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G3BP1 domain-dependant recruitment of cytoplasmic RNA to stress granules

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RNA-binding RAS-GTPase G3BP1 is a multi-functional protein that is known to be one of the key components of stress granules — non-membrane ribonucleoprotein cell compartments that are formed by liquid-liquid phase separation. With its ability to mediate both protein-protein and protein-RNA interactions, G3BP1 is perceived as a key regulator of the stress granule assembly. **Aim.** Explore the influence of G3BP1 domains and the consequence of SG-associated RNA binding protein G3BP1 interaction on mRNA recruitment and stress granule assembly.

Methods. Molecular cloning, Microtubule-bench assay coupled with HCS analysis, RNA *in situ* hybridization, and quantitative fluorescence.

Results. In order to assess the mRNA recruitment by G3BP1, we coupled the MT-bench assay with the RNA *in situ* hybridization method. We discovered that full length G3BP1 and G3BP1–230–466 aa (DN-term) retain their ability to bind RNA, while 1–230 aa (ΔC-term) and G3BP1DPRM deletion constructs lost their ability to bind RNA. Previously we have shown that G3BP1 interaction level with RNA binding proteins involved in stress granule assembly is at highest when full length G3BP1 is present, and drops significantly in cases of ΔC, DN and DPRM deletion constructs. Following these findings, we decided to emulate the G3BP1-induced RNA recruitment process in the stress granules themselves. We opted for measuring the fluorescence intensity of transfected G3BP1 and its deletion constructs precisely in stress granules. In order to achieve that, we performed RNA *in situ* hybridization and thus located the stress granules within cells, measured the fluorescence intensity of tracked RNA, and compared the intensity values of RNA and G3BP1 and its deletion constructs in stress granules. In addition, we measured the surface area of stress granules and calculated the RNA enrichment value in stress granules per cell. We found out that transfection of deletion constructs of G3BP1 leads to a decrease of 68 % of the SG area compared to G3BP1 WT. Additionally, the intensity levels ratio between G3BP1 and tracked RNA neared 1 for G3BP1 WT and dropped significantly for the deletion constructs, 0.6 for ΔC, 0.62 for DN and 0.75 for DPRM, which points to the importance of full-length G3BP1 protein in the stress granules assembly.

Conclusions. The stress granules assembly and consequent/parallel RNA recruitment to SGs relies on full-length G3BP1 protein and loss of either C-terminal, N-terminal domains or proline-rich motif result in smaller stress granules and overall decrease in their number per cell.

Analysis of gene expression of TKS family in different breast cancer subtypes

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Aim. Breast cancer displays substantial heterogeneity and poses challenges in terms of treatment. Our investigation focuses on the potential use of TKS4, TKS5 and isoforms as oncomarkers in this type of cancer. These proteins are identified as pivotal factors in the formation of invadopodia, governing the metastatic properties of cells.

Methods. The National Cancer Institute provided the breast tumor samples, from which RNA was extracted and used in this study. The TKS4 common isoform pool and the expression levels of TKS4-L, TKS4-b, and TKS5-L isoforms were determined using the RT-qPCR method with the fluorescence-labeled Taq-Man probes. For the assessment of relative gene expression, the ΔCt method was employed on the experimental qPCR data. Additionally, the RNA-seq gene expression data for common isoform pools of TKS5 and TKS4 were obtained from the Cancer Genome Atlas Breast Invasive Carcinoma (TCGA-BRCA) dataset. The statistical analysis phase was performed using RStudio and encompassed correlation analysis, the Kruskal-Wallis H test, and the Wilcoxon rank-sum test. The survival analysis was conducted using the UCSC Xena Explorer.

Results. A strong positive correlation was observed between the expression rates of TKS5-L and TKS4 (PCC = 0.625, $P < 0.001$), as well as TKS4-L (PCC = 0.736, $P < 0.001$), which is the main isoform of TKS4. This finding aligns with the correlation data between TKS5 and TKS4 from the TCGA-BRCA dataset (PCC = 0.6562, $P < 0.001$).

In the luminal A and luminal B HER2- subgroups, there was an elevated expression of TKS4-L ($P < 0.05$) compared to the adjacent tumor tissues. Similarly, TKS5-L exhibited increased expression in the HER-enriched and luminal B HER2- subgroups ($P < 0.05$) compared to adjacent tumor tissues, and in the triple-negative subgroup compared to the HER-enriched ($P < 0.05$). However, there was no significant difference in the expression of TKS4 and TKS4-b in the samples.

When investigating the TCGA-BRCA dataset, the TKS4 expression showed a significant increase in the triple-negative subgroup ($P < 0.001$) compared to all other subgroups except for HER-enriched. Notably, a higher TKS4 expression in the triple-negative subgroup correlated with improved survival ($P < 0.01$).

Conclusions. Using the findings of our study, we propose that TKS4-L emerges as the main isoform of TKS4, playing a role in the carcinogenesis of breast cancer cells. Notable differences in expression across molecular subgroups are observable for both TKS4, TKS5 and their isoforms. The data regarding the expressions of TKS4 and TKS5 hold potential as prognostic and oncomarkers in breast cancer.

New approaches to the therapy of chronic myeloid leukemia: modeling the level of Bcr-Abl by inhibiting the deubiquitinating activity of USP1

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Aim. Chronic myeloid leukemia (CML) is myeloproliferativea neoplasm arising as a result of a reciprocal translocation between chromosome 9 and 22, which leads to the formation of the Bcr-Abl oncoprotein. A characteristic feature of Bcr-Abl is its constitutive tyrosine kinase activity, which causes malignant transformation of cells. The main therapeutic strategy involves the use of tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib, bosutinib, ponatinib), but a third of patients develop drug resistance, which causes an urgent need to find new approaches to the treatment of CML. An alternative strategy to combat CML is to model the level of the Bcr-Abl oncoprotein, which involves a detailed screening of its partner proteins in order to find a therapeutic target. We believe that USP1, due to its deubiquitinating activity, stabilizes the level of the oncoprotein Bcr-Abl, which leads to its accumulation in the cell and the progression of CML.

Methods. K562 cells were chosen for the experiment. The interaction of proteins was investigated by the methods of co-immunoprecipitation and pull-down analysis. The subcellular localization of the protein complex was determined by immunofluorescence analysis followed by confocal microscopy. The method of co-immunoprecipitation and immunofluorescence analysis was used to study USP1 protein phosphorylation. The expression level of Bcr-Abl was studied by Western blot analysis using specific antibodies. The results were analyzed quantitatively and statistically.

Results. The formation of the nuclear protein complex Bcr-Abl/USP1 was established in K562 cells. It was found that the oncoprotein Bcr-Abl interacts with USP1 using the PH domain. The presence of phosphorylated forms of the USP1 protein at tyrosine sites in K562 cells was shown. The relationship between the tyrosine kinase activity of the oncoprotein Bcr-Abl and the phosphorylation of deubiquitinase USP1 was revealed. The effect of deubiquitinase on the level of the Bcr-Abl oncoprotein in K562 cells was determined.

Conclusions. We believe that the formation of the Bcr-Abl/USP1 protein complex is a key event for the stabilization of Bcr-Abl levels in cells, which contributes to the malignant transformation of cells and the progression of CML. Uncontrolled tyrosine kinase activity of Bcr-Abl can be one of the factors in the deregulation of USP1 functions and can contribute to the disruption of downstream signaling pathways and affect the proteosomal degradation of the oncoprotein itself. Based on the obtained results, we identify deubiquitinase USP1 as a promising therapeutic target for the development of a new strategy for the treatment of CML by modulating the level of Bcr-Abl in the cell.

Multifunctional response of PBMC from patients with COVID-19 to the immunomodulatory capability of hUC-MSC

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Aim. During COVID-19, the SARS-CoV-2 assaults lymphocytes, causing initial damage to the immune cells. Although UC-MSCs are known for their immunomodulatory and protective abilities, the molecular basis of their impact on impaired immune cells is understudied. The transcriptome, cell surface immunophenotype, and cytokine release of PBMC from patients with COVID-19 co-cultured with and without UC-MSC were compared to identify target genes, pathways, and processes.

Methods. Human PBMC (n = 6) were isolated from peripheral blood samples of patients with COVID-19 and resuspended in an RPMI-1640 in the presence of Dynabeads® Human T-Activator CD3/CD28. Human UC-MSCs (n = 3) were isolated via explant methods and cultured up to passage 3. PBMC/UC-MSC co-culture was performed at a 5:1 ratio; the cells were cultured for six days before being harvested for FACS and RNAseq analysis. The conditioned media from UC-MSC/PBMC were collected for further ELISA. Total RNA was isolated from PBMC. Novogene Co., LTD prepared and sequenced the RNA-seq libraries using an Illumina HiSeq2000. Clean data are deposited in the NCBI Sequence Read Archive with the accession number PRJNA929329.

Results. mRNA-seq revealed 4172 differentially expressed genes in PBMC after co-culture with UC-MSCs. GO biological process analysis revealed that the genes involved in leukocyte migration, immunological defense (especially in neutrophil-mediated immunity), angiogenesis, and extracellular matrix remodeling were overrepresented among 2993 upregulated genes. A GO biological process analysis of 1179 downregulated genes found a decrease in cell division and DNA reparation-related genes. KEGG annotation revealed an increase in inflammatory-associated B-cell receptor and NF-kappa B signaling pathways). Furthermore, the enrichment of genes associated with phagosomes, cytokine–cytokine receptor interaction, protein digestions, and ferroptosis suggest the activation of the immune response. In addition, after co-culture with UC-MSC, the expression of apoptosis-associated genes in PBMC reduced. The analysis of CD surface receptors expression demonstrated an increase in the early T cell markers (CD25 and CD69) within the CD3 cells subpopulation. In addition, the percentage of effector T cells, senescent effector CD8 and CD 4 T cells, and memory CD8 T cells decreased. UC-MSC did not affect the maturation of CD4 Th cells, cytotoxic CD8 T cells, and regulatory T cells when co-cultured with PBMC. Furthermore, UC-MSC significantly stimulated MCP-1, IL-6, and G-CSF while remarkably attenuated MIP-1α, IL-10, and IL-12p70 secretion by PBMC.

Conclusion. Thus, UC-MSCs enhance immune defense machinery by augmenting the expression of genes involved in neutrophil and T cell activation, decreasing the expression of apoptosis-associated genes, and restricting effector T cell maturation under co-culture conditions.

Spectral studies of ligand-protein interactions of oligoribonucleotides

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Aim. Understanding the interaction between S protein and nucleic acids, specifically of yeast oligoribonucleotides (ORNs), yeast ORNsNasalt (ORNsNa), can provide insight into the development of novel therapeutics and vaccines against coronavirus infections.

Methods. To explore the binding of RNA with proteins, we investigated the effect of yeast oligoribonucleotides (ORNs) and their sodium salt form (ORNsNa) on fluorescence quenching and conformational changes of the coronavirus recombinant S-protein isolated from eukaryotic cells.

Results. The study showed that S protein interacts with both the acidic and salt forms of oligoribonucleotides, with a slightly stronger binding to the acidic form. The binding constants were measured as $24.16*10^{-6}$ M and $26.85*10^{-6}$ M, respectively, indicating a low-affinity interaction of the protein with the ligands.The Gill coefficient, which measures the degree of cooperativity in ligand-receptor interactions, was found to be 0.9 for the interaction of S protein with acidic ORNs and 0.81 for the interaction with salt forms of ORNs, indicating a negatively cooperative binding. This means that once one ligand molecule binds to the protein, the affinity of the protein for other ligand molecules decreases. This effect was more pronounced in the case of interaction with salt forms of ORNs.

The binding constant obtained in our experiment suggests that the S protein interacts with both the acidic and salt forms of oligoribonucleotides with a low affinity but with slightly stronger binding to the acidic form. The Gill coefficient, which is a measure of cooperativity, indicates a negatively cooperative binding of the protein with both forms of ORNs, with a more pronounced effect observed with the salt form.

An important observation from our experiment is the increase in melting point, which suggests stronger stabilization of the S protein when bound to the acidic form of ORNs. This could have important implications for understanding the role of ORNs in the stability of the S protein in various conditions.

Furthermore, our results show that the secondary structural elements of the protein change most intensively in a concentration range of 1 to 10 μM for acidic ORNs and 20 to 150 μM for salt forms, with a return to the initial composition. This periodic change in the secondary structure indicates a non-cooperative protein-ligand interaction.

Conclusions. Overall, our experiment provides valuable insights into the interaction between the S protein and oligoribonucleotides, highlighting the importance of considering the different forms of ORNs and their effects on protein stability and structure.

STARD8 and STARD9 as DSD Candidates: Unveiling Novel Mutations in 46,XY Gonadal Dysgenesis Patients

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Introduction. Investigating gene mutations that affect gonad development is crucial for understanding the genetic mechanisms behind Disorders/Differences in Sex Development (DSD). This research aims to identify novel DSD genetic variants using whole exome sequencing (WES).

Methods. WES was performed on two unrelated 46,XY *SRY*-positive patients with gonadal dysgenesis. A mutant fly line model was established and tested, and the molecular modeling of mutant proteins was conducted.

Results. In the first patient, a hemizygous missense mutation NM_001142503.2 c.2659 C>T (p.Arg887Cys) (rs766188656) in the *STARD8* gene (MAF = 0.0000251) was identified and confirmed as pathogenic one using bioinformatic tools.

In the second patient, two different mutations in a compound heterozygous state were identified in the *STARD9* gene: NM_020759.3 c.5585_5590del (p.Ser1862_Thr1863del) (rs528276071) — an in-frame deletion (MAF = 0.0019), combined with NM_020759.3 c.3514 C > T (p.Arg1172Cys) (rs12594837) — a missense mutation (MAF = 0.00837).

The analysis of the genetic background of both patients did not reveal any pathogenic variants implicated in the DSD phenotype. All detected mutant variants were inherited from healthy parents — heterozygous carriers and were not previously implicated in the pathogenesis of any disease.

Bioinformatic analysis revealed that the mutant variant in the *STARD8* and both mutations in the *STARD9* genes were located in positions that are conserved in primates.

The *D. melanogaster* mutant line was established, proving the impact of the p.Arg887Cys mutation in the STRAD8 ortholog on the early development of gonads.

Molecular modeling showed that the protein's regions carrying STARD9 mutations can accomplish competitive interaction with the interface of other proteins and allosteric modulation (distal binding).

Conclusions. Based on the results obtained in this study, previous reports of the *STARD* gene family mutations in DSD patients, the expression patterns of the *STARD8* and *STARD9* genes, and the steroidogenic properties of their protein products, we conclude that *STARD8* and *STARD9* are considered as 46,XY DSD causing genes.

Study of the interaction of the implanted collagen scaffold, seeded with human umbilical cord MSCs, with the surrounding cells of the body

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The use of MSC-seeded scaffolds is a promising approach for effective MSC delivery to the site of tissue damage. **The aim** of the research was to study the interaction of the implanted collagen scaffold, seeded with both original MSCs and MSCs that were preconditioned with a low concentration of H_2O_2, with the surrounding cells of the animal body.

Methods. The Balb/c mice were divided into three groups: the first group was a negative control, an "empty" collagen scaffold was implanted subcutaneously in the mice, a scaffold seeded with original MSCs in the amount of $1x10^5$ cells/piece of scaffold was implanted in the second group, and the third group was implanted with a scaffold seeded with preconditioned MSCs H_2O_2.

Results. In 5 days, the scaffolds were removed from the implantation sites for further analysis with histological and molecular methods. After removing the samples, it was found that the negative control scaffold didn't implant under the animal's skin without apparent signs of inflammation. In the second group, the implantation of a scaffold seeded with original MSCs was observed, which was evidenced by the formation of blood vessels at the scaffold implantation site, as well as fibroblasts, lymphocytes *etc.* which were detected on its periphery. Intensive angiogenesis at various stages was observed in the animals, in which a scaffold with preconditioned MSCs was implanted under the skin. The migration of various cells throughout the scaffold, including a large number of MSCs, was detected. The presence of human alpha-satellite DNA sequences was confirmed by expensive PCR analysis of DNA isolated from those scaffolds seeded with initial and preconditioned MSCs.

Conclusions. The results obtained indicate a better therapeutic effect of MSCs preconditioned with H_2O_2 on the target tissue/organ, which was manifested in intensive angiogenesis, higher survival of preconditioned MSCs and their prolonged storage in the implanted scaffold, which ensures long-term secretion of various growth factors and cytokines, which contribute to more successful recovery of the target tissue/organ.

Synthesis, purification and interaction of oligonucleotides with recombinant signaling proteins and their receptors

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The aim of the study was to synthesize, purify, and study the interaction of oligonucleotides (OLNs) with recombinant signaling proteins and their receptors. The fluorescence analysis and the Stern-Volmer equation were also used to conduct a comparative analysis of the level of this interaction by determining the Stern-Volmer constants of protein-nucleic acid binding.

Methods. The synthesis of oligonucleotides, in particular OLN-1, OLN-2, OLN-3, OLN-4, OLN-5, OLN-6, OLN-7 and OLN-8, was performed using the solid-phase phosphoamidite method on an H-8 oligo synthesizer (K&A Laborgeraete, Germany). Oligonucleotides were purified by low-pressure chromatography using P-8 cartridges (K&A Laborgeraete, Germany) with polymerized dextran (MicroPure II Column, USA). The homogeneity and purity of the synthesized oligonucleotides were verified by high-performance liquid chromatography (HPLC) (Shimadzu Prominence, Japan). Fluorescence analysis was performed by titration of the following proteins: insulin (Farmak, Ukraine), interferon α2-β (SPC "INTERFARMBIOTEK", Ukraine), insulin and interferon receptors (CLOUD-CLONE CORP. (CCC), USA) and somatotropin (S.A. Balkan Pharmaceuticals, Moldova) on a spectrofluorimeter (Horiba, France) at an excitation wave of Ex=295 nm, emission range Em=305–405 nm, 5 nm slit. In the experiment, 50 pM protein was used in Tris-HCl buffer (50 mM/L, pH 7.4) with a volume of 200 μL, and 20 μL of OLN at a concentration of 2 nM/ml were added to a final volume of 400 μL.

Results. The results of fluorometric titration of OLNs with the studied proteins indicate their static binding of A+B=AB to one ligand to form a non-fluorescent complex, except for OLN-3 with INS and OLN-2 with INF α2-β, where mixed binding is observed. The results obtained indicate that the studied proteins interact with OLNs at very low concentrations. The most active were OLN-1 (STP — 7.51 mol-1/l, INSR — 11.05 mol-1/l, INFR — 9.73 mol^{-1}/l, INF α2-β — 10.95 mol^{-1}/l), OLN-6 (STP — 20.09 mol^{-1}/l, INSR — 13.40 mol^{-1}/l, INF α2-β — 14.56 mol^{-1}/l), OLN-7 (STP — 12.8 mol^{-1}/l, INSR — 14, 50 mol^{-1}/l, INS — 23.81 mol^{-1}/l, INF α2-β — 27.35 mol^{-1}/l) and OLN-8 (STP — 12.17 mol^{-1}/l, INSR — 30.50 mol^{-1}/l, INS — 10.59 mol^{-1}/l, INFR — 10.52 mol^{-1}/l, INF α2-β — 35.60 mol^{-1}/l), which bound to all proteins.

However, in some cases, two-ligand positive cooperative binding 2A+B=A2B occurs, when 2 ligands are attached to the protein, and the attachment of one OLN molecule facilitates the attachment of the next one due to conformational changes. The most active in this case were OLN-3 (INF α2-β 13.45–2.94 mol^{-1}/L (1 and 2 ligands)) and OLN-4 (INF α2-β 20.11–2.59 mol^{-1}/L (1 and 2 ligands)).

Conclusions. The results of our experiments indicate a high degree of interaction of OLNs with recombinant signaling proteins and their receptors, which may be accompanied by changes in their conformation and biological activity, which, in turn, may be important for their therapeutic application.

Scaffold protein ITSN1 associates with the SUMO modification system

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Aim. Changes of scaffold protein's ITSN1 expression were associated with several neurodegenerative disorders (Down syndrome, Alzheimer, Parkinson's and Huntington's diseases) and certain types of cancer. A number of posttranslational modifications (PTMs) are known to affect ITSN1 functions in cell and add layers to its regulation. Our investigation centers ITSN1 association with SUMOylation, a well-known PTM, that promotes changes in protein localization and tightly linked to the stress response in both physiological and pathological conditions.

Methods. Full-length human SUMO1, SUMO2 and SUMO3 sequences were cloned in pEGFP-C vector for co-expression with Omni-ITSN1-S in MCF-7 cell line and used in co-immunoprecipitation with anti-Omni antibody. Lysates of 293 cells with transiently expressed GFP-SUMO3 were prepared for GST-binding assay with GST-tagged domains of ITSN1 (EH1/2, CCR, SH3). To confirm the ability of ITSN1 to undergo SUMOylation we used immunoprecipitation of endogenous ITSN1 from lysates of SH-SY5Y cells and mouse brain followed by detection of anti-SUMO2/3 and anti-ITSN1 antibodies.

Results. Overexpressed short isoform of ITSN1-S precipitates with SUMO1, SUMO2 and SUMO3. Both ubiquitously expressed and brain specific isoform of ITSN1-S equally associated with recombinant GFP-SUMO proteins. Further analysis revealed that SH3D and EH1/2 domains of ITSN1 are sufficient to precipitate SUMO3 *in vitro*. Additionally, our immunoprecipitation results suggest that endogenous ITSN1 undergoes SUMOylation since SUMO2/3-antibody immunoreactive bands were detected in immunoprecipitates of endogenous ITSN1.

Conclusions. Based on the results of our study, we conclude that ITSN1 associates with the proteins of SUMO modification system. SUMO1, SUMO2 and SUMO3 proteins were identified as novel protein partners for scaffold protein ITSN1. Considering that SUMO-modification plays an important role in many cell functions by controlling proteins activity and its subcellular localization we suggest that this PTM can contribute to the nuclear localization of ITSN1, mechanism of which is still unclear. However, considering a scaffold nature of ITSN1 protein and its ability form complexes with SUMO proteins, further research into conditions under which these interactions occur as well as their mutual localization is required to obtain a clear picture of its physiological role.

Comparison of new non-nucleoside MGMT inhibitors' effectiveness in HEp-2 and T98G cancer cells

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Aim. New low-weight non-nucleoside MGMT inhibitors are an alternative to the standard inhibitor O^6-benzylguanine (BG) in the combination alkylating chemotherapy. The analysed compounds have already been proven to be low cytotoxic in previous studies *in vitro*. Our goal is not just to investigate and analyze the previous and new data concerning the inhibitors' efficacy, but also to compare the impact of the compounds on cancer cells of different types.

Methods. Both lines HEp-2 and T98G were treated by new inhibitors only and with using the combination of new inhibitors and an alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). To assess the inhibiting activity of the compounds, Western-blot analysis was used for measurement of the level of MGMT in both cell lines after treatment. Different methods have been used to evaluate the effectiveness of the inhibitors. Clonogenic analysis has been realized with HEp-2 cells to determine the ability of cells to the colony formation after the combined treatment. Whereas in T98G cells, autophagy level and number of alive and dead cells have been measured with fluorescence microscopy and fluorescence spectrophotometry. Monodansylcadaverine was used for dyeing autophagosomes. Meanwhile, for dyeing alive and dead cells was used Live-Dead Imaging Cell Kit, respectively.

Results. All analysed inhibitors reduced the MGMT level in both cell lines according to Western-blot analysis. However, in T98G glioma cells a decrease in the protein level was more significant than in HEp-2 laryngeal carcinoma cells compared to the controls. It should be noted that the inhibitor 41B has shown the greatest inhibitory activity in both cell lines. We cannot correctly compare the results concerning inhibitors' effectiveness obtained by different methods: one measures the number of dead cells, and the other measures the number of cells not capable to colony formation. However, in general, there is a tendency that the glioma cells are more sensitive to the combine action of inhibitors and alkylating compound, even at the lowest concentrations of MNNG. This observation is also evidenced by the autophagy data. The high level of autophagy in treated cells indicated that the cytotoxic effect of MNNG is enhanced in the presence of inhibitors, whereas MNNG by itself at investigated concentrations did not induce autophagy in glioma cells.

Conclusions. Thus, the low-weight non-nucleoside compounds exhibited greater inhibiting activity in the T98G gliomacells than in the HEp-2 cells. The inhibitor 41B demonstrated the greates tinhibiting activity in both T98G and HEp-2 cell lines compared to the controls. In general, the glioma cell line was more sensitive to the cytotoxic effect of MNNG in combination with inhibitors, even at the lowest concentrations of MNNG.

Eukaryotic translation elongation factor eEF1Bβ is responsible for binding valyl-tRNA synthetase

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Valyl-tRNA synthetase (ValRS) is an indispensable enzyme that catalyzes conjugation of valine to its cognate tRNA. In higher eukaryotes, this enzyme is associated with translation elongation factor complex, eEF1B, comprising three different subunits α, β, and γ. The exact site of interaction remains obscure. Up to now, the ValRS interaction site on eEF1B remains unknown. However, it has been hypothesized that ValRS most probably binds the β subunit of the eEF1B complex. Thus, **the aim** of our work was to study the interaction between the recombinant N-terminal domain of ValRS and full-length eEF1Bβ.

Methods. Recombinant fusion proteins GST-eEF1Bβ (full-length and Δ77 truncated form) and His-ValRS N-terminal domain were expressed in *E.coli* and purified on affinity columns. Complex formation between His-ValRS N-terminal domain and eEF1Bβ (full-length and Δ77 truncated form) was examined by pull-down assay. Recovered fractions were concentrated and analyzed by SDS-PAGE.

Results. It was shown that concentrated fractions after His-ValRS/ eEF1Bβ FL pull-down contained two distinct bands compared to His-ValRS/ eEF1Bβ Δ77, where only 1 band for valyl-tRNA synthetase domain was present.

Conclusions. According to the obtained results, we can conclude that ValRS N-terminal domain and eEF1Bβ FL form a stable complex. The putativeValRS interaction site on eEF1Bβ will be discussed.

Prospective DNA markers for the identification of hazelnut varieties of Ukrainian selection

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Aim. The aim of this study is to review the current data on the DNA markers used for the identification of hazelnut varieties and to identify the ones useful for practical use in hazelnut selection in Ukraine.

Methods. Analysis of special literature and Internet resources.

Results. Hazelnut (Corylus spp.) is a valuable crop of global importance, which is grown as a source of nuts, wood and decorative elements. Accurate identification of the genetic composition of varieties is of crucial importance for breeding work, as it allows obtaining new offspring with desired traits. Various molecular markers are widely used for the identification of varieties all over the world, in particular SSR markers, which play a key role in the identification of hazelnut varieties, and are currently a certain "gold standard", which makes this system of markers promising for use in the identification and certification of Ukrainian varieties selection.

Other molecular markers potentially suitable for identification of hazel varieties are iPBS markers. iPBS markers are easier to use and do not require complex and expensive equipment compared to SSR. To date, many studies have been conducted on the use of iPBS markers for the identification of varieties of many plants, where these markers have shown good results. Considering the low requirements in respect to special equipment and reagents associated with the development and analysis of iPBS markers and their previously demonstrated efficiency, the development of additional marker system for the identification of Ukrainian hazel varieties seems quite possible.

Conclusions. The well-known system of SSR markers has been already proven as an effective method of accurate and reliable identification and certification of hazel varieties. Thus, the SSR marker system is a promising option for identification of hazelnut varieties of Ukrainian selection. Furthermore, a system of iPBS markers can also be an effective alternative to SSR-fingerprinting under conditions of limited resources and equipment.

Computer modeling and molecular dynamics of the complex of C-module of human tyrosyl-tRNAsynthetase with dextran-70 polysaccharide

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Aim. Protein-based pharmaceuticals are among the fast growing categories of therapeutic agents in the clinic and as commercial products, and typically target high-impact areas such as various cancers, auto-immune diseases, and metabolic disorders. Recently the isolated C-module of human tyrosyl-tRNAsynthetase (C-TyrRS) was discovered as a novel cytokine. The aim of this work was to explore the possibility of reducing the level of aggregation and to improve the stability of the C-TyrRS in combination with dextran-70 polysaccharide for the structural-functional research and the development of new sustainable biomedical products.

Methods. The spatial structure of C-TyrRS complex with dextran-70 was modeled using the AutoDock software. Molecular dynamics (MD) simulation of the complex was carried out at 37 °C in the environment of 150 mMNaCl solution in 200 ns time interval. All MD calculations and trajectory file conversion were performed with GROMACS 2023.2 software using CHARMM36 force field. VMD and Chimera software were used for trajectory visualization and analysis.

Results. As a result of molecular docking investigations, the spatial structure of the C-TyrRS — dextran-70 complex was obtained and its binding energy was taken as the evaluation criterion. Analysis of the data obtained by MD simulation of the C-TyrRS — dextran-70 complex showed a significant decrease of RMSD (root mean square deviation of Ca atoms) and RMSF (root mean square fluctuation of Ca atoms) values of C-TyrRS atoms, which indicates a higher stability of protein in the complex comparing to the free state C-TyrRS.

Conclusions. The data obtained using molecular docking allow us to reasonably assume the binding interface of C-TyrRS with the dextran molecule. The analysis of the MD trajectories shows the general stabilization of the C-TyrRS protein by polysaccharide molecules in the complex.