizes Coenzyme A. The specific recognition of BSA-S-S-CoA was demonstrated by ELISA and western blot analysis. The Endogenously CoAlated proteins in the HEK293/Pank1b cell line were detected by western blot and immunofluorescent analyses. A range of immunoassays showed that A11 antibody has the same antigenic epitope specificity as the existing anti-CoA mAb (1F10). **Conclusions.** Usage of reversible conjugation of CoA to KLH via thiol group by SPDP crosslinker instead of irreversible conjugation of CoA to maleimide-activated KLH via thiol group (used for generation of 1F10 antibody) did not affect the antigenic epitope specificity of newly generated anti-CoA antibodies A11. It can be explained by probable existence of only one immunogenic epitope in CoA molecule.

Tristetraproline in breast cancer

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Introduction. Tristetraproline (TTP, ZFP36) is an RNA-binding protein known to regulate negatively many oncogenic transcripts. It is a promising biomarker because its expression altered in multiple types of cancer and frequently is associated with the clinical features and patient prognosis. **Aim.** To analyze the *ZFP36* expression levels in clinical samples of different subtypes of breast cancer (BC) and to investigate the effects of doxorubicin and temozolomid on the *ZFP36* methylation and expression in MCF-7 cell line. **Methods.** Bioinformatics analysis, RT-qPCR, qMSP, cell culture. **Results.** Our findings showed that in BC specimen of all subtypes the *ZFP36* expression levels are significantly higher compared to control adjacent tissues. Interestingly, in HER+ BC subtype the expression was significantly increased compared to all other subtypes, which allows us to consider this gene as a HER+ BC biomarker candidate. The analysis of survival rates (overall, disease-free and separately for all 4 subtypes) in cohorts of patients with high and low *ZFP36* expression showed that in general the overall survival rate in cohort in the high *ZFP36* expression group was increased compared to the low *ZFP36* group. However, if to analyze every subtype separately, the HER+ cohort patients from the high *ZFP36* group showed significantly decreased survival rate compared to the low *ZFP36* group (100 and 200+ months, respectively). In contrast, in the cohort with luminal B subtype, the tendency was the same as overall, which highlights the need of individual approach to each tumor. We also analyzed the effects of doxorubicin and temozolomid on the *ZFP36* methylation and expression in MCF-7 cell line. Our findings showed that only the 150 μM temozolomid concentration led to the reduction of *ZFP36* promotor methylation (namely 2-fold), but this did not affect the *ZFP36* expression. In contrast, doxorubicin did not affect the *ZFP36* methylation status, but had an effect on expression levels: 0.1 μM doxorubicin exposure led to 2-fold decrease of expression level, and 0.5 μM and 1.0 μM exposure led to 2.2 and 2.7-fold increase. Therefore, we can make a suggestion that there is a mechanism, different from a change of methylation status, via which doxorubicin affects the *ZFP36* expression. As it is known that in MCF-7 cells doxorubicin increases activity of NFkB, and considering that NFkB induces the *ZFP36* expression via binding to its promoter we suggest it as a potential mechanism of the observed effects. **Conclusions.** The *ZFP36* expression altered not only in tumor tissues compared to the control group, but also within the subgroups with significant increase in HER+ samples compared to other tumor specimens. Despite of being considered as an anti-oncogene, the patients with HER+ BC in cohort with high ZFP36 expression showed a decreased survival rate compared to the low expression cohort. Furthermore, the ZFP36 expression is increased under doxorubicin treatment with no change in its methylation status.

Research of antistaphylococcal activity to Staphylococcus aureus strains with different phenotypes of resistance to antibiotics

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Aim. Research of two compounds - the derivatives of acridin-9-yl-(1H-benzoimidazol-5-yl)-amine and 2-phenyl-quinazoline, which possess antistaphylococcal activity toward multidrug resistant *Staphylococcus aureus (S. aureus)* strains. **Methods.** The compounds: **1** (the derivatives of acridin-9-yl-(1H-benzoimidazol-5-yl)-amine) and **2 (**2-phenyl-quinazoline) were extensively screened for the antistaphylococcal activity with following evaluation of the minimal inhibitory concentration (MIC) to 29 multidrug resistant *S. aureus* strains isolated in Ukrainian hospitals. A sample of each culture (were cultured in Cation-adjusted Mueller Hinton broth at 37 °C overnight) was then diluted 40-fold in fresh broth and in-cubated at 37 °C for 1.5–3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600). Growth inhibition of all bacteria was determined measuring absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The bacterial strains were investigated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommendations. **Results.** The minimum inhibitory concentration (MIC) values for compounds **1** and **2** toward *S. aureus* isolates. The MIC values ranged between 78.12 and 312.5 mg/L for compound 1 and between 156.2 and 312.5 mg/L for compound 2. Compound 1 was more active than compound 2 on 12 of the 29 bacterial strains, whereas compound 2 had a higher antimicrobial effect on 3 strains. Compound 1 is more profitable than compound 2 and reveals the antimicrobial activity toward *S. aureus* strains which have different profiles of antibiotic sensitivity with MIC values in the range from 78.12 to 312.5 mg/L, whereas compound 2 demonstrates antimicrobial activity with MIC values in the range from 156.2 to 312.5 mg/L. Compound 1 possesses the highest antibacterial activity with MIC value of 78.12 mg/L against the multidrug resistant *S. aureus* strains. All these strains have susceptibility to chloramphenicol, moxifloxacin and linezolid. Vice versa, it was revealed that the compound 1 has the lowest antibacterial activity against *S. aureus* isolates which have resistance to chloramphenicol; two of them have resistance to moxifloxacin and one of them has resistance to linezolid. Compound 2 has antibacterial activity against tested *S. aureus* strains, mostly with a MIC value of 156.2 mg/L, with the exception of three isolates, which are susceptible to linezolid. **Conclusion.**. The compounds 1 and 2 possess the effectiveness toward *S. aureus* strains with various antibiotic resistance profiles and can be useful for the development of new compounds with antistaphylococcal activity against the multidrug resistant *S. aureus* strains.

Impact of new MGMT inhibitors on autophagy in glioma cells *in vitro* at combined action with alkylating agent N-methyl-N'nitro-N-nitrosoguanidine

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Aim. The new low-weight non-nucleoside inhibitors of repair enzyme MGMT (O^6-methylguanine-DNA methyltransferase) have shown high level of efficacy and low level of cytotoxicity in human cancer cells *Hep-2*. However, it is important to investigate the impaction of new inhibitors in others human cancer cells: especially, to compare the influence of new inhibitors on the cells, which have different level of the *MGMT* gene expression. That is why the study was performed using two glioma cell lines: *T98G* with constative high level of the *MGMT* expression and *U251MG* with methylated promoter in the *MGMT* gene. **Methods.** Both lines *T98G* and *U251MG* were treated by new inhibitors only and with using combination of new inhibitors and alkylating agent N-methyl-N'nitro-N-nitrosoguanidine (nitrosoguanidine). The autophagy level and number of alive and dead cells were measured with fluorescence microscopy and fluorescence spectrophotometry after Monodansylcadaverine dying for autophagosomes and dying by Live-Dead Imaging Cell Kit respectively. Also with Western-blot analysis the level of MGMT was measured in both cell lines after treatment. **Results.** The efficiency of new MGMT inhibitors was analysed by Western blotting and compared to O^6-benzylguanine inhibitor. As expected, the level of MGMT protein was high in the *T98G* cells and it was undetectable in the *U251MG* cells. The analysed inhibitors 41 and 41B reduced the MGMT level in *T98G* cell line and this effect was similar to the effect of O^6-benzylguanine inhibitor after 24 h of treatment. However, at the later time points (48 h and 72 h) the level of MGMT protein in cells after the 41 and 41B treatment was higher than after O^6-benzylguanine. It is supposed that O^6-benzylguanine has a prolonged inhibiting effect due to its chemical structure and the mechanism of action that may also explain its higher cytotoxicity. The impact of new inhibitors on the autophagy level was investigated in glioma cells.The absence of autophagy effects is observed after the treatment with] O^6-benzylguanine and analyzed inhibitors. However, the level of autophagy is significantly elevated in the *T98G* cells after combined treatment by inhibitors and nitrosoguanidine. Thus, inhibition of MGMT makes the cells more sensitive to the alkylating agent since the level of autophagy was much lower when the cells were treated with nitrosoguanidine only. This finding was supported by the data obtained by analysis of the live-dead cell numbers with Live-Dead Imaging Cell Kit. Indeed, 41 and 41B inhibitors at the studied concentration do not increase the level of dead cells in both cell lines. However, the posttreatment with nitrosoguanidine raises the number of dead cells, but only in *T98G* cells. **Conclusions.** Thus, new non-nucleoside inhibitors of MGMT significantly reduce the MGMT level, but this effect could be observed only after 24h treatment. Longer treatment by new inhibitors has a weaker inhibiting effect comparing with O^6-benzylguanine. The combined treatment by inhibitors and nitrosoguanidine induces high autophagy level in *T98G* cell line. However, a low level of autophagy is observed at the same conditions in the *U251MG* cells. The analysed inhibitors do not elevate the level of dead cells in both cell lines. Though, combined treatment leads to high death rate only in the *T98G* cells.

Synthesis and purification of homopolymeric oligonucleotides

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Our study **aimed** to prepare, identify and purify homopolymeric oligonucleotides (oligoribonucleotides and deoxyribonucleotides). **Methods.** In order to synthesize oligonucleotides, we used the amidophosphite method. According to this method, the synthesis is performed by stepwise condensation of the building blocks to the 5'-end of the growing chain, cycle by cycle until the target product is obtained. The synthesis was carried out in 200 nmol (1000 Å) columns filled with a carrier - glass with a controlled pore size. For each synthesis, the corresponding column was used. For DNA synthesis, these were the columns with: 5'-DMT-dA(Bz), 5'-DMT-dC(Ac), 5'-DMT-dG(iBu), and for RNA synthesis - 5'-DMT-Bz-rA and 5'-DMT-3'-ibu-rG (Merck), as well as the corresponding phosphoamidites: DMT-dC(bz), DMT-dA(bz), DMT-dG(ib), Bz-rA, rG(ib) (Merck). Vertical gel electrophoresis, (15 % w/v polyacrylamide) 8M urea, 150 V, was used to analyze the obtained product. Spectral methods were also used (including the acquisition of an emission-excitation matrix). The oligonucleotides were purified by high performance liquid chromatography, HPLC (reversed-phase chromatography). Ion-pair HPLC was performed using a Discovery® HS C18 column (particle size 3 μm, 15 cm × 4.6 mm). The mobile phase consisted of: A, 0.1 M TEAA, pH 7; B, ACN. A gradient from 10 to 14.5 % B in 30 min, 0.5 ml/min, 51.5 °C was used. **Results.** Homopolymeric oligodeoxynucleotides were synthesized: GGGGGGGGGGGGGGGGGGG (G19), CCCCCCCCCCCCCCCCCCCCCC (C24), AAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAA (A24) and oligoribonucleotides AAAAAA (rA5), GGGGG (rG5). As a result of the synthesis, we obtained a high yield (according to the third monitor) of about 98.5 % step from step (overall about 70 %). **Methods** for electrophoresis and high-performance liquid chromatography were selected and developed. According to the chromatograms, the approximate elution time of the obtained substances was obtained: G19–3.25 min, A24–10.25 min, C24–12.75 min, rA5–4.25 min, rG5–4 min. The purity of the obtained products was proved by spectral methods. **Conclusion.** A complete cycle of the preparation and purification of homopolymeric oligonucleotides has been developed, which allows us to scale up the process and study biological activity.

Cyanine-based fluorescent probes for biomolecule detection and cell imaging

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Aim. Biological research involves the study of a wide range of biomacromolecules, cellular processes, and compartments. Therefore, searching for simple and convenient methods to analyze and study the biological systems based on fluorescent probes is important for various research and practical purposes. Cyanine dyes have outstanding advantages as fluorescent probes due to their good optical properties, biocompatibility, and low toxicity. Therefore, this research aimed to identify new fluorescent probes among cyanine dyes for the detection of proteins and nucleic acids in solution and visualization of cells by microscopy across all visible diapason of the light spectrum. **Methods.** Fluorescence and UV-Vis absorbance spectroscopy to investigate the photophysical properties of cyanine dyes both free and in the presence of different biomolecules. The dyes' suitability as probes for cell imaging was evaluated using fluorescence microscopy. The selectivity of the dyes for specific organelles was examined through the colocalization analysis with standard dyes and antibodies. The study also included an assessment of the dyes' cytotoxicity for long-term imaging. **Results.** 32 cyanine dyes were examined as fluorescent probes for detecting biomolecules in solution and for imaging the compartments of eukaryotic cells. The fluorescence spectroscopy study has shown that positively charged unsymmetrical monomethine cyanines are more sensitive to RNA than DNA with intercalation binding mode. Trimethine cyanine dyes were sensitive to nucleic acids regardless of their type. Bridged penthamethine cyanine dyes bearing N-sulfonate group are the most sensitive to serum albumins. The quantum yield values and detection range were estimated for the most promising dyes. It was shown that the studied cyanine dyes possess high values of quantum yields in the presence of biomolecules (up to 44 %) and high sensitivity to low biomolecules concentration. Five binding sites for the dyes were identified on the albumin globule. Moreover, the model of complexation for HSA/pentamethine cyanine dye has been proposed with a previously undescribed binding site. Fluorescence microscopy has shown that all studied dyes possess high permeability to biological membranes and are effective in low working concentrations. Depending on the dyes' structure and charge, they possess different selectivity to cell compartments. Most of the studied dyes possess high photostability and low cytotoxicity and therefore are suitable for long-term live-cell imaging. **Conclusion.** As a result of the study, new fluorescent probes were discovered that emit at various parts of the spectrum ranging from blue to far-red. These probes can be used to detect biomolecules in solution and visualize cellular compartments by microscopy.

Treatment of acute respiratory distress syndrome caused by COVID-19 with human umbilical cord mesenchymal stem cells

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Aim. The study aims to determine the effect of mesenchymal stem cells on changes in microRNA and cytokine levels after MSC transplantation in patients with severe COVID-19 pneumonia and associated fibrotic lung changes. The study included 15 patients who received a standard treatment protocol (control group) and 13 patients who received a combination treatment with three consecutive doses of MSC (MCS group). **Methods.** Real-time qPCR was performed to assess miRNA levels, ELISA determined cytokine levels, and pulmonary computed tomography (CT) was used to assess lung fibrosis. Data were obtained on the day of the patient's hospitalization (day 0) and on the 7th, 14th, and 28th days of observation. CT of the lungs was performed at 2, 8, 24, and 48 weeks after hospitalization. Correlation analysis was used to study the association between the levels of biomarkers in peripheral blood and lung function indicators. **Results.** Triple MSC transplantation has been proven safe and does not lead to serious adverse reactions in patients with severe COVID-19. For the first time, MSC transplantation has been shown to increase plasma levels of IP-10, MIP-1, G-CSF, and IL-10 in patients with severe COVID-19. On the other hand, indicators of inflammation such as IL-6, MCP-1, and RAGE did not differ between groups. The total CT score in the MSC group was 12-fold lower ($p<0.05$) than in the control group at week 48. The level of surfactant D, a type II alveocyte damage marker, decreased in plasma four weeks after MSC transplantation, whereas it increased in the control group. In the MSC group, inflammatory parameters such as ESR and CRP decreased faster than in the Control group. MSC therapy accelerated the recovery of lymphocytes and reduced the percentage of banded neutrophils compared to the Control group. MSC transplantation did not affect the expression levels of miR-146a, miR-27a, miR-126, miR-221, miR-21, miR-133, miR-92a-3p, miR-124, or miR-424. MSCs enhanced neutrophil activation, phagocytosis, and leukocyte migration, activated early T cell markers, and reduced effector and senescent T cells' maturation in PBMCs *in vitro*.

The squaraine derivatives as potential photosensitizers in photodynamic therapy of cancer

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Aim. Photodynamic therapy (PDT) is one of the promising cancer treatment methods. The advantage of using PDT is the selective destruction of cancer cells by activating photosensitizers (PS) upon light irradiation. The research aims to investigate the photodynamic properties of squaraine dyes with different N-substituents. **Methods.** Organic synthesis, fluorescence spectroscopy, UV-VIS spectroscopy, laser scanning confocal microscopy, cytotoxicity assay. **Results.** The spectral-luminescent properties of studied dyes in the methanol, DMSO, aqueous solution, and in the presence of albumin were investigated. All studied dyes in complexes with serum albumins demonstrated an increase in fluorescence emission intensity compared to the free state in an aqueous solution. The maxima of absorption spectra are located at 657–677 nm. The fluorescence emission maxima lie in the far-red spectrum between 670–690 nm, making dyes suitable for photodynamic therapy. The efficacy of the studied dyes as photosensitizers for photodynamic therapy (PDT) was evaluated *in vitro*. The samples demonstrate negligible dark toxicity but show cytotoxicity after irradiation. Binding to albumins reduced the cytotoxic effect of dyes with hydrophilic groups. At the same time, a dye with a hydrophobic structure retains PDT properties both in an aqueous medium and in the presence of albumin. A breast cancer cell line (MCF-7) was used to study the ability of the studied dyes to penetrate the cell membrane and their distribution inside the cells. The blue fluorescent standard dye Hoechst binding to nuclear DNA was used for the co-staining. All studied dyes can penetrate through the cellular membrane, stain the cell components in the cytoplasm, and do not accumulate in nuclei as shown by co-staining with Hoechst: no co-localization with nuclear DNA dye is observed. **Conclusions.** All synthesized dyes exhibited maximum absorption in the "therapeutic window" area, making them suitable for bioimaging *in vivo* and PDT. It was shown that all studied dyes had low dark toxicity but showed toxicity after irradiation with red light. It was demonstrated that binding to albumin and aggregating of dyes can have both negative and positive effects on photosenitizers' phototoxic property.

Properties of endogenous retroviral env genes and patterns of their expression during early embryogenesis of *Gallus gallus*

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Introduction. Endogenous retroviral *env* genes were detected in all studied representatives of mammalian species, as well as in some avian, fish and reptiles. However, the studies on their functioning have been conducted only in mice. Therefore, their role in muscle development during embryogenesis was not well recognised and requires in-depth research. **Aim.** The aim of this study was to determine the endogenous retroviral *env* representatives in the genome of *Gallus gallus* and their properties *in vitro* and expression patterns in the early stages of embryonic development. **Methods.** The search for retroviral *env* genes was carried out in *Gallus gallus* genome assembly V5.0 (NCBI database). All open reading frames (ORF) longer than 450 bp were selected from downloaded genomic sequences using Unipro UGENE 1.30 and checked against the HMM model of TLV-coat motif. Then all TLV-contained ORFs were checked to contain canonical Env domains and motives using HMM search. The predicted *env's* were obtained by PCR and cloned into pGEMT-Easy, phCMV-VSVG and pCDH-CMV-MCS-EF1-copGFP vectors for further research. All sequences were confirmed by sequencing. WISH was performed with corresponding DIG-labelled RNA probes. Determination of the results of transfection of eukaryotic cells was performed by fluorescent immunohistochemistry. Expression of putative *G. gallus env* genes in embryonic chicken myocytes was studied by qPCR. **Results.** We identified the endogenous retroviral *env* genes in *G. gallus* the proteins of which have all the canonical structural characteristics of the syncytins. The chicken *env* genes had intracellular localization similar to human *Syncytin-1*, and their overexpression led to the cell-cell fusion of different cell lines, such as HEK 293T, DF-1, MEF-1, CHO-K1. All of them were expressed in derma-myotome of chicken embryo from 3 to 5 days of development. The level of *ALV-Env* expression in the leg buds of chicken embryo from 4 to 10 days was higher at day 5 when there were active processes of somite mesoderm cell migration and fusion. **Conclusions.** We have shown that the endogenous retroviral *env* genes of the *G. gallus* were expressed in derma-myotome. *ALV-Env* was expressed in the hindlimbs on day 5 of embryogenesis. Chicken Env's excellently induce cell-cell fusion in eukaryotic cultures of various origins. It can be assumed that they are involved in other cellular processes, such as migration or proliferation.

ПЕРЕДПЛАТА

2023 рік

Передплату на журнал можна оформити через передплатне агентство «Укрінформнаука».

Для оформлення передплати необхідно заповнити бланк-замовлення (бланк) і відправити його на адресу Агенції електронною поштою (innovation@nas.gov.ua).

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Під час заповнення бланку-замовлення просимо уважно заповнювати всі його пункти.

Рахунок-фактура

На підставі отриманого замовлення буде оформлено рахунок-фактуру. Рахунок буде надіслано Вам для оплати по факсу (або електронною поштою), оригінал рахунку буде відправлено на вашу поштову адресу. Після отримання рахунку-фактури Вам необхідно оплатити його через відділення банку. Оплату необхідно виконати в терміни, визначені в рахунку-фактурі шляхом безготівкового перерахування на банківський рахунок, вказаний у рахунку-фактурі.

Договір

При необхідності Ви можете укласти договір з Агенцією «На поставку періодичних видань». Договір вступає в силу після його підписання і діє до моменту виконання зобов'язань. Після закінчення строку дії договору на Вашу вимогу Вам видадуть акт виконаних робіт.

Податкові накладні

Якщо Вам потрібна податкова накладна, то в спеціальній графі бланку-заявки обов'язково вкажіть необхідні дані на підставі свідоцтва платника податку.