Ukrainian Society of Cell Biology
Institute of Cell Biology NAS of Ukraine
Ivan Franko National University of Lviv

7th Congress of the All-Ukrainian Public Organization «Ukrainian Society of Cell Biology» with international representation

Program and the Book of Abstracts

11 – 13 September 2024
Lviv, Ukraine

Book of Abstracts contains the materials of the **7th Congress of the All-Ukrainian Public Organization «Ukrainian Society of Cell Biology»** with international representation which focuses on novel insights in Cell Biology and Biotechnology in Ukraine and abroad. The authors are solely responsible for the content of the abstracts.

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7th Congress of the All-Ukrainian Public Organization «Ukrainian Society of Cell Biology» with international representation

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CONGRESS PROGRAM

PROGRAM
of the 7th Congress of the All-Ukrainian Public Organization «Ukrainian Society of Cell Biology» with international representation Lviv, September 11-13th, 2024 (USCB) http://www.cellbiol.lviv.ua/ 2024

September 11th (Wednesday)

09:00 – 10:00 Registration of participants, Conference hall of the Scientific Library of Ivan Franko National University of Lviv

10:00 – 10:30 Congress opening, press release for the media

Speakers: A. Sibirny (Chair of the Organizing Committee), V. Melnyk (Rector of Ivan Franko National University of Lviv), R. Hladyshevsky (Vice-Rector of Ivan Franko National University of Lviv), O. Sedlyar (Director of Scientific Library of Ivan Franko National University of Lviv), Lviv city council and Lviv regional administration.

10:30 – 13:50 Session "**Cell signaling mechanisms, autophagy and apoptosis**" ***Chair: Rostyslav Stoika (Lviv)***	
(Conference hall of the Scientific Library of Ivan Franko National University of Lviv)	
10:30 – 10:50	Lecture 1. Andriy Sibirny (Lviv). Non-conventional yeasts in cell biology and biotechnology.
10:50 – 11:10	Lecture 2. Alex Rayevsky (Kyiv). Structural preconditions for the ATG8 functioning and the effect of lipidation and acetylation on its regulatory mechanism.
11:10 – 11:30	Lecture 3. Kostyantyn Dmytruk (Lviv). Specific degradation of cytosolic proteins in the methylotrophic yeast *Komagataella phaffii* upon carbon source replacement.
11:30 – 11:50	Lecture 4. Oleh Stasyk (Lviv). Anticancer therapy based on arginine deprivation: old cons and new developments.
11:50 – 12:10	Lecture 5. Yurii Bandura (Lviv). Mitophagy as an adaptive process in streptozotocin-induced diabetes mellitus.
12:10 – 12:30	**Coffee break**
12:30 – 12:50	Lecture 6. Rostyslav Panchuk (Lviv). Functional reactivation of mutant *TP53* gene by novel thiosemicarbazones leads to enhanced cell death induction in colon cancer cells.
12:50 – 13:10	Lecture 7. Olena Kravets (Kyiv). Autophagic flux detection in angiosperm microsporogenesis.
13:10 – 13:30	Lecture 8. Rostyslav Horbay (Ottawa, Canada). SMAC mimetics and macrophage-derived exosomes eradicate tumor cells. (*online*)
13:30 – 13:50	Lecture 9. Natalya Finiuk (Lviv). New efficient waterborne systems for delivery of water-insoluble thiazolidinone derivatives based on

	branched polymeric surfactants with side polyoxazoline chains.
13:50 – 14:50	**Lunch** (see the nearest possible locations at the end of the Program, marked *).
14:50 – 15:20	*Presentation of conference sponsors: ТОВ «Шимюкрейн», ТзОВ «АЛСІ» ЛТД*
15:20 – 16:20	Session **«Biology of eukaryotic and prokaryotic cells»** ***Chair: Kostyantyn Dmytruk (Lviv)***
15:20 – 15:40	Lecture 1. Yaroslav Blume (Kyiv). Paradigma for eukaryotic cell division: lucky 13 protofilaments of microtubules
15:40 – 16:00	Lecture 2. Oksana Stoliar (Ternopil). Metallothionein related redox shift as the strategy of stress response in facultative anaerobes on the model of bivalve molluscs.
16:00 – 16:20	Lecture 3. Yurii Yusypovych (Lviv). Identification of ophiostomatoid fungi vectored by *Ips acuminatus* infesting *Pinus sylvestris* in the Lviv region (*online*)
16:20 – 17:40	Session **«Biology of animal and plant cells»** ***Chair: Yaroslav Blume (Kyiv)***
16:20 – 16:40	Lecture 1. Rostyslav Blume (Kyiv). Genetic heterogeneity and population structure of *Camelina microcarpa* cytotypes in Ukraine, a hotspot of the species genetic diversity.
16:40 – 17:00	Lecture 2. Dmytro Novozhylov (Kyiv). Development of molecular markers for identification stem rust resistance gene *SR39*
17:00 – 17:20	Lecture 3. Galina Shevchenko (Kyiv). Photosynthetic apparatus of Chernobyl plants is tolerant to heavy metal stress. (*online*)
17:20 – 17:40	Lecture 4. Юлія Білоножко (Kyiv). Цитогенетичні особливості *Viscum album* L., що зростає на різних видах рослин-господарів. (*online*)
17:40 – 18:40	**Poster session №1** (sections: "*Cell signaling mechanisms, autophagy and apoptosis", "Biology of eukaryotic and prokaryotic cells", "Biology of animal and plant cells")*
19:00 – 21:30	**Get together party** (cafeteria of House of Scientists, Lystopadovoho Chynu Str. 6)

***Lunch** – the nearest possible locations:

- Restaurant "Puzata Khata", Shevchenka Ave., 10, Lviv;
- Restaurant "SteakHause", Shevchenko Ave., 25, Lviv;
- Restaurant "Beer Garden", Ivan Franko Str. 29, Lviv;
- Restaurant "Budmo", Stefanyka Str. 19, Lviv;
- Restaurant "Caucasus", Shota Rustaveli Str. 2, Lviv;
- "PastaCafe" – Dudayeva Str. 3, Lviv;
- Cafe "Lviv Croissants", Shota Rustaveli St, 6, Lviv,
- McDonald's, Shevchenka Ave., 7, Lviv;
- Bussines Hub "Black Honey", Shota Rustaveli Str., 12, Lviv.

September 12th (Thursday)

09:00 – 15:40	Session **«Medical problems of cell biology»** ***Chair: Oleh Stasyk (Lviv)***
(Conference hall of the Scientific Library of Ivan Franko National University of Lviv)	
09:00 – 09:20	Lecture 1. Ihor Shymanskyi (Kyiv). Mechanisms of neuroprotective action of vitamin D3 in glucocorticoid-induced neurotoxicity.
09:20 – 09:40	Lecture 2. Valentyna Chopyak (Lviv). Long-COVID: the role of NK cells and herpes virus type 6 activation
09:40 – 10:00	Lecture 3. Sergiy Shulga (Kyiv). Application of liposomal form curcumin and microRNA in Alzheimer's disease therapy.
10:00 – 10:20	Lecture 4. Igor Kaidashev (Poltava). PPAR- γ agonist Pioglitazone restored mouse liver, kidney medulla and lung mRNA expression of clock genes and Inflammation-Related genes disrupted by reversed feeding.
10:20 – 10:40	Lecture 5. Anna Havrylyuk (Lviv). Changes of expression of regulatory and inhibitory receptors on CD8 T cells in long-COVID patients.
10:40 – 11:00	Lecture 6. Valeriia Ustymenko (Kyiv). Morphological and quantitative analysis of conduit for 3d scaffolds to repair injured peripheral nerves.
11:00 – 11:20	**Coffee break**
11:20 – 11:40	Lecture 7. Arsen Ishchuk (Kyiv). The role of TRPV4 in the colon of rats with 6-OHDA-induced parkinsonism.
11:40 – 12:00	Lecture 8. Mariya Sabadashka (Lviv). Corrective effect of grape pomace extract in oxidative-nitrative stress in diabetes mellitus.
12:00 – 12:20	Lecture 9. Maya Vergolyas (Kyiv). Toxic effect of drinking water by using *in vitro* methods.
12:20 – 12:40	Lecture 10. Anna Moroz (Lviv). Functional state of erythrocytes in rats with streptozotocin-induced diabetes mellitus and its' correction under administration of fruit extract of hybrid *Cornus mas* × *Cornus officinalis*
12:40 – 13:00	Lecture 11. Віталій Шейко (Nizhyn). Вплив набутої короткозорості на системний імунітет та показники нейродинамічних функції.
13:00 – 14:20	**Lunch** (see the nearest possible locations at the end of the Program, marked *).
14:20 – 14:40	Lecture 12. Iryna Byelinska (Kyiv). Hematopoiesis and chemical skin burns: what is the difference in the blood markers of the healing process?
14:40 – 15:00	Lecture 13. Maksym Hutsaliuk (Kyiv). Effect of translocation t(8;21) on cytomorphological characteristics of blasts and neutrophils of pediatric acute myeloid leukemia.
15:00 – 15:20	Lecture 14. Halyna Hachkova (Lviv). Redistribution of

	sialylated membrane glycoconjugates and erythrocyte stabilization with biologically active compounds *G. officinalis* in type 1 diabetic rats.
15:20 – 15:40	Lecture 15. Tetiana Petryn (Lviv). Functional state of erythrocytes after the administration of the medicinal mushroom *Ganoderma lucidum* (*Agaricomycetes*) to animals with experimental metabolic syndrome.
15:40 – 16:00	*Presentation of conference sponsor: ПРАТ "КОМПАНІЯ ЕНЗИМ"*
16:00 – 16:40	**Reporting and election meeting of the All-Ukrainian Public Organization «Ukrainian Society of Cell Biology»**
16:40 – 17:40	**Poster session №2** (section "*Medical problems of cell biology").*
17:40 – 18:10	Lviv tour or walking excursion in Library
18:10 – 22:00	**Banquet (**Vienna Coffee House Lviv, 12 Svobody Ave., Lviv)

***Lunch** – the nearest possible locations:

- Restaurant "Puzata Khata", Shevchenka Ave., 10, Lviv;
- Restaurant "SteakHause", Shevchenko Ave., 25, Lviv;
- Restaurant "Beer Garden", Ivan Franko Str. 29, Lviv;
- Restaurant "Budmo", Stefanyka Str. 19, Lviv;
- Restaurant "Caucasus", Shota Rustaveli Str. 2, Lviv;
- "PastaCafe" – Dudayeva Str. 3, Lviv;
- Cafe "Lviv Croissants", Shota Rustaveli St, 6, Lviv,
- McDonald's, Shevchenka Ave., 7, Lviv;
- Bussines Hub "Black Honey", Shota Rustaveli Str., 12, Lviv.

September 13th (Friday)

09:00 – 11:40	Session **«Biotechnology and metabolic engineering»** ***Chair: Dariya Fedorovych (Lviv)***
(Conference hall of the Scientific Library of Ivan Franko National University of Lviv)	
09:00 – 09:20	Lecture 1. Rostyslav Stoika (Lviv). Structure-function interrelations in drug design and activity: Novel hybrid benzoisothiazole-1,2,3-triazole-4-carboxamides for treatment of human breast carcinoma.
09:20 – 09:40	Lecture 2. Dariya Fedorovych (Lviv). *Candida famata* cell factories for production of vitamin B2.
09:40 – 10:00	Lecture 3. Roksolana Vasylyshyn (Lviv). Development of *Ogataea polymorpha* strains capable of high-temperature cellobiose alcohol fermentation.

10:00 – 10:20	Lecture 4. Justyna Ruchala (Rzeszów, Poland). Non-traditional yeast in the pursuit of sustainable development - microbial production of riboflavin from renewable resources.
10:20 – 10:40	Lecture 5. Olena Tigunova (Kyiv). Metabolomic analysis of the *Clostridium* sp. Ucm b-7570 deletion mutant model (*online*)
10:40 – 11:00	**Coffee break**
11:00 – 11:20	Lecture 6. Andriy Zakalskiy (Lviv). Recombinant creatinine deiminase from *Corynebacterium glutamicum*: overexpression, isolation, characterization, and bioanalytical application
11:20 – 11:40	Lecture 7. Oleksandr Petrenko (Kharkiv). Three-dimensional constructs based on MSCs with different cell organization: bioengineering and safe storage.
11:40 – 12:20	Session **«Bioanalytics»** ***Chair Mykhailo Gonchar (Lviv)***
11:40 – 12:00	Lecture 1. Mykhailo Gonchar (Lviv), Microbial enzymes and recombinant cells coupled with nanozymes as sensing elements of amperometric biosensors.
12:00 – 12:20	Lecture 2. Nataliya Stasyuk (Lviv), Bioanode based on genetically engineered bacterial cells enriched with creatinine deiminase and N-methylhydantoin-sensitive bionanocomposite in construction of self-powered biosensors
12:20 – 12:40	*Presentation of conference sponsor ТОВ «Логіклабгрупа»*
12:40 – 13:40	**Lunch** (see the nearest possible locations at the end of the Program, marked *).
13:40 – 14:40	**Poster session №4** (sections *"Bioanalytics", "Biotechnology and metabolic engineering")*
14:40 – 15:40	Short oral presentations of 6 best posters of young scientists (under 35 years) - 5 min presentations.
15:40 – 16:30	Conference closing ceremony. Young Scientists Award for the best poster presentation.

***Lunch** – the nearest possible locations:

- Restaurant "Puzata Khata", Shevchenka Ave., 10, Lviv;
- Restaurant "SteakHause", Shevchenko Ave., 25, Lviv;
- Restaurant "Beer Garden", Ivan Franko Str. 29, Lviv;
- Restaurant "Budmo", Stefanyka Str. 19, Lviv;
- Restaurant "Caucasus", Shota Rustaveli Str. 2, Lviv;
- "PastaCafe" – Dudayeva Str. 3, Lviv;
- Cafe "Lviv Croissants", Shota Rustaveli St, 6, Lviv,
- McDonald's, Shevchenka Ave., 7, Lviv;
- Bussines Hub "Black Honey", Shota Rustaveli Str., 12, Lviv.

Session 1

Cell signaling mechanisms, autophagy and apoptosis

NON-CONVENTIONAL YEASTS IN CELL BIOLOGY AND BIOTECHNOLOGY

Andriy Sibirny **Lecture 1**

Andriy Sibirny[1,2]

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Non-conventional yeasts (those different from *Saccharomyces cerevisiae*) show many interesting and even unique properties like capability to utilize unusual carbon sources (methanol, xylose, L-arabinose, lactose) or overproduce important commodities like microbial oils or riboflavin. We work with several species of non-conventional yeasts, mainly with *Komagataella phaffi, Ogataea polymorpha* and *Candida famata.*

K. phaffi is used mostly by us for studying autophagy, both selective peroxisome autophagy (pexophagy) or selective cytosolic enzyme degradation. Earlier, we identified new genes involved in autophagy, *ATG26, ATG28, ATG35, GPR1, GSA2, GSS1, ACG1*. Currently, we look for mechanisms of selective autophagic degradation of the cytosolic enzymes of methanol metabolism, like fructose-1,6-bisphosphatase, formaldehyde dehydrogenase and formate dehydrogenase as well as heterologous enzyme beta-galactosidase expressed under methanol-induced *FLD1* promoter. In the thermotolerant yeast *O. polymorpha*, we study alcoholic fermentation of the lignocellulosic sugars: xylose, L-arabinose and cellobiose. We constructed strains with 50 times elevated ethanol production from xylose, those capable of cellobiose fermentation and started engineering L-arabinose fermentation. The recombinant strains of *O. polymorpha* were constructed producing lactic acid due to expression of lactate dehydrogenase genes of different origins and peculiarities of yeast lactic acid synthesis were studied. The yeast *C. famata* naturally overproduces riboflavin under iron starvation. Using combination of random selection and metabolic engineering, strains of *C. famata* were constructed which accumulate more than 16 g riboflavin/L or overproduce riboflavin on cheese whey and lignocellulosic hydrolysates. Besides, unique overproducers of flavin coenzymes FMN and FAD were constructed. Using *K. phaffi* and *C. famata*, the yeasts overproducing bacterial antibiotics (roseoflavin, aminoriboflavin) were constructed for the first time. Further perspectives of non-conventional yeast application in basic and applied science will be discussed.

STRUCTURAL PRECONDITIONS FOR THE ATG8 FUNCTIONING AND THE EFFECT OF LIPIDATION AND ACETYLATION ON ITS REGULATORY MECHANISM

Alex Rayevsky **Lecture 2**

Alex Rayevsky[1], Elijah Bulgakov[1], Yaroslav Blume[1]

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Autophagy is a highly orchestrated, dynamic process that relies on the autophagy proteins (ATGs) to regulate the formation of the autophagosome. To date, nearly half of the more than 40 identified autophagic proteins are part of the basic autophagy machinery that is conserved across all kingdoms of life and is exemplified by the common model organism *A. thaliana* [1]. The ubiquitin-like ATG8 proteins, which are responsible for the modification of membranes, via the process of 'Atg8ylation'[2], serve to transmit signals of membrane stress, damage and remodeling. ATG8 is initially activated by the E1 enzyme ATG7 and subsequently transferred to the E2 enzyme ATG3, where it is finally conjugated to PE via a ligase complex that includes the ATG12-ATG5 and ATG16 conjugate [3].

It is well known that canonical site on ATG8 share two common features: first, they adopt an extended β conformation and form an intermolecular β-sheet with the β2 strand of Atg8, and second, they leverage two conserved hydrophobic residues in the W/F/YxxL/I/V motif to bind to the W and L sites of Atg8, strictly speaking [4]. Based on the sequence-based alignment we reconstructed all the plant ATG8s from *A. thaliana* and performed a series of molecular dynamics simulations. In attempt to understand structural features responsible for the PPI selectivity, we analyzed the output trajectories applying both the RMSD-based clustering approach. These allowed us to suggest that despite the very similar binding site, the internal rigidity/flexibility of the protein scaffold defines its functionality at different stages of autophagy development. It was confirmed with a different result of protein-protein docking and further simulation of a set of ATG8s and cognate adaptors and receptors. Selecting three the most representative isoforms (A, E, H) from each group of ATG8 family, we introduced post-translational modifications, lipidation and acetylation, into the structures. Our modeling resulted in a structure-based comparison, showing a complete hindering of the PPI interface, along with a decrease of structural motility, as a result of decoration with acetyl groups at K49 and K51. This was estimated and illustrated by means of PCA analysis and comparison with the unmodified models. Regarding the lipidation, we generated and parametrized a PTM residue using R.E.D.III server and Ambertools. But the output is less pronounced, we obtained a well-equilibrated models of plant ATG8s, serving as a fine-adjusted tool for the next step of the investigation.

1. Yemets, A., et al., Autophagy formation, microtubule disorientation, and alteration of ATG8 and tubulin gene expression under simulated microgravity in Arabidopsis thaliana. npj Microgravity, 2024. 10(1): p. 31.
2. Deretic, V. and D.J. Klionsky, An expanding repertoire of E3 ligases in membrane Atg8ylation. Nature Cell Biology, 2024. 26(3): p. 307-308.
3. Liu, X.-M., et al., Lipidation-independent vacuolar functions of Atg8 rely on its noncanonical interaction with a vacuole membrane protein. eLife, 2018. 7: p. e41237.
4. Johansen, T. and T. Lamark, Selective Autophagy: ATG8 Family Proteins, LIR Motifs and Cargo Receptors. Journal of Molecular Biology, 2020. 432(1): p. 80-103.

SPECIFIC DEGRADETION OF CYTOSOLIC PROTEINS IN THE METHYLOTROPHIC YEAST KOMAGATAELLA PHAFFII UPON CARBON SOURCE REPLACEMENT

Kostyantyn Dmytruk **Lecture 3**

Kostyantyn Dmytruk[1], Olena Dmytruk[1], Ulyana Kyryliv[1], Andriy Sibirny[1,2]

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Exploring the mechanisms of cytosolic protein degradation holds significant importance both in terms of fundamental understanding and in terms of practical applications. The study investigated the decrease in the specific activity of fructose-1,6-bisphosphatase (Fbp), formaldehyde dehydrogenase (Fld1) and formate dehydrogenase (Fdh1) in several strains of *Komagataella phaffii*. These strains comprised the wild-type strain GS200, a strain SMD1163 with a defective autophagy pathway and a strain with the *GSS1* hexose sensor gene deleted. The investigation focused on short-term and long-term induction with methanol, followed by a shift to glucose, with or without the addition of MG132, a proteasome degradation inhibitor. It was shown that the duration of cell incubation on methanol had no particular effect on the inactivation of enzymes. The effect of the proteasome inhibitor MG132 was insignificant. Fbp, Fld1 and Fdh1 undergo degradation through the vacuolar pathway irrespective of the duration of methanol induction. This conclusion was verified through Western blot analysis and fluorescence microscopy studies. The disruption of Atg1 and Atg6, which are involved in autophagy initiation and autophagosome formation, does not affect the degradation of Fld1 and Fdh1. In contrast, the Atg15 lipase, responsible for breaking down the membranes of autophagic bodies in the vacuole, is essential for the degradation of these enzymes in *K. phaffii*. The absence of Fld1 and Fdh1 degradation in *atg15Δ* after the shift from methanol on glucose suggests the protection of the enzymes as autophagic cargos in autophagic bodies, retaining their membrane integrity. *K. phaffii* strain expressing heterologous β-galactosidase under the Fld1 promoter has been constructed and used as a parental strain for insertional tagging of genes involved in cytosolic protein degradation. In one of the resulting strains, the insertion cassette disrupted the gene encoding β-1,6-N-acetylglucosaminyltransferase.

ANTICANCER THERAPY BASED ON ARGININE DEPRIVATION: OLD CONS AND NEW DEVELOPMENTS

Oleh Stasyk **Lecture 4**

Olena Vovk, Galyna Shuvayeva, Dmytro Demash, Nikita Polishchuk, Oleh Stasyk

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Anticancer amino acid arginine (Arg) deprivation therapy (ADT) has been proposed based on pegylated forms of two alternative recombinant arginine-degrading enzymes, human arginase I (ARGI) and bacterial arginine deiminase (ADI). ADT has been shown to be very effective *in vitro* abrogating proliferation of cultured tumor cells irrespective of the status of their Arg biosynthetic pathway. In fact, we were first to demonstrate that cultured malignant cells cannot convert ornithine to Arg due to deficiency in mitochondrial enzyme ornithine transcarbamylase (OTC). However, the downstream Arg precursor, citrulline, is present in human blood stream and can be converted to Arg by sequential action of argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL), of which the former is a rate-limiting and is reversibly downregulated in many tumors. Therefore, it is status of ASS which determines sensitivity of malignant cells to ADT *in vivo*.

Despite promising results in pre-clinical studies and initial clinical trials, ADT has not reached the clinics yet. This is mainly due to its several intrinsic limitations associated with the induction of ADT-resistant phenotype and possible adverse effects for the organism caused by the prolonged exposure to low Arg levels needed to affect tumor growth.

We and others proposed several combination modalities that include ADT and chemotherapeutic drugs or irradiation aimed to enhance ADT effectiveness that will be discussed.

Acknowledgement. This study was partially supported by “Presidential Discretionary-Ukraine Support Grants” from Simons Foundation, Award No 1030281. This work was also supported in part by the NRFU project N 2023.04/0048, State registration number: 0123U103586, «Development of a broad-spectrum antimicrobial agent based on the extract of the leguminous plant *Indigofera spicata*».

MITOPHAGY AS AN ADAPTIVE PROCESS IN STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

Yurii Bandura **Lecture 5**

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Type 1 diabetes mellitus (T1D) is an autoimmune disease that destroys beta cells in the pancreas, leading to a decrease in insulin production. The resulting hyperglycemia can lead to excessive formation of free radicals in the mitochondria and, as a result, to the development of nitrate-oxidative stress. Excessive free radicals can also damage the mitochondria themselves. The accumulation of damaged mitochondria has serious consequences, namely, a decrease in ATP production, the formation of even more reactive oxygen species (ROS), the release of pro-apoptotic factors such as cytochrome C, and impaired calcium ion transport with subsequent cell death. Therefore, an important criterion for the functional state of a cell is to assess the degree of mitophagy. Mitophagy is a selective type of autophagy that leads to the degradation of damaged mitochondria. Mitochondria-associated endoplasmic reticulum membranes (MAMs) play an important role in the connection between mitochondria and the endoplasmic reticulum (ER), as well as in the regulation of mitophagy. MAMs serve as a platform for the interaction between mitochondria and autophagosomes and contain a number of proteins responsible for the process of mitophagy activation. One of these important proteins is the Parkin ligase, which is responsible for ubiquitination.

The aim of our study was to investigate changes in the size and length of MAMs and the content of Parkin/LC3 proteins in pancreatic cells under streptozotocin-induced diabetes. Using the electron microscopy method and Fiji software, we evaluated the main criteria of MAMs in normal and T1DM conditions. Thickness of contact between the ER and mitochondria is approximately 11-34 nm in the normal state, which ensures the normal functioning of both organelles. In streptozotocin-induced diabetes, the formation of denser contacts was shown: the width of which was 8-17 nm, which ensures a more intense flow of Ca^{2+} ions into the mitochondria. Excessive Ca^{2+} can lead to mitochondrial dysfunction, namely, disruption of the electron transport chain and overproduction of ROS, which in turn can cause apoptosis. Using Western blot analysis, we analyzed the levels of two key autophagy proteins, Parkin and LC3, and recorded an increase in their levels in the experimental group compared to the control group. Thus, our data indicate the formation of closer contacts between MAMs and an increase in mitophagy, which may indicate a disruption of the Ca^{2+} homeostasis system, which can lead to a violation of the cell's energy balance.

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Session 1: Cell signaling mechanisms, autophagy and apoptosis / Сигнальні механізми клітини, автофагія та апоптоз

FUNCTIONAL REACTIVATION OF MUTANT *TP53* GENE BY NOVEL THIOSEMICARBAZONES LEADS TO ENHANCED CELL DEATH INDUCTION IN COLON CANCER CELLS

Rostyslav Panchuk **Lecture 6**

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Colorectal cancers with high *TP53* mutation rates pose significant therapeutic challenges, constituting 10% of all cancer cases worldwide. The *TP53* gene encodes p53 tumor suppressor protein, and mutations in this gene, particularly at hot-spot codons, contribute to the progression of more than 60% of colorectal cancers. Therefore, identifying specific vulnerabilities associated with these mutations for targeted therapies is of paramount importance.

Recently it was demonstrated that several small molecules, which belong to the class of Michael acceptors (e.g. MIRA-1, PRIMA-1, STIMA-1 and APR-246), can form covalent bonds with cysteine residues in mutant p53 molecule. This results in an enhanced thermal stability of reactivated mutant p53 in a wild-type p53-like folding state and tumor growth inhibition. Unfortunately, all these compounds possess low selectivity of action and strong side effects *in vivo* due to their thiol-binding properties, which excludes their use in clinical practice. Novel thiosemicarbazone derivative COTI-2 possessing strong [Zn^{2+}] chelating properties seems to be a much more promising candidate for this role. However, exact mechanisms underlying mutp53 reactivation by COTI-2 are still poorly understood, and monotherapy by COTI-2 was usually not enough to achieve full tumor remission. In order to overcome these challenges, novel COTI-2 derivatives – COTI-NMe2 and KP1550 were synthesized, possessing 20-40-fold higher anticancer activity *in vitro* and intrinsic ability to overcome acquired resistance of tumor cells to COTI-2.

It was revealed that isogenic HCT-116 colon cancer cells harboring most frequent contact (R175H) and structural (R273H) *TP53* mutations, especially generated by us for this task, are hypersensitive to COTI-2, COTI-NMe2 and KP1550 compared to parental cell line with intact *TP53* gene. Cytomorphological studies had revealed that none of compounds induced apoptosis, as there were no signs of chromatin hypercondensation. Instead of it, studied thiosemicarbazones led to formation of massive amount of vesicles in cytosol and specific changes in cellular morphology, which are considered as a main hallmark of paraptosis – another form of cell death. Western-blot analysis had revealed specific fluctuations in key proteins, involved in cell cycling, ER stress and apoptosis depending on dosage of these compounds. In particular, low doses of COTI-2, COTI-NMe2 and KP1550 (up 10 μM, 24h incubation) led to overexpression of p21Waf1 and increase in level of phosphorylated Erk 1/2, with no changes in activity of caspase-3. Further increase of their concentration up to 25 μM led to disappearance of p21Waf1 and ERK 1/2, while total level of p53 gradually increased altogether with massive cleavage of PARP-1 – another marker of apoptosis. No changes in Beclin-1 and BiP expression were observed thus excluding autophagy as a mode of action of studied compounds.

Summarizing, hypersensitivity of colon cancer cells harboring R175H and R273H mutations of *TP53* gene to novel thiosemicarbazones seems to be caused by two deferent mechanisms. In low concentrations they led to classical ER stress induction (mediated by Erk 1/2), while further increase of their dose led to activation of apoptotic signaling pathways, mediated by reactivated p53. Further studies of their molecular mechanisms of action are in progress.

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AUTOPHAGIC FLUX DETECTION IN ANGIOSPERM MICROSPOROGENESIS

Olena Kravets **Lecture 7**

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Autophagy ensures the fertility of reproductive organs, affects pollen and seed productivity, yield, including under unfavorable conditions [1-3]. The critical role of autophagy in the degradation of the tapetum (the lining layer of the anther) has been confirmed, where it is involved in the regulation of anther lipid metabolism [4-5]. However, the question of the role of autophagy in meiosis, in particular microsporogenesis of angiosperms, remains insufficiently studied. It is assumed that autophagy is involved in the degradation of important regulators of division, in progression and termination of meiosis [6]. The aim of the current research is to determine the autophagic flux in microsporogenesis in various representatives of monocots and dicots using methods of light and luminescent (confocal) microscopy.

At the beginning of microsporogenesis, in the zygotene-pachytene prophase, autophagosomes heterogeneous in size and shape are visualized in the periplasmic cytoplasm of microsporocytes. Appearance of autophagosomes at the beginning of meiosis may indicate the degradation of proteins that initiate microsporogenesis and segregation (synapsis) of chromosomes. Autophagy activity does not change significantly during microsporogenesis. Cases of intense staining of some microsporocytes with a marker for autophagosomes (LysoTracker™ Red) are obviously related to programmed cell death (PCD) for the maintenance of anther tissue homeostasis.

Active processes of autophagy unfold at the finish of microsporogenesis. Moreover, in some species (*Hosta* and *Lilium* with secretory type of tapetum) autophagosomes accumulate in the locular fluid and tapetum, triggering PCD of tapetal cells. Whereas in other species (*Capsella* with secretory type and *Tradescantia* with periplasmodial type), autophagosomes accumulate in tetrads of microspores, which probably indicates degradation regulators of meiosis, which are localized exactly in microsporocytes. Differences in tissue localization autophagosomes at the end of microsporogenesis in the studied species are probably related to different localization of regulators meiotic division, namely, internal – in microsporocytes and tetrads in *Capsella* or *Lilium* and external – in sporophyte, tapetum – in *Hosta* and *Tradescantia,* as well as differences in PCD types of tapetal tissue.

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SMAC MIMETICS AND MACROPHAGE-DERIVED EXOSOMES ERADICATE TUMOR CELLS

Rostyslav Horbay **Lecture 8**

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Introduction. SMAC mimetics (SMCs), drugs that antagonize members of the Inhibitor of Apoptosis protein family, synergize with immune ligands and immunostimulatory agents to eradicate tumors [1,2]. We have demonstrated that SMCs triggers release of 30-150 nm small extracellular vesicle (sEV) that carry inflammatory cytokines from immune and cancer cells, that later synergize with SMCs to eradicate tumor cells.
Methods. sEV isolation and validation was in accordance with the latest ISEV recommendations (extracted with differential ultracentrifugation and validated via Zetaview NTA, ONI Nanoimager, and Western blotting) [3,4]. We used cell culture approaches to study the impact of SMC-induced sEVs on cancer and immune cells, including viability assays, direct and transwell co-culture conditions, ELISA, and flow cytometry.
Results. SMC treatment increased the rate of sEV release in macrophages by ~5X (t-test, $P<0.05$, $n=4$), but does not significantly alter the secretion rate of sEVs in cancer cells. SMC treatment of macrophages leads to packaging of proinflammatory cytokine into sEVs, particularly TNFα. The biogenesis of these TNFα-bearing sEVs was done in an ESCRT-dependent manner, as proteins such as Syntenin and Alix were present on the sEV surface. On the contrary, tumor cell-derived were missing these key ESCRT proteins, but had elevated levels of HSC70. Immune-cell derived sEVs eradicated tumor cells in the presence of a SMC (ANOVA, $n=4$, $P<0.0001$). Furthermore, SMC induced packaging of the positive TNFa regulator LITAF into macrophage-derived sEVs, but reduced the presence of TACE (TNFa-converting enzyme) in macrophages. In addition, the Syntenin-antagonist KSL128114 synergized with SMCs to induce death of cancer cells (67% viability SMC only compared to 14% of the combination, $n=2$), but did not induce the death of immune cells. Lastly, RIG-I, a cytosolic pattern recognition receptor that induces a Type I interferon response, was detected within tumor-derived sEVs from interferon- and SMC- treated cancer cells, which implicates that SMCs stimulate tumor cell-derived sEV release that would activate an innate immune response.
Conclusion. Our data demonstrates that SMC treatment increases cytokine-carrying sEV release from immune cells, which then eradicates cancer cells in a therapeutic favorable manner.

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NEW EFFICIENT WATERBORNE SYSTEMS FOR DELIVERY OF WATER-INSOLUBLE THIAZOLIDINONE DERIVATIVES BASED ON BRANCHED POLYMERIC SURFACTANTS WITH SIDE POLYOXAZOLINE CHAINS

Natalya Finiuk **Lecture 9**

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Several polymeric nanomedicines were approved by the FDA or are either undergoing clinical trials [1]. Here, novel branched polyoxazoline (pOX)-containing polymer poly(VEP-*co*-GMA)-*graft*-pOx (P1) was synthesized *via* controlled synthesis consisting of two steps: 1) polymerization and obtaining the backbone with side epoxide groups of monomer units of glycidyl methacrylate (GMA); 2) the reactions of the latter with the terminal hydroxyl group of monosubstituted pOX oligomer molecules. The copolymers combining hydrophobic backbone and hydrophilic side pOX chains are the surface-active substances forming micelle-like structures capable of forming stable waterborne systems that were used for immobilization of water-insoluble thiazolidinone Les-6294 derivative (1-(4-chlorophenyl)-3-[5-[2-chloro-3-(4-nitrophenyl)prop-2-enylidene]-4-oxo-2-thioxothiazolidine-3-yl]pyrrolidine-2,5-dione). The size and morphology of the initial polymeric micelles and derivative-polymer complexes were studied using DLS, TEM, and SAXS methods. The Les-6294 compound and its complexed form possessed high cytotoxic and antiproliferative activity toward breast (MCF-7, MDA-MB-231), colorectal (HCT116, HT-29, DLD-1), gastric (AGS) carcinoma cells, and glioblastoma (A172) cells with IC_{50} values in a range of 0.56-6.85 μg/mL. The complexation of Les-6294 with P1 resulted in the 4-6-fold elevation of anticancer activity of this derivative. The P1 polymer possessed low toxicity towards studied cell lines. The Les-6294 and Les-6294/P1 inhibited the formation of colonies and DNA biosynthesis in HT-29 and DLD-1 cells. These agents showed low toxicity towards the pseudo-normal cells of MCF-10A, Balb-3T3, and C8 lines. They demonstrated selectivity towards studied breast, colorectal, gastric, and glioblastoma cells. The Les-6294 and Les-6294/P1 decreased the mitochondrial membrane potential and elevated caspases 3/7, 8, 9, and 10 activities resulting in apoptosis *via* extrinsic and intrinsic pathways in HT-29 and DLD-1 cell lines. The Les-6294 and Les-6294/P1 affected the S transition checkpoint in these cells. Thus, the new polymer with a grafted polyoxazoline is a promising carrier for the delivery of water-insoluble agents that act by improving their solubility in aqueous media and enhancing antiproliferative action towards tumor cells.

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THE INHIBITORY EFFECT OF THIACALIX[4]ARENE C-1193 ON THE PLASMA MEMBRANE Na^+,K^+-ATPase OF UTERINE MYOCYTES

Tetyana Veklich **Poster 1**

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Na^+,K^+-ATPase is an electrogenic Mg^{2+},Na^+,K^+-ATP-dependent transport system of the plasma membrane (PM) that actively transports monovalent Na and K ions and thereby maintains their electrochemical gradients, which are essential for normal cell functioning. Moreover, Na^+,K^+-ATPase is important for the regulation of intracellular Ca concentration due to the physical connection between Na^+,K^+-ATPase, NCX and Ca stores in the sarcoplasmic reticulum. The function of the sodium pump is susceptible to alterations in various pathological conditions, including diabetes and ischemia. Therefore, it is promising to search for a compound that would allow changing the activity of the PM sodium pump. From this point of view, the calixarenes are very interesting and perspective compounds. Calix[4]arene possess anti-virus, bacterocyde anti-swelling and anti-trombotic features.

Thiacalix[4]arene were synthesized and characterized using NMR and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry, NASU (head of the department - Academician of NASU, prof. V.I. Kalchenko). All enzymatic activities were assayed in cell suspension perforated with 0.1 % digitonin. The methods of enzymatic and kinetic analysis were used to demonstrate that thiacalix[4]arene-bis-hydroxymethylphosphonic acid C-1193 had a more efficient inhibitory effect on Na^+,K^+-ATPase activity in the plasma membrane of myometrium cells ($I_{0.5}$=42.1±0.6 nM) as compared to calix[4]arene-bis-hydroxymethylphosphonic acid C-99, and had almost no effect on relative activities of other ATPases localized in this subcellular structure.

The method of confocal microscopy and Ca^{2+}-sensitive fluorescent probe fluo-4 were used to demonstrate that thiacalix[4]arene C-1193 increased the intracellular concentration of Ca ions in the immobilized uterine myocytes. Having tested the affinity of this enzyme for ATP and Mg ions depending on the concentration of C-1193, as well as its effect on the cooperative effect and on the maximum rate of ATP hydrolysis, we found that in both cases a significant decrease in the maximum rate of ATP hydrolysis was observed, which, in combination with the absence of an effect on the affinity constants, indicates a non-competitive mechanism of inhibition of Na^+,K^+-ATPase activity by thiacalix[4]arene C-1193.

Obtained results are important for understanding and subsequent investigation of mechanisms of Na^+,K^+-ATPase inhibition by calixarene C-1193 and can be used to create new more effective inhibitors of mentioned enzyme and new uterotonics for medicine, based on the calixarene core.

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THE STATE OF VITAMIN D_3 AUTO-/PARACRINE SYSTEM IN RAT KIDNEYS DEPENDING ON THE VITAMIN D_3 STATUS

Olha Lisakovska **Poster 2**

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Kidneys plays a crucial role in the classical pathway for the activation of vitamin D_3 (D_3). In the liver, D_3 precursors are hydroxylated to 25-hydroxvitamin D_3 ($25D_3$). $25D_3$-vitamin D_3 binding protein (VDBP) complex uptakes into the kidney, where $25D_3$ is converted by 1α-hydroxylase (CYP27B1) to the hormonally active form – 1,25-dihydroxyvitamin D_3 ($1,25D_3$). In turn, $1,25D_3$ isinactivated by 24-hydroxylase (CYP24A1). The current study aims to examine the relationship between D_3 status and the state of the D_3 auto-/paracrine system in the rat kidneys.

Female Wistar rats (132.33±6.99 g) were divided into 3 groups depending on their D_3 status: 1) control rats on conventional diet; 2) rats with nutritional D_3 deficiency (VDD, 8 weeks); 3) rats treated with vitamin D_3 (1000 IU/kg of b.w. per os, 4 weeks) after 8 weeks of VDD diet. Serum $25D_3$ level was monitored by ELISA. Protein and mRNA levels of the key components of vitamin D_3 auto-/paracrine system were measured by western blotting and/or RT-qPCR.

In VDD group, we observed a dramatic decline of $25D_3$ serum level from 118.27±18.05 nmol/L in control rats to 16.45±1.52 nmol/L. At the same time, these rats demonstrated reduced final body weight (198.00±20.54 g) compared to control (245.67±13.87 g), that was associated with a concurrent decrease in the kidneys weight (1.37±0.25 g) vs. 1.74±0.05 g in the control rats. There was no statistically significant difference in the kidney-to-body weight ratio between studied groups. D_3 administration elevated $25D_3$ level to 78.24±22.57 nmol/L and restored body weight gain (247.75±5.19 g) to control values. We found an increase in *Vdr* (by 2.2 folds) and *Cyp24a*1 (by 12.7 folds) and a decrease in *Cyp27b1* (by 4.8 folds) gene expression in VDD rats. After $25D_3$ restoration we observed a dramatic decrease in *Vdr* (by 19.7 folds vs. VDD group, by 9.1 folds vs. control) and an increase in *Cyp27b1* level (by 14 folds vs. VDD group, and by 2.9 folds vs. control) with simultaneous normalization of *Cyp24a1* content. At the protein level, we found an elevation of VDR and VDBP content in kidneys of VDD rats (by 1.9 and 2.3 times respectively) and a reduction of their protein contents (by 2.9 and 3.2 folds vs. VDD rats) after D_3 repletion. At the same time, CYP27B1 and CYP24A1 content decreased by 1.6 and 2 times respectively in VDD rats, suggesting impaired both synthesis and catabolism of D_3 hormonally active form. After D_3 supplementation, no corrective effect was observed on the protein levels of these enzymes.

In summary, depletion of $25D_3$ circulating pool led to a remarkable imbalance of vitamin D_3 auto-/paracrine system on the transcriptional and translational levels in rat kidneys. However, these changes were mostly reversible, especially at the transcriptional level, following normalisation of vitamin D_3 status.

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STUDY OF CHITOSAN BASED HYDROGEL FILMS *IN VITRO* AND *IN VIVO*

Nazar Manko **Poster 3**

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Chitosan and its derivatives attract an increasing attention in biomedicine. Chitosan in various forms has long been used as an emergency hemostatic agent in modern military conflicts. Our study is addressed to investigation of the possibility of new chitosan-based hydrogels films to use it for wound dressing and healing.

We studied chitosan-based hydrogels films *in vitro* and *in vivo*. Initially, we evaluated the biodegradation and biocompatibility of the films. Secondary we tested films in vivo, by ethical proved wound healing protocols on mice C57\Black.

At *in vitro* part of study, we observed that that the **CHT film** didn't have negative effect on fibroblasts under 24 h treatment. Studied sample kept their appearance and shape in Physiological saline by incubation for 14 days Degradation of sample **CHT film** was observed in DMEM and FBS, the time of complete degradation was less than 72 hours **CHT film** did not cause significant changes in Balb/c-3T3 cells morphology, only small number of cells with condensed nucleus was observed in control (untreated) cells and in studied sample. Also, we observed lysosome activation in Balb/3T3 cells by CHT Film after 24 h incubation. Such activation of lysosomes by experimental samples may be an indicator that the gel is mostly degraded by lysosomal enzymes.

At *in vivo* part of study was managed faster wound closing by using the **CHT film,** in comparison to control and Gel (commercial version of wound healing gel) by one-time treatment. We observed increasing wound healing by using **CHT film,** in particular at 3 and 9 days after treatment. Namely, on the 3 day, in the test samples, there were more pronounced areas of re-epithelialization at the edges of the wound. At 9-day samples after CHT film treatment demonstrate the best results of skin recovery: complete epithelization, the structure of the epithelial plate approaches the structure of the epidermis. There was a contraction of the connective tissue: the distance between the edges of the wound sharply decreased, in the deeper layers of maturing connective tissue (the extracellular matrix is coarse-fibrous, disordered placement of fibroblasts), subepithelial areas of granulation tissue with moderate cellularity.

Summarizing, chitosan-based gel films show high biocompatibility, non-toxicity, and promote wound healing even with single use compared to a commercial sample under the same conditions.

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RESEARCH OF MECHANISMS OF NON-COENZYMIC ACTION OF THIAMINE: NEW PROTEIN TARGETS AND SIGNIFICANCE FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

Olha Mezhenska **Poster 4**

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The study is devoted to the identification of nerve tissue proteins that exhibit affinity for thiamine. The proteins from nerve tissue extracts that specifically bind thiamine [1] for the first time have been identified by mass spectrometric analysis and Western blot analysis, in particular, the LRP4-Agrin protein complex, which is a component of the nAChR cluster and a series of signaling cascades [2]. The amino acid residues of both Agrin and LRP4 polypeptide chains involved in thiamine binding were determined using the molecular docking. Comparative analysis of the binding sites of thiamine and thiamine phosphorous esters with these proteins and with known thiamine-dependent proteins revealed their high similarity and low likelihood of replacement of thiamine with adenosine compounds or acetylcholine, as well as explaining the causes of lower affinity for thiamine phosphate esters compared to thiamine. Bioinformatics tools suggested the possible biological roles of thiamine and its biologically active derivatives when binding to the Agrin-LRP4 complex.

The results obtained in these studies are important as they expand the current understanding of cell-molecular mechanisms of vitamin B_1 functions realization [3]. This further substantiates the advisability of using thiamine and its pharmacological forms for the prevention and/or treatment of pathologies that are induced or accompanied by thiamine deficiency, in particular, Wernicke-Korsakov encephalopathy, Alzheimer's, Parkinson's and Huntington's disease, amyotrophic sclerotic disease, fronto-temporal dementia, diabetes and others.

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INDOSPICINE SELECTIVELY TRIGGERS CANCER CELLS DEATH UNDER ARGININE DEPRIVATION

Galyna Shuvayeva **Poster 5**

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Arginine-deprivation therapy is an actively developing metabolic anticancer approach. To overcome the resistance of some cancer cells to such a monotherapy, rationally designed combination modalities are needed. We evaluated for the first time indospicine, an arginine analogue of *Indigofera* leguminous plant genus origin, as potential enhancer compound for the arginine-deprivation therapy based on recombinant human arginase I. We demonstrated that indospicine at low micromolar concentrations is selectively toxic for human colorectal cancer cells only in the absence of arginine. In arginine-deprived malignant cells indospicine deregulates some prosurvival pathways (PI3K-Akt, MAPK), activates mTOR signaling, exacerbates endoplasmic reticulum stress and triggers caspase-dependent apoptosis. These effects are reversed by the exposure to translation inhibitors. Importantly, indospicine is not degraded by rhARG and does not inhibit this enzyme at its effective doses. Thus, the obtained results emphasize the potential of arginine structural analogues as components for combinational anticancer therapy based on enzymatic arginine deprivation.

ARGININE-DEPRIVATION THERAPY ENCHANCERS: 3-BROMOPYRUVATE AND GEMCITABINE

Dmytro Demash **Poster 6**

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Amino acid deprivation and arginine deprivation therapy (ADT) in particular is a promising strategy of metabolic antitumor therapy. ADT may be an additional option for the treatment of refractory tumors, especially for patients with multidrug-resistant tumors. Despite promising results in pre-clinical and clinical trials, ADT has several limitations associated with the induction of ADT-resistant phenotype and adverse effects for the organism caused by prolonged exposure to low arginine levels.

Our study was designed to investigate the potential of enhancing ADT, induced by proprietary recombinant human arginase 1 (rhARG1), by 3-bromopyruvate (3-BrPyr), a known modulator of energetic homeostasis, or gemcitabine (GEM), a deoxycytidine analog.

For the first time, we determined the cytotoxicity parameters for combinations of ADT+3-BrPyr and ADT+GEM for head and neck cancer cells (FaDu, SAS). We showed that GEM prevented the growth recovery of both cell lines after reintroduction from arginine-depleted into the complete medium.

We also demonstrated the additive effects of ADT and 3-BrPyr on cell proliferation and the significant changes in cell signaling cascades, particularly the FAK-PI3K-Akt pathway. Observed effects also included aberrant expression of cell adhesion molecules and impaired cell migration as compared to respective controls.

Thus, both GEM and 3-BrPyr showed promising results in combination with ADT. These preliminary results underscore the potential of modifying and enhancing ADT to increase its antitumor activity and reduce unwanted side effects. Our further research will focus on a deeper understanding of the cellular mechanisms involved in the mentioned processes and on finding optimal combinations of agents.

IDENTIFICATION OF THIAZOLE/THIAZOLIDINONE DERIVATIVES AS INHIBITORS OF MUTANT CALRETICULIN FOR THE TREATMENT OF MYELOPROLIFERATIVE NEOPLASMS

Iryna Ivasechko **Poster 7**

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Calreticulin (CALR) frameshift mutations represent the second cause of myeloproliferative neoplasms and have been identified as a main disease driver, suggesting that the development of drugs targeting mutant CALR is of great significance. Previously, it was hypothesized that a small molecule targeting the N-glycan binding domain of CALR might inhibit the oncogenicity of the mutant CALR, and Hematoxylin was identified as a candidate binder [1]. In our present work we studied the selective growth inhibitory effect of 75 compounds, thiazole/thiazolidinone derivatives, with similar structural components as in Hematoxylin on the BaF3 wt cell line and model cells of myeloproliferative neoplasms with mutations in the *CALR* gene (CALR del52 and CARL ins5). The selection of compounds was made on the basis of structural 3D-similarity to Hematoxylin from our in-home library of various synthesized heterocyclic compounds that have experimentally established antitumor activity. BaF3 is a murine interleukin-3 dependent pro-B cell line, a popular system for exploring protein function and their inhibitors because some proteins can render BaF3 cells to be independent of IL-3 (like mutated CALR del52 and ins5), while their inhibitors antagonize this effect. Six of the 75 compounds (Gor-20102, Gor-20103, Les-6650, Les-6557, Les-5463, and Les-5421) exerted the growth-inhibitory effect in both mutated cell lines (CALR del52 and CALR ins5) with IC_{50} values ranging from 2.68 μM to 38.91. Three compounds (Gor-20097, Les-6638, and Les-5580) had an inhibitory effect only on CALR del52 cells (IC_{50} values from 7.93 to 10.55 μM), while seven compounds (Les-557, Les-5418, Les-6134, Les-5581, Les-5577, Les-4353, Les-4339, and Les-1495) had an inhibitory effect only on CALR ins5 cells (IC_{50} values from 9.27 to 37.82 μM). It is important to note that these compounds did not have a significant effect on the viability of other cancer and pseudonormal cell lines (IC_{50} > 50 μM), including MDA-MB-231, HT-29, HCT-116, Balb-3T3, HEK-293, and others.

The identified compounds might serve as a basis for further optimizing and developing effective drugs targeting mutant CALR. In addition, validating the anticancer activity and mechanisms of their action is necessary.

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RESPONSE OF ISOLATED LYMPHOCYTES OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA TO PYRROLIDINEDIONE-THIAZOLIDINONE HYBRID LES-6287 COMPOUND

Natalya Finiuk **Poster 8**

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Chronic lymphocytic leukemia (CLL) is widely heterogeneous in terms of progression and therapeutic efficiency. The isolated CLL cells are more pertinent to the clinical setting and serve as a valuable model for drug development research.

Here we evaluated the toxicity of Les-6287 compound (1-(4-hydroxyphenyl)-3-[5-[2-chloro-3-(4-nitrophenyl)prop-2-enylidene]-4-oxo-2-thioxothiazolidine-3-yl]pyrrolidine-2,5-dione) towards isolated peripheral blood cells of patients with CLL and healthy human donors. The lymphocytes were isolated in a density gradient of Gradisol-G from the blood of patients with CLL before chemotherapy. They were found to be sensitive to the Les-6287 treatment with the IC_{50} of 9.35 ± 0.45 μM. The Les-6287 generated mitochondria-dependent apoptosis, DNA damage *via* single-strand breaks and fragmentation, and inhibition of PARP1 reparation enzyme in lymphocytes of CLL patients. The lymphocytes isolated from the blood of healthy donors were much more resistant (IC_{50} >96.87 ± 1.23 μM) toward the toxic action of the Les-6287 compound. The effect of Les-6287 on the phagocytic activity of normal polymorphonuclear (PMN) cells was investigated. Yeast cells of *Debaryomyces hansenii* strain (VKM Y-102) were used as an object of phagocytosis [1]. We did not find changes in the phagocytic activity of the PMN of healthy donors under Les-6287 treatment at 10 μM (54.65±5.92) and 100 μM (54.15±5.32) doses compared to the untreated cells (53.04±6.10). However, other ways of targeting the immune functions by the Les-6287 compound in CLL patients cannot be excluded.

Thus, compound Les-6287 possessed pronounced toxicity for isolated cells from patients with chronic lymphocytic leukemia *via* mitochondria-dependent apoptosis.

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CYTOTOXIC AND PROAPOPTOTIC ACTIVITY OF NEW THIOPYRANO[2,3-D]THIAZOLE-BASED DERIVATIVES AGAINST COLORECTAL ADENOCARCINOMA CELLS

Yuliia Kozak **Poster 9**

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Colorectal cancer (CRC) is the third most common cancer globally and the second leading cause of cancer-related deaths, following lung cancer [1]. High mortality rates are largely attributed to the limited treatment options for patients with advanced CRC [2]. Developing more effective and tolerable therapies remains a critical goal in modern medicine. Considering the successful use of thiazole-containing drugs in CRC treatment, new thiopyrano[2,3-d]thiazoles show potential as promising candidates for further therapeutic development.

The aim of the study was to evaluate the cytotoxic and proapoptotic activity of new thiopyrano[2,3-d]thiazole-based derivatives (Les-6547, Les-6557) against HT-29 and DLD-1 colorectal adenocarcinoma cells.

The IC50 value of Les-6547 was 4-7 μM for HT-29 and DLD-1 colorectal cancer cells, compared to 32-90 μM for pseudo-normal human keratinocytes and mouse fibroblasts, respectively. IC50 of Les-6557 was 5-10 μM for cancer cell lines (HT-29, DLD-1) versus 40-68 μM for pseudo-normal cell lines (Balb/c 3T3, HaCat). Thus, human colorectal adenocarcinoma cells are more sensitive to the action of Les-6547 and Les-6557 compared to pseudo-normal cells, indicating the selectivity of their action. Les-6547 and Les-6557, at a 1 μM dose, completely inhibit the ability of single colorectal HT-29 cancer cells to form colonies, similar to doxorubicin. Apoptotic cell numbers increased approximately ~6-fold and cells with reduced mitochondrial membrane potential increased ~5-fold after 24 hours of exposure with compared to control cells ($P \leq 0.001$). The percentage of cells with active caspase-8, -9, and -3/7 increased by more than 10-fold in HT-29 cells treated with Les-6547 and Les-6557 versus control cells ($P \leq 0.001$).

Les-6547 and Les-6557 exhibited pronounced cytotoxic and pro-apoptotic effects against colorectal adenocarcinoma HT-29 cells. The studied compounds induced apoptosis through both intrinsic and extrinsic pathways.

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SCREENING FOR MUTANT STRAINS WITH AUTOPHAGY DEFECTS OF CYTOSOLIC PROTEIN β-GALACTOSIDASE IN THE METHYLOTROPHIC YEAST *KOMAGATELLA PHAFFII*

Zuo Mingxing **Poster 10**

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The investigation of the mechanisms of cytosolic protein degradation is of great fundamental and applied importance. The β-galactosidase is most probably inactivated due to degradation occurring in vacuoles by autophagy mechanism. In our previously study, a developed system for the selection of recombinant strains of *Komagatella phaffii* with impaired autophagic degradation of the heterologous model cytosolic protein (yeast β-galactosidase) was constructed. Based on this system, the β-galactosidase of methanol-grown *K. phaffii* transformants can be assayed directly on plates using X-Gal staining which opens opportunity to isolate the mutants defective in degradative inactivation of cytosolic proteins in *K. phaffii*.

In this study, the N-methyl-N'-nitro-N-nitrosoguanidine was used for mutagenesis in order to obtain autophagy defective mutants. After several rounds of mutagenesis and using YPD with X-Gal as the selective medium, the mutant strains which showing blue color in plate were obtained. After shift from methanol to glucose medium, the β-galactosidase activity of the mutant colonies was higher than that of the original strain. It was also observed under fluorescence microscope that mutant colonies, after shift from methanol to glucose, showed stronger fluorescence as compared to the parental strain. The common phenotypes of the mutants defective in general non-selective autophagy is the loss of viability that occurs during incubation in starvation medium. Therefore, the viability assay in nitrogen starvation medium become evident to monitor autophagy-deficient mutants. The results indicate that the analyzed mutant strain have growth defects under starvation conditions as compared to the original strain. The presented results suggest that the obtained mutant has defects in autophagy. In the future studies, the relative enzymes, such as alcohol oxidase, will be measured after mutant cells shift from methanol to glucose medium.

Despite a large array of information on the mechanisms of degradation of proteins and cellular organelles, the mechanisms of selective degradation of cytosolic proteins remain unclear. Therefore, the analysis of the mechanisms of degradation of cytosolic proteins in methylotrophic yeast, the selection of mutants with damage to the relevant mechanisms has significant scientific interest and application.

NOVEL BRANCHED POLYMER DRUG CARRIERS CONTAINING GRAFTED SIDE POLYETHYLENE GLYCOL (MPEG) OR POLYOXAZOLINE (POX) CHAINS: BIOCOMPATIBILITY *IN VIVO*

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Development and application of effective biocompatible nanoplatforms for drug delivery is a crucial task in modern pharmacology and medicine. They enhance the antitumor drug action, decrease their toxicity, as well as overcome an acquired resistance of tumors to chemotherapy, and reduce adverse side effects towards normal cells of the body. Here, we discussed the results of comparative study of branched polymeric carriers: 1) poly(VEP-co-GMA)-graft-mPEG; 2) poly(VEP-co-GMA)-graft-pOX; 3) poly(PEGMA-co-DMM) containing units of poly(ethylene glycol)-methyl ether-methacrylate (PEGMA) and dimethyl maleate (DMM) synthesized via polymer analogous transformation and copolymerization of the macromer PEGMA. Different compositions and approaches of synthesis provide control of structural and molecular weight characteristics of new branched copolymers, their micellization ability and colloidal-chemical properties that affect the biocompatibility.

Their biocompatibility in C57/BL6 female mice was evaluated taking into account hematological and biochemical profiles of blood in treated animals. There was no mortality or significant changes in the body weight detected in mice that received the nanomaterials under study. The total dose of 664 mg/kg of those materials (quantity of polymer carrying 20 mg/kg dose of immobilized drug) was not lethal to studied animals. However, when mice received a sublethal dose (20 mg/kg) of doxorubicin (Dx), a decrease in body weight was observed compared to the healthy animals, and 60% of the treated mice died during the experiment. There were also changes in blood formula of mice detected under their treatment with a sub-lethal dose of Dx (20 mg/kg). That led to a decrease in the white blood cell count, neutrophilia and lymphocytopenia, with a simultaneous increase in the level of monocytes. In addition, a decrease in hemoglobin and red blood cell (RBC) levels was recorded in animal group treated with Dx. In contrast to Dx's action, the polymeric nanocarriers did not affect the hematopoietic system of treated mice. A decrease in the activity of liver function tests was observed in mice treated with the PC pEtOx on day 70, compared to day 30. In the group injected with PEG, an increase in ALT activity by 4.14 times was detected on day 30 of the experiment and by 2.96 times on day 70. The activity of alkaline phosphatase was found to be elevated in between the 30th and 70th day. Urea content showed similar dynamics of changes (decrease) in between the 30th and 70th day. Creatinine level was decreased by 23.5% on the 70th day compared to the initial day of blood sampling.

Summarizing, the highest biosafety potential was demonstrated by the polymeric nanocarrier poly(VEP-co-GMA)-graft-pOX for its use as a platform for drug delivery.

The in vivo studies were conducted under the approval of the BioEthics Committee of the Institute of Cell Biology, NAS of Ukraine (protocol N2/2024 dated by 19.03.2024).

HYPOXIA-INDUCED INSULIN LIKE GROWTH FACTOR AS REGULATOR OF CELLULAR SENESCENCE

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Purpose. Hypoxia is known as a factor affecting life expectancy, and stimulating cellular growth and proliferation, in particular, through the induction of growth factors. However, the question of how hypoxic induction of genes influences cellular senescence is not fully elucidated. The aim of the work was to establish the features of insulin-like growth factor IGF-1 expression in response to different modes of hypoxia and the effects of this factor on cells with low proliferative activity.

Methods. The influence of various modes of moderate hypoxia (chronic, periodic, acute) on Wistar rats or FVB mice was carried out by keeping on the middle altitude or by "lifting" in barochamber. Expression of IGF-1 protein and activation of proliferative proteins in tissues was assayed by immunoblotting. The cells of retina pigment epithelium RPE were cultivated in the presence of different concentrations of IGF-1 within 24-72 h. The proliferative response was evaluated by determining the expression and phosphorylation of the ATR/CHK pathway kinases and detection of EdU accumulation by fluorescence-activated cell sorting.

Results. The acute hypoxia is found to cause transient induction of IGF-1 protein within 24-72 h after exposure, which does not lead to proliferative response in postmitotic cells (cardiomyocytes). During periodic hypoxia sessions, the response of IGF-1 expression to each hypoxia séance was gradually decreased, and in chronic hypoxia the protein expression is markedly reduced compared to control. In the last cases, hypertrophic changes in myocardial cells were observed, which could be reversed after stopping the short-term hypoxia seances, but had become stable with prolonged (chronic) hypoxia action. In RPE cells, an increase in proliferative response was found when exposed to IGF-1 within 24-48 h, but with a longer exposure or increasing IGF-1 concentration, this response has inhibited.

Conclusion. Thus, the stimulatory effects of hypoxia on cellular growth/proliferation are short-term and reduced with prolonged hypoxia, in particular, due to the inhibition of expression of growth factors; as well as the effect of these factors on the cell can inhibit as their concentration increases. In turn, IGF-1 is able to cause proliferative response and counteract cellular senescence in cells with low proliferative activity, which can be important for the restoration, in particular, the retina pigment epithelium in its age degeneration. Therefore, the hypoxia and IGF-1 demonstrate counteraction of cellular senescence in the short-term period and limitation of cellular growth/proliferation – in prolonged.

RADIOBIOLOGICAL RESEARCH OF NON-TUMOR CELLS IN BLOOD OF CERVICAL CANCER PATIENTS

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Introduction. The use of new sources of ionizing radiation in oncological practice makes it possible to implement a conformal strategy of radiation therapy (RT) of a tumor and thus reduce the radiation load on healthy cells from its surroundings. However, even under these conditions of RT, it is hard to avoid damage of healthy tissue. Therefore, improvement of RT efficiency should take into account the ability to predict development of radiation complications, that worsen the life quality of cancer patients.

Goal of the work. To develop an adequate scheme for radiobiological support of radiation therapy of cervical cancer (CC) patients to predict radiation complications with use of highly radiosensitive cells that pass-through radiation zone. It is important for CC patients with locally advanced forms, who are treated according to the program of radical chemo-radiotherapy.

Materials and methods. The object of study is peripheral blood, as an integral indicator of homeostasis, and T-lymphocytes as the most radiosensitive cells of the human body. A panel of molecular, biochemical and cytological methods was used before and after radical chemo-radiotherapy program for 41 patients with locally advanced CC.

Results. Examination of CC patients before the RT was compared with healthy controls, and showed moderately increased (1.36 times) the pro-antioxidant ratio (PAR) in the blood hemolysate, an increased content of malondialdehyde (MDA, 3.08 times) and decreased of SH-groups concentration (1.31 times) in the blood plasma. The transmembrane potential (TMP) in mitochondria's as well as formation of reactive oxygen species in peripheral blood lymphocytes was decreased (1.46 and 3.36 times, respectively) but generation of superoxide anion radical ($O_2^{\cdot-}$) was increased (1.7 times) along with an increased amount of DNA double-strand breaks (2.33 times) and level of spontaneous apoptosis (2.84 times). The severity of redox balance disturbances in blood of CC patients were dependent on the extent of tumor process. Thus, an increase in tumor size and metastases to regional lymph nodes correlated with a higher level of TMP, (1.25 and 1.41 times, respectively), and with slight increase of $O_2^{\cdot-}$ generation in lymphocytes. Complex treatment with radiation and chemotherapy significantly affect the studied radiobiological parameters of CC patients. In the blood plasma we observed a further increase in the MDA content (1.42 times) and a comparable decrease in the concentration of SH-groups. Also we registered increase of the PAR in the blood hemolysate (1.28 times), intensification of $O_2^{\cdot-}$ generation and TMP in lymphocytes (1.41 and 1.26 times, respectively).

Conclusions. Analysis of preliminary results justifies the need to continue research in order to improve radiobiological support of RT for gynecological cancer patients. This will help to increase the effectiveness of RT, reduce radiation complications, and improve the quality of life of CC patients.

Session 2

Biology of eukaryotic and prokaryotic cells

Session 2: Biology of eukaryotic and prokaryotic cells/Біологія еукаріотичної та прокаріотичної клітини

PARADIGMA FOR EUKARYOTIC CELL DIVISION: LUCKY 13 PROTOFILAMENTS OF MICROTUBULES

Yaroslav Blume **Lecture 1**

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A little more than 125 years ago (1898), Kyiv University professor Sergei Navashin (1857-1930) discovered double fertilization in plants. Against the background of this discovery, the fact remained in the shadows that, while describing double fertilization, S. Navashin made significant efforts to study mitotic division in the generative cells of the pollen tube of the lily (*Lilium martagon* L.). These studies coincided in time with the works of the Polish-German scientist Eduard Strassburger (1844-1912), who introduced the terms "prophase", "metaphase", "anaphase" and "meiosis" into scientific circulation (1884). In particular, he, like a number of other researchers, demonstrated the same role of mitosis in the division of the generative nucleus as in the division of meristematic cells. However, for a long time, researchers could not visualize and identify microtubules, the main structural element of the mitotic spindle.

Only later, already in the second half of the 20th century, in the wake of the formation of a new biological discipline called "cell biology," it became clear that microtubules are an essential component of the cytoskeleton of any eukaryotic cell, in which they form not only the mitotic spindle, but also the interphase microtubular networks. Due to basic structural conservation (the formation of polymer filaments of the heterodimeric protein tubulin) along with a high level of dynamic plasticity (thanks to associated proteins), microtubules not only ensure mitotic division, but also maintain cell shape, intracellular transport and motility of flagella and cilia, and participate in the positioning of organelles and provide fine regulation these processes via signal transduction. The basic structure of any microtubule consists of 13 protofilaments, each of which is formed from tubulin heterodimers. There are also deviations from this magic number (and exceptions such as microtubular doublets in flagella and cilia), but the rule of the "lucky" number 13 is observed quite strictly, especially in plant cells.

A high level of structural and functional plasticity of microtubules is ensured by: peculiarities of the expression of different tubulin genes at different stages of ontogenesis and in different cell types; post-translational modifications of tubulin; the presence of structural and motor proteins associated with microtubules. The now established combination of modern research methods underlying potential of cell biology, genomics, bioinformatics and structural biology has made it possible to take a step forward in understanding the fine details of the mechanisms of microtubule functioning. Therefore, our presentation will review the main milestones of our own research from the production of mutant plants with altered tubulin and their somatic hybrids to the production of transgenic plant lines with mutant tubulin and the use of mutant tubulin genes as selective marker genes in biotechnology.

Separately, we will cover the issues of our discovery of such post-translational modifications of plant tubulin as phosphorylation at tyrosine residues and nitrotyrosylation, as well as the study of their functional significance. The possibilities of using big data analysis for structural biological analysis of the molecular structure of plant tubulin in comparison with tubulins of other origin and their functional significance will be analyzed. The possibilities of modeling the structure of intact microtubules will also be considered to elucidate their dynamic properties and the specifics of interaction with antimicrotubule drugs and the relationship with cellular components, in particular, those involved in the development of autophagy.

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METALLOTHIONEIN RELATED REDOX SHIFT AS THE STRATEGY OF STRESS RESPONSE IN FACULTATIVE ANAEROBES ON THE MODEL OF BIVALVE MOLLUSCS

Oksana Stoliar **Lecture 2**

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The redox state and metal chelator ability make low-molecular-weight (LMW) thiols the major cellular redox buffers and central link in the distribution of the essential metal zinc (Zn) [1]. Particularly, glutathione (GSH) is the major thiol-disulphide redox buffer of the cell among the eukaryotes. Another ubiquitous cytosolic LMW thiol, metallothionein (MT), is scarcely discussed as a thiol-disulfide buffer [1], despite its thiols comprise ~ tenth part of GSH level [2].

The goal of our research was to elucidate the relations between the responses of MT on the one hand, and oxidative stress, redox balance, metal (Zn,Cu) accumulation, and cytotoxicity manifestations on the other hand, in the facultative anaerobe under the environmentally relevant combine effects of micropollutants. Two experimental sets were accomplished utilizing the swollen river mussel *(Unio tumidus*) as the model organism in the exposures for 14 days. The groups were: I. microplastics (MP, 1 mg L^{-1}, size 35-50 μm), psychoactive substances caffeine (Caff, 20 μg L^{-1}) and chlorpromazine (Cpz, 12 ng L^{-1}) or their mixture (MixI); II. MP (1 mg L^{-1}, size 0.1–0.5 mm), nonsteroidal anti-inflammatory drug ibuprofen (IBU, 0.8 μg L^{-1}), or their combination (MixII). The digestive gland tissues were analyzed. The results have shown an almost unified response of MT and GSH: the increase of MT and its apo-form and GSH concentrations. The NADH/NAD^+ balance increased simultaneously, indicating the metabolic shift favorable for the redox state of thiols. The GTPase activation and decrease of the Zn/Cu ratio in the tissues were also common responses. The lipid peroxidation and caspase-3 activity were more dependent on the LMW thiols state than on the activities of antioxidative enzymes. Some specific manifestations for each exposure were abolished under the multi-stress effect. Generally, the molluscs demonstrated sensitive and unified redox-dependent responses of LMW thiols that can be the decisive adaptations of the facultative anaerobes under the influence of micropollutants.

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IDENTIFICATION OF OPHIOSTOMATOID FUNGI VECTORED BY *IPS ACUMINATUS* INFESTING *PINUS SYLVESTRIS* IN THE LVIV REGION

Yurii Yusypovych **Lecture 3**

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Ophiostoma species include causal agents of blue-stain in timber as well as some important tree pathogens [1]. The spores of these fungi are carried by bark beetles and mites. In recent years, outbreaks of the bark beetle *Ips acuminatus* (Gyll.) have been observed in the pine forests of Ukraine, which have been accompanied by massive tree deaths [2]. More than 130 species are currently recognized in the genus *Ophiostoma*, but despite the richness of species, only several species associated with *I. acuminatus* have been identified in Ukraine among them *O. ips, O. cf. rectangulosporium, O. minus, O. pallidulum, O. piceae* and *O. olivacea* [3]. These fungal species were found in pine stands in Eastern Ukraine; however, ophiostomatoid fungi associated with bark beetles and colonizing pine in the forests of Western Ukraine have not been described.

The aim of the current research is to identify the species of ophiostomatiod fungi vectored by *I. acuminatus* infesting Scots pine stands in Lviv region. *I. acuminatus* imagoes were collected from under the bark of the colonized pine tree. After sterilization of the insects' surface, the digestive tract was removed and homogenized. The resulting homogenate was used to isolate the fungi. Two isolates morphologically similar to ophiostomatoid fungi were obtained in pure culture. Fungi were identified by sequencing amplicons of ITS regions and the beta-tubulin gene. BLASTN search and phylogenetic analysis showed that both isolates belong to *O. clavatum* complex fungi [4]. The isolate named *Ophiostoma* sp. B-0922 showed 99,67 % ITS sequence and 100% beta-tubulin gene sequence similarity to *Ophiostoma clavatum*. The species identity of the other strain named *Ophiostoma* sp. C-1022 is being clarified. ITS sequences of the isolates *Ophiostoma* sp. B-0922 and *Ophiostoma* sp. C-1022 were placed in the Genbank under accession numbers QR799511.1 та QR799512.1, respectively

Thus, for the first time, we isolated ophiostomatoid fungi of the *O. clavatum* complex associated with *I. acuminatus,* which caused blue-stain disease in Scots pine trees growing on the western of Ukraine. A more detailed genetic analysis will be conducted to further clarify their taxonomic position.

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PLANT GROWTH-PROMOTING TRAITS OF ENDOPHYTIC BACTERIAL STRAINS ISOLATED FROM THE SCOTS PINE TISSUES

Valentina Kovaleva **Poster 11**

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Endophytic bacteria inhabiting plant organisms play a decisive role in helping their hosts adapt to biotic stresses and unfavorable environmental conditions [1]. These bacteria interact with their host plants, eliciting positive responses such as promoting plant growth, enhancing plant resistance to stress, and boosting plant immunity [2]

Most of our knowledge about bacterial endophytic microbiomes and their role in plant physiology comes from studies on crop plants and the model species *Arabidopsis thaliana*. In contrast, much less is known about endophytic bacteria associated with long-lived forest plants, particularly conifers. Scots pine is a long-lived plant (averaging 200 years) that experiences significant fluctuations in climatic conditions throughout its ontogeny and inhabits areas with poor soils. Therefore, the search for strains of endophytic bacteria from Scots pine with growth-promoting abilities is promising for sustaining the growth of pine trees under nutrient-poor edaphic conditions in the context of global climate change [3].

The aim of the current research is to isolate, identify, and characterize the plant growth-promoting (PGP) properties of endophytic bacteria of the genus *Pseudomonas* from Scots pine sapwood. Of more than sixty isolates, only four exhibited morphological and physiological characteristics typical of bacteria from the genus *Pseudomonas*. Through 16S rRNA gene sequencing, the bacterial isolates were identified as *P. putida*, *P. lurida*, and *Stenotrophomonas maltophilia*. All isolates demonstrated PGP activities, including the production of indole-3-acetic acid (IAA), ammonia, and siderophores. Additionally, two bacterial isolates of *P. lurida* showed nitrogen-fixating and phosphorus-solubilizing properties.

Bioinoculation of pine seeds with *Pseudomonas* strains increased seed germination by 35-45% (depending on the strain) under field conditions. It also altered the morphology of seedling root systems, leading to increased branching, enhanced lateral root formation, and greater root hair production. Furthermore, the dry biomass of inoculated seedlings increased by 80%. These results indicate the potential of using the strains characterized in this study for cultivating Scots pine planting material.

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ЗМІНИ МЕТАБОЛІЗМУ ГЛЮКОЗИ В КЛІТИНАХ РМЗ ЛЮДИНИ ПІСЛЯ ЇХ СПІВКУЛЬТИВУВАННЯ З *BIFIDOBACTERIUM ANIMALIS IN VITRO*

Тамара Козак **Poster 12**

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Сьогодні відомо, що представники мікробіоти людини, що являються компонентом пухлинного мікрооточення, знаходяться у тісній взаємодії з процесами обміну організму та ініціюють метаболічні зміні в пухлинних клітинах [1].

Метою роботи була оцінка впливу живих *Bifidobacterium animalis* на поглинання глюкози, продукцію лактату та експресію білків, асоційованих з регуляцією метаболізму глюкози (GLUT-1 та STAT-6, PI) в клітинах РМЗ людини *in vitro*. Об'єкти дослідження: клітинні раку молочної залози людини ліній T47D, MCF-7 і MDA-MB-231 та ліофілізовані *Bifidobacterium animalis subsp. lactis BB-12*. Методи: культура клітин, біохімічні методи, проточна цитометрія, імуноцитохімічний та статистичний аналіз.

Було виявлено посилення поглинання глюкози і продукції лактату клітинами ліній T47D та MCF-7 (люмінальний підтип) за умови їх співкультивування з *B. animalis*. В клітинах MDA-MB-231 (базальний підтип) за таких же умов спостерігали підвищення поглинання глюкози на фоні зменшення продукції лактату.

Співкультивування T47D з *B. animalis* супроводжувалась статистично достовірним зниженням експресії рецептора інсуліну (PI), в порівнянні з контролем, на 30%. Разом з тим, в клітинах MCF-7 та MDA-MB-231 спостерігали підвищення експресії PI на 15-20% відносно контролю клітин. Також було показано, що біфідобактерії спричиняють підвищення експресії транспортера глюкози GLUT-1 в клітинах T47D, MCF-7 та MDA-MB-231 на 55%, 90% і 70%, відповідно. Слід відмітити, що базальний рівень експресії GLUT-1 в досліджених клітинах суттєво відрізнявся: клітини T47D-133,3±15,3 бали, MCF-7 - 99,7±1,5 бали, у порівнянні з MDA-MB-231 - 26,3±7,5 балів. Окрім того, *B. animalis* призводили до суттєвого підвищення експресії активатора транскрипції генів, що кодують гліколітичні ферменти, STAT-6 у всіх досліджених клітинах.

Висновки: Співкультивування клітин РМЗ з *Bifidobacterium animalis* призводить до змін метаболічного профілю злоякісно трансформованих клітин, які свідчать про посилення гліколізу. Дані зміни більш виражені в клітинах РМЗ люмінального підтипу.

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МОДУЛЯЦІЯ ШЛЯХІВ МЕТАБОЛІЗМУ ГЛЮКОЗИ В КЛІТИНАХ РАКУ МОЛОЧНОЇ ЗАЛОЗИ ЛЮДИНИ *IN VITRO* ВНАСЛІДОК ДІЇ УМОВНО-ПАТОГЕННИХ ПРЕДСТАВНИКІВ МІКРОБІОТИ

Дмитро Кукурудза **Poster 13**

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Відомо, що умовно-патогенні представники мікробіоти людини (УПБ) можуть спричиняти значний вплив на окисно-відновний потенціал злоякісних клітин [1]. Одним із механізмів, які використовують злоякісні клітини для подолання оксидативного стресу – посилення гліколізу [2].

Для більш глибокого розуміння механізмів взаємовпливу УПБ та пухлинних клітин було досліджено вплив інактивованих теплом бактерій видів *Staphylococcus aureus B-918, Staphylococcus saprophyticus,* і *Pseudomonas syringae* на продукцію АФК, лактату та споживання глюкози клітинами РМЗ людини (T47D та MCF-7) *in vitro*.

Було показано, що експозиція клітин РМЗ з УМБ призводить до статистично достовірного збільшення продукції АФК у всіх досліджених клітинах. Так, в клітинах T47D найбільші зміни рівня АФК виявляли після їх культивування з *Staphylococcus aureus* – на 25% більше ніж в контролі. Найбільшу кількість АФК в клітинах MCF-7 виявляли після їх експозиції з *Pseudomonas syringae* – на 61% більше ніж в інтактних клітинах.

Основним продуктом гліколітичного шляху метаболізму глюкози клітинами є лактат. В клітинах T47D спостерігали посилення продукції лактату на 50-70%, а найвищий рівень продукції лактату відмічали після експозиції їх з *Pseudomonas syringae.* Разом з тим, саме в цій групі клітин зміна продукції АФК, порівняно з контролем, була найменшою (на 11%). Подібну закономірність спостерігали і в клітинах MCF-7 після їх культивування з УПБ: експозиція клітин з бактеріями видів *Staphylococcus aureus* та *Staphylococcus saprophyticus* призводила до значного підвищення продукції лактату (на 95- 115%), що супроводжувалось збільшенням продукції АФК лише на 20-35%. Тоді як обробка клітин MCF-7 *Pseudomonas syringae* спричиняла підвищення продукції лактату на 44%, а збільшення АФК на 61%.

Висновки: Отримані результати дозволяють припустити, що досліджені бактеріальні клітини індукують в клітинах РМЗ людини люмінального підтипу оксидативний стрес, однак клітини РМЗ використовують гліколіз і ефект Варбурга для нейтралізації такого впливу мікроорганізмів.

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EFFECT OF POTASSIUM DICHROMATE ON CELLS OF METAL-RESISTANT PLANT GROWTH-PROMOTING BACTERIA ISOLATED FROM DIFFERENT BIOTOPES

Solomiia Komplikevych **Poster 14**

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The search for and application of strains of plant growth-promoting bacteria is relevant because of the current challenges. It is also important for these organisms to resist environmental conditions, as this will ensure their long-term survival under stressful conditions and better efficiency of preparation use [1]. The most common environmental pollutants are heavy metal compounds. The positive effect on plants is provided not only by improving morphometric parameters, and increasing yields, but also by preventing the penetration and accumulation of metal compounds in plant tissues [2].

The study of changes occurring in the bacteria *Rhodopseudomonas yavorovii* IMV B-7620, isolated from Yavorivske Lake (Lviv region, Ukraine); *Ochrobactrum rhizosphaerae* IMV B-7956, isolated from the filtrates of the Lviv solid waste landfill; *Paenibacillus tundrae* IMV B-7915, isolated from a sample containing soil, moss, *Deschampsia antarctica* (Barcelot Island, Maritime Antarctica), under the influence of potassium bichromate. It was found that these bacteria have plant growth-promoting properties on wheat cultivar Tybalt (improve seed germination, increase root and shoot length, and chlorophyll content).

Potassium dichromate affected bacterial growth (biomass accumulation, duration of lag phase and specific growth rate in the exponential phase of batch culture changed) and caused free radical damage in *R. yavorovii* IMV B-7620, *P. tundrae* IMV B-7915 and *O. rhizosphaerae* IMV B-7956 cells, that was detected by the increase in the content of lipid peroxidation (LPO) products and carbonyl groups in proteins. The severity of damage is strain-specific. The enzymes of the antioxidant defense system are involved in protecting the studied bacteria (catalase, superoxide dismutase, glutathione peroxidase, glutathione S-transferase and glutathione reductase activities changed). Under the influence of $K_2Cr_2O_7$, the qualitative and quantitative composition of pigments in *R. yavorovii* IMV B-7620 changed. The content of exopolysaccharides in *R. yavorovii* IMV B-7620 increased under the influence of $K_2Cr_2O_7$. In *P. tundrae* IMV B-7915 extracellular polymers are important for protection against $K_2Cr_2O_7$. The bacteria *O. rhizosphaerae* IMV B-7956 produce exopolysaccharides under the influence of $K_2Cr_2O_7$, while the content of extracellular proteins is slightly different from the control.

Based on the results of factor analysis and considering the deviations from the control of antioxidant-prooxidant balance, it was found that superoxide dismutase, catalase activity, thiols, and extracellular polymeric substances were important in neutralizing LPO products formed under the influence of $K_2Cr_2O_7$ in *R. yavorovii* IMV B-7620 cells. Exopolysaccharides and enzymes of the glutathione system are important for the survival of *P. tundrae* IMV B-7915 under the influence of $K_2Cr_2O_7$. Catalase and superoxide dismutase activities contributed to the protection of *O. rhizosphaerae* IMV B-7956 cells from direct or indirect effects of LPO products under the influence of $K_2Cr_2O_7$.

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RECOMBINANT HUMAN ALPHA-SYNUCLEIN INFLUENCES THE SENSITIVITY OF *OGATAEA POLYMORPHA* CELLS TO THE EXCESS OF EXOGENOUS Mn^{2+} IONS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with abnormal aggregation of alpha-synuclein protein with the formation of insoluble amyloid fibrils. The tendency of alpha-synuclein to aggregate depends on the specific conditions of the cellular microenvironment (pH level, temperature, ionic strength), macromolecular environment, and the presence of other ligands/partners (amyloidogenic proteins, metal ions, intermediate toxic compounds, specific lipid molecules). Numerous epidemiological studies have demonstrated the relationship between PD and individual or combined exposure to such heavy metals as iron, mercury, manganese, copper and lead. Despite significant progress achieved through numerous studies, the molecular mechanisms of metal-induced abnormal properties of alpha-synuclein remain incompletely elucidated. Studying the mechanisms of alpha-synuclein cytotoxicity using different model organisms, such as the yeast *Ogataea polymorpha*, can significantly expand understanding of pathogenesis and the influence of various exogenous and endogenous factors on disease progression, as well as expand the possibilities for finding potential approaches to the therapy of neurodegenerative diseases.

The aim of the research was to study the effect of Manganese ions on the physiological properties of the recombinant yeast strain *O. polymorpha* with constitutive expression of human alpha-synuclein. In particular, it was established that with an increase in the concentration of Mn^{2+} in the culture medium, the growth rate of the wild-type strain noticeably decreased, while the toxic effect of Mn^{2+} ions was less pronounced for the recombinant cells. The percentage of dead cells in the alpha-synuclein producing strain cultured on the medium with 40 mM Mn^{2+} was the lowest compared to other culture conditions and compared to the wild-type strain. An excess of Mn^{2+} contributed to an increase in the number of reactive oxygen species (ROS)-positive cells in the both studied yeast strains. However, the proportion of ROS-positive cells in the model strain was lower even when grown with high concentrations of Mn^{2+}. Also an extra of Mn^{2+} ions led to a more pronounced increase in the activity of antioxidant enzyme catalase in the both strain, while the presence of the recombinant protein did not affect the activity of this enzyme. The content of secondary products of lipid peroxidation in the cells of the yeast model strain was lower at cultivation on the medium without and with an excess of ions of this metal. Therefore, we showed for the first time that recombinant human alpha-synuclein contributes to reducing the negative effect of manganese ions on cells of a model yeast strain.

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THE ROLE OF THE HIGH-AFFINITY GLUCOSE SENSOR Gcr1 AND PEROXINE Pex3 IN THE UTILIZATION OF CITRIC ACID BY METHYLOTROPHIC YEAST *OGATAEA POLYMORPHA*

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Among the approximately 600 species of yeast known today, only about 50 species are capable of assimilating methanol. All methylotrophic yeasts are facultative methylotrophs, as their growth can be supported by other compounds. About half of them have strains that are able to use citrate as a source of carbon and energy.

The aim of the research was to study the ability of strains of the methylotrophic yeast *Ogataea polymorpha*, defective in glucose repression and peroxisome biogenesis, for the utilization of citric acid as the only source of Carbon, and in a mixture with other sources of Carbon. In particular, it was established that the ability to utilize citrate is inherent only to cells of the methylotrophic yeast *O. polymorpha*, in which the processes of glucose catabolite repression are damaged, while the wild-type strain is unable to use citric acid as a growth substrate. However, impairment of peroxisome biogenesis impairs the utilization of citrate as a carbon substrate by *Δgcr1Δpex3* cells during the first 18 h of cultivation, but has practically no effect on the growth of this strain as a whole. When the *O. polymorpha Δgcr1* strain was grown on a medium with a mixture of citrate and glucose, diauxic growth was observed, while the *Δgcr1Δpex3* mutant was characterized by both ways of utilization of the substrate mixture: diauxia before 18 hours of cultivation, and mixotrophy after 18 hours. However, mixotrophy was observed when the *O. polymorpha* strain defective in glucose repression was grown on a medium with a mixture of methanol and citrate. In addition, the phenomenon of ethanol repression of genes encoding exogenous citrate metabolism enzymes in the *Δgcr1* strain and the ability of the wild type to utilize citrate only under conditions of growth in a medium containing ethanol, but not glucose, were discovered for the first time.

Thus, it was established that the deletion of the *Δgcr1* gene leads to damage of glucose repression of genes encoding citrate metabolism enzymes, and ethanol in strains defective for glucose repression causes inhibition of the expression of citric acid metabolism genes. In addition, the absence of peroxisomes in cells of the *Δgcr1Δpex3* strain did not significantly affect the efficiency of citrate utilization as the only Carbon source.

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CADMIUM TELLURIDE-BASED NANOPARTICLES ARE BIOCOMPATIBLE WITH HUMAN NEUROBLASTOMA CELLS SH-SY5Y AND CAN BE USED IN BIOMEDICAL STUDIES OF THIS MALIGNANCY

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Today, nanomedicine is undergoing rapid development, which requires the creation of biologically relevant nanostructures, including semiconductor nanoparticles, magnetic nanoparticles, nanostructures based on carbon and metals. Research on semiconductor nanocrystals (NCs), also known as quantum dots (QDs), and their applications in nanomedicine has greatly intensified over the past few decades. In addition, semiconductor QDs are also becoming valuable analytical tools for nanomedicine and nanobiotechnology, as they enable the creation of fluorescent probes for labeling intracellular components of malignant and other pathologically changed cells, imaging and sensing *in vitro* and *in vivo* with high throughput.

The aim of the research was to study the biocompatibility of cadmium telluride nanoparticles with human neuroblastoma cells SH-SY5Y and the possibility of their use as a fluorescent label for *in vitro* imaging intracellular components of cells. The nanoparticles of cadmium telluride with a diameter of 2.9 nm with spherical symmetric shape was synthesized by chemical colloidal method.

Using the MTT test, it was established that, depending on the concentration, cadmium telluride nanoparticles have a cytotoxic effect. In the concentration range from 300 to 10 μM, IC50 was determined as 30 μM. In the range of cadmium telluride concentrations from 30 to 300 μM, nanoparticles slightly inhibited the growth of SH-SY5Y neuroblastoma cells, while concentrations higher than 30 μM led to the death of these cells. In addition, the possibility of using cadmium telluride nanoparticles as a potential fluorescent label for imaging human neuroblastoma cells SH-SY5Y was tested. Only after 48 h of incubation of malignantly transformed cells with the investigated nanoparticles in the concentration range from 20 to 300 μM in DMEM medium with glutamine, fetal bovine serum and gentamicin, it was possible to visualize the fluorescent signal. As the content of cadmium telluride in the culture medium increased, the ability of cells to absorb these nanoparticles was not suppressed. Thus, cadmium telluride nanoparticles can be used to visualize malignantly transformed neuroblastoma cells in concentrations that practically do not affect the viability of cells during long-term incubation with them.

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TRANSCRIPTIONAL PROFILING OF SCOTS PINE DEFENSINS UNDER BIOTIC AND ABIOTIC STRESS

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Plant defensins represent a family of small cationic proteins sharing a common scaffold, which is known as the cysteine-stabilized αβ (CSαβ) fold [1]. The spectrum of plant defensin activities is quite broad, ranging from protection against bacteria, fungi, and viruses, to inhibition of protein synthesis, α-amylases, proteases, or ion channels. They are produced as the first line of defense in response to invading pathogens. In addition, some plant defensins are also induced in response to environmental stress such as drought and salinity. Genome-wide analysis of defensin-like (DEFL) genes in some angiosperm plants revealed that these genes are arranged in polymorphic multigene families. This gene family is poorly understood in gymnosperms. Recently, several pine defensin genes were cloned by us and the properties of their protein products were studied [2, 3].

Global climate change and related shifts in temperature and hydrological regimes disrupt the stable functioning of forest ecosystems, ultimately leading to reduced vitality in pine stands. The basic approach to increasing the resilience of forest ecosystems to stressors is the selection of genotypes with high adaptive potential, using candidate genes, specifically defensin genes.

The aim of the current research is to elucidate the transcriptional patterns of Scots pine defensins *PsDef1-4* in response to biotic (phytopathogenic fungi *Ophiostoma* sp. and *Fusarium* sp.) and abiotic (heat, salt, drought, flood, etc.) stress. The expression levels of defensin genes in Scots pine seedling tissues were assessed using qRT-PCR. The expression patterns of Scots pine defensin genes were investigated during the early stages of the infection process. *PsDef*s exhibited differential expression at the tested time points, depending on the nature of the pathogen. Abiotic stress modulated the expression of defensin genes: heat stress significantly increased transcript levels of all four genes; drought induced *PsDef3* expression; *PsDef1-3* genes responded to excess cadmium; and acid and water stress induced *PsDef1* and *PsDef2* expression, respectively. The obtained results indicate the potential of using pine defensins as candidates for the selection of multi-stress resilient pine genotypes.

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MARKERS OF OXIDATIVE STRESS IN *STREPTOMYCES* SP. BACTERIA ISOLATED FROM THE SPOIL HEAP OF CENTRAL ENRICHMENT FACTORY "CHERVONOHRADSKA", UNDER THE INFLUENCE OF LEAD NITRATE

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Heavy metals have a largely negative effect on microbial cultures, leading to the occurrence of oxidative stress and inhibition of reparative antioxidant systems in microbial cells [1]. We studied the effect of lead nitrate on markers of oxidative stress in *Streptomyces* sp. CEF-7 bacteria isolated from the spoil heap of Central Enrichment Factory "Chervonohradska".

Cells of microorganisms sensitive to the presence of heavy metal salts in the environment are prone to oxidative stress, one of the main consequences of which is the process of lipid peroxidation [2-3]. Analyzing the content of diene conjugates in cells of bacteria Streptomyces sp. CEF-7, it can be concluded that their content after adding lead nitrate to the medium did not differ reliably from the content in the control.

The most common cellular targets for reactive oxygen species (ROS) are proteins. Different AFOs and their derivatives cause specific types of damage to individual amino acids in the polypeptide chain. In the presence of oxidative stress, SH-groups are blocked due to the breaking of intramolecular bonds, in the formation of which SH-groups participate [2-3]. A decrease in the content of total thiols by 63%, compared to the control, was revealed during the cultivation of *Streptomyces* sp. CEF-7 bacteria with 1.6 mM $Pb(NO_3)_2$. At a concentration of 0.35 mM, no changes in the content of thiols were observed.

Based on the obtained results, it can be assumed that *Streptomyces* sp. CEF-7 isolate has antioxidant mechanisms that protect cells from the formation of AFOs and, accordingly, make them resistant to heavy metal salts in the environment.

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APS-REDUCTASE ACTIVITY OF *DESULFOTOMACULUM* SP. AR1 UNDER DIFFERENT CONDITIONS OF CULTIVATION

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Sulfate-reducing bacteria reduce inorganic sulfate as the final electron acceptor during the oxidation of organic substances to hydrogen sulfide. These microorganisms are widespread in marine sediments, silt of reservoirs and sewage treatment plants, rust layers on metal surfaces. Sulfate-reducing bacteria also have high hydrogenase activity, which indicates their high potential in the field of energy production. They play a key role in the reducing part of the sulfur biogeochemical cycle; cause microbial corrosion of metals, they can be used for the purpose of bioremediation of wastewater for precipitation of heavy metal ions with hydrogen sulfide of biogenic origin.

Assimilative reduction of sulfate has been described in many living organisms [1], and the process of dissimilatory sulfate reduction is unique only to sulfate-reducing bacteria [2]. The process common to these two pathways is the activation of sulfate ions by ATP-sulfurase (EC 2.7.7.4) to adenosine phosphosulfate (APS). During dissimilatory sulfate reduction, APS is reduced by APS reductase (EC 1.8.99.2) to sulfite, which is converted to sulfide with the participation of sulfite reductase (EC 1.8.99.1) [3]. The activity of these enzymes differs depending on the type of bacteria and the conditions of their cultivation.

The aim of the current research was to study the APS-reductase activity of bacteria *Desulfotomaculum* sp. AR1, isolated from the domestic and industrial wastewater treatment system of the city of Lviv, under different cultivation conditions.

A relatively high APS-reductase activity (8.03 ± 0.52 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ of protein) was revealed in cell-free extracts of *Desulfotomaculum* sp. AR1, obtained as a result of treatment of cells with ultrasound. The dependence of the APS-reductase activity of bacteria on the concentration of sulfate ions in the medium was found. An increase in the sulfate content to 20 mM/L is accompanied by an increase in enzymatic activity. The highest APS-reductase activity was found at a temperature of 30°C and pH 8.0. Durng the cultivation of *Desulfotomaculum* sp. AR1 in media with oxygen and toluene as carbon sources, the enzymatic activity was 2.2 and 2.5 times lower, respectively, compared to controls. High APS-reductase activity of bacteria *Desulfotomaculum* sp. AR1 indicates their important role in the processes of accumulation of hydrogen sulfide in water bodies enriched with sulfates.

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IDENTIFICATION OF FtsZ EFFECTORS TARGETING INTER-DOMAIN CLEFT BASED ON PHARMACOPHORE SCREENING AND MOLECULAR DOCKING

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The identification of new antibiotics are usually starts with determination of molecular targets and sites associated with critical and vitally processes in bacteria [1, 2]. Therefore, when we develop new methods and strategies combating bacterial diseases, effectors of cell cycle and binary fission are in the prime grope of interest. FtsZ (**F**ilamenting **t**emperature-**s**ensitive mutant **Z**) is the key component of Z-ring that recruits binary fission proteins and representing the most promising molecular target for ligand-induced inhibition of bacterial reproduction. At present, we have wide and heterogeneous grope of experimentally confirmed compounds, demonstrating direct interaction with FtsZ [3]. At the same time, despite pronounced effect, the number of compounds is toxic for mammalian cells. This mainly pertaining to GTP-competitive effectors of bacterial FtsZ [4]. Therefore, identification of effectors with alternative mechanisms of protein-ligand interaction, are in the frontier of this topic. The most promising hopes are now associated with FtsZ effectors, provoking diverse inner allosteric shifts.

The object of the current study was effectors of the Inter-Domain Cleft (IDC) site. Earlier, we studied fluctuations of IDC pocket and ranked its conformations, shaped under pressure of protein-ligand adaptation [2].

Our revision of the ChEMBL database (www.ebi.ac.uk/chembl/) identified 379 FtsZ effectors. At the same time, the binding sites for most of these compounds remain unknown. Based on ChEMBL and RCSB Protein Data Bank data, we managed conformers library adopted for pharmacophore screening with Pharmit (https://pharmit.csb.pitt.edu/), which is now available on the Pharmit server as IFBG: Public Library “PDB+ChEMBL” under the actual code: VBS30O74UAMW00AL8EML. Subsequent pharmacophoric screening and processing of the hit-list, recognize 89 individual compounds, potentially associated with IDC site.

The molecular docking of selected compounds were performed with CCDC GOLD 2023.2.0 and iGEMDOCK v2.1 programs. Based on pharmacophore search and two molecular docking protocols, we analyze selected compounds as allosteric effectors of IDC site. Among them, 88 fully fit pharmacophore descriptors, steric constraints, fitness functions of docking, its settings and constraints, applied under our screening protocol. One more compound - curcuminoid CHEMBL116438, was recognized as potential effector of the IDC site, and its IDC-dependent action should be examined confirmed with molecular dynamics simulations.

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Session 3

Biology of animal and plant cells

GENETIC HETEROGENEITY AND POPULATION STRUCTURE OF *CAMELINA MICROCARPA* CYTOTYPES IN UKRAINE, A HOTSPOT OF THE SPECIES GENETIC DIVERSITY

Rostyslav Blume **Lecture 1**

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False flax, or gold-of-pleasure (*Camelina sativa* (L.) Crantz), is a cutting-edge biofuel oilseed crop, which has gained increasing attention due to a number of beneficial traits, including desirable fatty acid content, reduced vegetation cycle, high genetic transformation amenability, etc. However, cultivated *C. sativa* demonstrates restricted genetic variation across the existing cultivars, which significantly limits breeding potential of this crop [1]. At the moment, little-pod false flax (*C. microcarpa* Andrz. ex DC.), the closest non-domesticated relative of camelina, is viewed as a promising candidate for boosting genetic polymorphism of existing *C. sativa* germplasm [1, 2]. Recent studies, suggest that Eastern Europe, particularly Ukraine, appears to the hotspot of the genetic diversity of hexaploid *Camelina* species [2, 3]. Therefore, the aim of this study was to assess genetic diversity and population structure of *C. microcarpa* in Ukraine and adjacent areas to uncover the distribution of cytotypes, which may be potentially used as wild germplasm donors for breeding.

For such investigation 105 *Camelina* sp. accession were investigated, using combination of TBP/cTBP- (length polymorphism of 1st and 2nd introns β-tubulin genes) and SSR-markers, capable of analyzing partially degraded DNA material from old herbaria specimens. Whole genome searches within *C. sativa* DH55 assembly revealed the presence of 34 homeologous β-tubulin genes, evenly allocated within almost each of chromosomes from different subgenomes [4]. TBP genotyping technique, implying analysis of these distinct loci, allowed dissecting *C. microcarpa* accessions of different ploidy and cytotype identity. Population analysis revealed the presence of three distinct populations (Central European, North and South Ukrainian) of the most widespread *C. microcarpa* (cyto)Type 2 (2*n*=38). At the same time, area of distribution (cyto)Type 1 *C. microcarpa* (2*n*=40) seems to restricted to Caucasian region. Clarified population structure and level of genetic polymorphism of *C. microcarpa* provide insights about the usage of crop wild relative germplasm for *C. sativa* improvement.

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DEVELOPMENT OF MOLECULAR MARKERS FOR IDENTIFICATION STEM RUST RESISTANCE GENE *SR39*

Dmytro Novozhylov **Lecture 2**

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Up to 60 stem rust resistance genes are known in wheat, but most of them are strain-specific. The Ug99 race group currently poses a significant threat. The first appearance of Ug99 was recorded in Uganda in 1999, during the following decades its spread throughout the world - North Africa, Middle East, South Asia, which poses threats to global food security. *Sr39* is a stem rust resistance gene in *Aegilops speltoides* that is effective against all Ug99 races. *Triticum aestivum* line LR5711 and its derivatives carry this gene as part of a translocated segment of chromosome 2S of *A. speltoides* to chromosome 2B of *T. aestivum*. The aim of this study was to identify possible loci that can encode *Sr39* and develop specific molecular markers.

Sequences were obtained from the NCBI GenBank The blastn tools of the NCBI Genome Workbench software package were used to identify homologous to deletion bin 2BS-0.53-0.75 *T. aestivum* genome fragments for *A. speltoides* and *A. taushii* (as a reference). Because of to the lack of data on the exact location in the genome and the sequence of the *Sr39* gene, and, moreover, the insufficiency of the data on *A. speltoides* coding sequences, a search for putative *Sr39* in *A. speltoides* genomic insertion was performed, based on the potential similarity of the domain architecture of the product of this gene to other Sr proteins, most of which are NB-LRR proteins. The domain architecture of such products was determined using SMART and InterPro services.

It was determined that there are three sequences potentially encoding CC-NB-LRR proteins in chromosome 2S of *A. speltoides*: CM038142.1:28520394-28524293, CM038142.1:29960732-29966088, and CM038142.1:29979171-29983853. They are homologs of RPM1-like (LOC109769301), RGA5 (LOC123496990 and LOC109767918) of *A. taushii*, respectively. All of them are clustered at the distal end of the deletion bin, which correlates with approximate location of the *Sr39* gene. Therefore, hypothetically, these RPM1-like and RGA5 of *A. speltoides* could be the *Sr39*. Molecular markers for these hypothetical *Sr39* genes have been designed and are currently undergoing validation. Thus, probable candidates for the role of stem rust resistance gene *Sr39* were determined, and the approximate boundaries of the sequences of these genes were determined, which allowed the development of potential specific molecular markers. The obtained data will help to solve the problem of development and identification of wheat varieties containing *Sr39* gene.

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PHOTOSYNTHETIC APPARATUS OF CHERNOBYL PLANTS IS TOLERANT TO HEAVY METAL STRESS

Galina Shevchenko **Lecture 3**

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During last decades, flora is found to be abundant and flourish in zone of the Chernobyl atomic plant, evidencing protective measures developed in plants since middle 80-ties. Main aim of our research is to find out mechanisms promoting plant survival in such adverse environment and in connection with the above we investigate functioning of photosynthetic apparatus in *Arabidopsis* seedlings. Effects of cadmium ions on the functional state of photosynthetic apparatus (PSA) of two natural ecotypes of *A.thaliana* (L.) (from Chernobyl zone (Che-07) and control area outside (Oasis)) as well as recovery from stress have been investigated. It was found that cadmium caused a decrease in maximum quantum yield of photochemical reactions in photosystems II (PSII) (F_v/F_m), quantum yield of PSII photochemistry in the light-adapted state (F'_v/F'_m), quantum yield of electron transport (φPSII), chlorophylls (Chl) and carotenoids (Car) content and an enhance in the level of non-photochemical quenching coefficient (qN) in leaves of both analyzed *Arabidopsis* accessions. Photochemical quenching of chlorophyll fluorescence (qP) was not affected by Cd^{2+} in seedlings of Chernobyl genotype. However, the significant reduction in qP was observed in *Arabidopsis* accession outside Chernobyl (Oasis). Lower absolute values of F_v/F_m, F'_v/F'_m, φPSII and qP in seedlings of Oas genotype after cadmium treatment indicate a lower photosynthetic efficiency and tolerance to Cd^{2+} ions. Large increase in qN value in the antenna of Oas ecotype indicates an increasing of energy dissipation in the light-harvesting complex. On the other hand, lower qN and higher φPSII levels in Che-07 show more efficient solar energy utilization in photosynthetic processes under cadmium stress. Elimination of Cd^{2+} ion impact in seedlings of Che-07 genotype, caused recovery of F'_v/F'_m values, and qP and φPSII parameters almost to the control levels and therefore, seedlings of Che-07 could be qualified as cadmium tolerant. On the contrary, Oas genotype, which did not recover, could be considered to be sensitive to cadmium. Higher tolerance of Che-07 ecotype to Cd^{2+} ions may be a consequence of prolonged adaptation to chronic ionizing radiation and development of nonspecific defense mechanisms against oxidative stress. Above evidences tolerance to heavy metals acquired by Chernobyl plants during long time growth in contaminated environment.

ЦИТОГЕНЕТИЧНІ ОСОБЛИВОСТІ *VISCUM ALBUM* L., ЩО ЗРОСТАЄ НА РІЗНИХ ВИДАХ РОСЛИН-ГОСПОДАРІВ

Юлія Білоножко **Lecture 4**

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Омела біла (*Viscum album* L., s.l.) є важливою складовою різних екосистем багатьох країн Європи в тому числі й України. Вона становить значний як науковий, так і практичний інтерес для фахівців різних галузей. В результаті еволюційних процесів, що відбуваються у взаємодії паразит-господар у середині виду *V. album*, спостерігається певна спеціалізація до виду (або роду) рослини-господаря. Наразі таксономія виду є не до кінця узгодженою. При зростанні на великій кількості різних видів дерев омела демонструє дуже широкий спектр морфологічного поліморфізму. За результатами генетичного аналізу виділяють окремі раси, підвиди або навіть окремі види [1-2]. Отже важливим науковим завданням є виявлення доказів процесу видоутворення всередині виду, що дозволить коректно класифікувати омелу білу. Для цієї мети важливо використовувати комплексний аналіз морфологічних,, анатомічних, цитологічних і генетичних показників.

Для проведення дослідження рослинний матеріал збирали з різних видів рослин-господарів. Використовували листя та бруньки омели під час росту. Вибірку насіння пророщували в чашках Петрі на середовищі Мурасіге-Скуга. Вивчали особливості клітин, ядер, ядерець, мікроядер та хромосом. З огляду на особливості рослини класичні протоколи цитогенетичних досліджень потребували певних модифікацій.

Результати каріологічного аналізу свідчать про те, що в диплоїдному наборі омели білої, яка зростає на різних видах рослин-господарів, наявно 20 хромосом (2n=20). Хромосоми *V. album* досить великі, добре фарбуються та чітко диференціюються на два плеча. Додаткових хромосом не виявлено. За результатом ана-телофазного аналізу встановлено, що більшість мітозів характеризуються проходженням без порушень. Патології мітозу зустрічаються із частотою 0,66±0,55%. Серед порушень відмічено хромосомні та хроматидні мости, відставання хромосом та мікроядра. Деякі клітини мали ядро неправильної форми. Інтегральний показник пошкодженості аберантних клітин низький. Мітотична активність становила в середньому близько 8% (без врахування телофази). В хромосомах омели білої спостерігається до двох вторинних перетяжок, що є характерною рисою хромосом багатьох видів листяних видів. У клітинах проростків насіння *V. album*, що зростає як на листяних породах дерев, так і на сосні звичайній, відмічено ядра з 1–4 ядерцями. Однак більшість клітин (73,85%) мали 1 ядерце.

У результаті проведеного аналізу меристематичних тканин гаусторій та листяної меристеми омели білої виявлені певні цитогенетичні особливості цього виду. В той же час якихось цитогенетичних особливостей, пов’язаних з рослинами-господарями, встановити поки не вдалося. В цілому показано доцільність залучення аналізу клітинних характеристк наряду з генетичними, анатомічними та біологічними показниками для комплексного дослідження еволюційних змін, спрямованих на видоутворення.

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SEELECTION OF NOVEL EFFECTORS OF MYCOBACTERIAL CYSK1 AND CYSM BASED ON STRUCTURAL ANALYSIS AND MOLECULAR DOCKING

Svitlana Spivak **Poster 15**

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The inhibition of enzymes, associated with the vitally important cascades of bacterial cell are the perspective strategy of modern anti-TB therapy. The objective of current research was identification of new molecular targets of anti-TB compounds and selection of their potential effectors. The bacterial *o-acetylserine sulfhydrylase* catalyze transformation of o-acetylserine to cysteine are conserved in all known Mycobacteria strains. At the same time, two functional homologes - enzymes CysK1 (P9WP55) and CysM (P9WP53), revile only 37% of sequence similarity. Based on our revision of RCSB Protein Data Bank we select several CysK1 structures (2Q3B, 3ZEI and 2Q3D, etc.) demonstrating 85-92% identity. Several x-ray-based models with < 2 Å resolution were selected for further analysis. Based on CysK1 complexes with triazolidine derivatives, we identify the key amino acids associated with ligand binding (Thr71, Ser72, Gln144, Phe145, Lys215, Gly222, Ala225 and Phe227) and composed site descripting file according with CCDC GOLD instructions for protein and ligand docking [1, 2].

Similarly, these residues form stable hydrogen bonds with the charged fragment of the ligand scaffold, and Thr81, Ser82, Gln154 form contact with the pyridoxal phosphate plane (PDBID: 5I7A). The CysK1 and CysM structural models were prepared according to a standard protocol (energy minimization, equilibration in aqueous environment, and structure relaxation). The CysK1 model have two constraints fixing aromatic fragments, associated with protein-ligand π-π interactions. The donors and acceptors of the site were grouped into two clusters (Thr78, Thr82 and Gln151, Asn221, Ala323), which should form at least one hydrogen bond with each group. The structurel model of CysM (PDB ID: 5I7A) was analyzed and prepared according to the standard protocol (energy minimization, equilibration in solvent and structure relaxation in Gromacs with Charmm36 ff) [3]. The cofactor was find as the necessary for correct docking process was left in the binding site. Based on docking pose and fitness scoring, we selected 150 lider-compounds for each molecular target. It was confirmed, that Gly73, Ser72, Thr71, Gln144 are capable to form stable network of h-bonds. On the other hand, we identify that terminal nitrogen of Lys215 associated with h-bonds and Re-cation interactions. In general, 25 potential inhibitors of CysK1 and 15 potential inhibitors of CysM were selected for upcoming *in vivo* screening.

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CATALASE AND ASCORBATPEROXIDASE ACTIVITY IN PLANT EXTRACTS

Olena Kuchmenko **Poster 16**

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During exposure to various biopathogens in plant organisms, the generation of reactive oxygen species starts, which in turn provokes the development of oxidative stress [1]. Mechanisms of damage to membrane lipids and proteins by active forms of oxygen are associated with lipid peroxidation processes [1].

Catalase and ascorbate peroxidase perform the function of direct neutralization of ozone and oxygen intermediators, which significantly slows down the formation of the toxic hydroxy radical OH • [2]. Their difference is that catalase breaks down hydrogen peroxide to H2O and O2, and ascorbate peroxidase reduces H2O2 to H2O.

Catalase is able to perform a catalytic function due to the features of its structure: the presence of four identical subunits, each of which has an iron-porphyrin complex as a cofactor. Ascorbate peroxidase also has a heme group in the active center, and to perform a full range of functions, it has multiple molecular forms, differing in substrate specificity, localization and optimal conditions necessary for catalysis of chemical reactions.

Ascorbate peroxidase is localized mainly in chloroplasts, while catalase is most often found in peroxisomes and mitochondria.

The aim of the work was to determine the activity of catalase and ascorbate peroxidase in aqueous extracts from robinia flowers, grape leaves, basil seeds and wheat grains.

The extracts were prepared by mixing water and dry plant material in a 7:3 ratio followed by extraction at 98 ° C for 40 minutes.

The principle of the method for determining catalase activity is the formation of a stable complex of hydrogen peroxide with ammonium molybdate. The principle of the method for determining ascorbate peroxidase activity is to study the reduction of H2O2 levels in phosphate buffer (pH 7.8) in the presence of ascorbic acid and EDTA.

As a result of the study, it was found that the catalase activity in extracts from grape leaves, robinia flowers and wheat grains is lower by 100%, 100%, and 95.5%, respectively, compared to the extract from basil seeds.

We also demonstrated that the activity of ascorbate peroxidase in extracts from robinia flowers and wheat grains is 40% and 66% lower, respectively, compared to extracts from grape leaves and basil seeds.

Such results may be due to the presence of high lipid content in basil seeds [3] and the fact that ascorbate peroxidase is a heat-resistant enzyme and catalase is thermolabile.

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REACTIVE OXYGEN SPECIES AND DROUGHT TOLERANCE OF LEGUME-RHIZOBIAL SYMBIOSIS

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The formation of legume-rhizobial symbiosis goes through a series of coordinated stages, the main of which is the distant interaction of symbionts, which is carried out through the exchange of molecular signals between macro- and microsymbionts. Clear regulation of precontact intermolecular events between both partners of symbiosis leads to the activation of the main pathways of symbiotic signals' transduction and the successful development of organogenesis programs of the nodule-epidermal and cortical [1]. The invasion of rhizobia into the cells of the root hairs of legumes, like the process of pathogenesis, causes the intensification of oxidative processes in plant cells accompanied by increased generation of reactive oxygen species (ROS), which induce a number of reactions in the plant [2]. Despite the widespread recognition of the role of ROS as regulatory and signaling molecules in plant cells, it remains unclear how exactly these signals are perceived, transmitted, and elicit a response. The study of the role of ROS in establishing symbiotic plant-microbial interactions is of particular scientific interest.

The intensity of drought-induced oxidative processes by the degree of superoxide anion radical generation and the activity of NADPH oxidase in soybean associated with different activity and virulence of *Bradyrhizobium* strains were studied [3]. The importance of active *Bradyrhizobium japonicum* for the formation of effective symbiosis with soybean to improve the realization of the adaptive potential of plants during drought and post-stress period has been established. Increased NADP oxidase activity in nodules, as well as excessive production of superoxide radical in nodules and roots of soybean in symbiosis with efficient *Bradyrhizobium* strains were observed during drought. However, the development of oxidative processes in effective soybean-rhizobial symbioses slowed down after exposure to stress. This indicates the adaptation of soybean plants associated with active *Bradyrhizobium* strains to growing conditions. In symbiotic systems with ineffective *Bradyrhizobium* strains, a significant increase in NADPH oxidase activity in nodules and intensification of superoxide radical formation in nodules and roots recorded both under drought and post-stress period. This indicates a significant development of oxidative processes and the inability to realize the adaptive potential of soybean plants in symbiosis with ineffective *Bradyrhizobium* strains. Thus, excessive formation of the superoxide radical and an increase in the activity of NADPH oxidase indicate the appearance of oxidative processes, which are a typical reaction in response to drought in soybean-rhizobial symbioses of varying efficiency. At the same time, the development of oxidative processes depends on the ability of soybean plants in symbiosis with *Bradyrhizobium japonicum* to realize their adaptive potential in during drought and post-stress period.

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INFLUENCE OF HEAVY METAL IONS ON PHYSIOLOGICAL PROCESSES IN THE PLANT ORGANISM

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The problem of plant resistance to adverse environmental factors has been one of the key issues in biotechnology and plant physiology for many years. Thanks to a large number of studies, it has now been proven that plant resistance to adverse abiotic and biotic factors is ensured by the functioning of a large number of different mechanisms operating at different levels of organisation. Scientists in many countries are particularly focused on studying the mechanisms of plant resistance to heavy metals, which is caused by a significant increase in environmental pollution, an increase in the number of vehicles, an increase in the amount of mineral fertilisers applied to the soil, etc [1, 4]. Among the most aggressive environmental pollutants that have a detrimental effect on the biosphere are heavy metal ions (HMI). The response of plants to the type and concentration of HMI is extremely diverse. The way in which plants absorb HMI can also vary. All plants growing under the influence of heavy metal ions accumulate toxic cations. In this case, hyperaccumulators are able to accumulate HMI in quantities that significantly exceed their content in the soil. The accumulation of heavy metal anions, similar to that of cations, is usually not observed, because in this case the toxicity of the metal is higher. The interaction of HMI with the cytoplasmic membrane activates lipid peroxidation. Numerous publications indicate the appearance of reactive oxygen species and activation of a number of enzymes. Heavy metal ions significantly affect photosynthesis, chloroplast structure, and pigments.

Cd^{2+}, Ba^{2+}, and Pb^{2+} ions cause changes in the lipid composition of thylakoid membranes. A typical phenomenon is a decrease in chlorophyll content, with chlorophyll b decreasing more than chlorophyll a. It has been established that excess heavy metals in leaves cause chlorophyll content, while their effect on the state of the pigment system is almost unknown. However, it is known that the pigment content and its state determine the development and activity of the photosynthetic apparatus [2, 3, 4]. Studies of the effect of heavy metal ions (HMI) on plant growth and development have shown that all pathological changes begin at the cellular level. Cell culture is the most convenient and promising system for studying the stressful effects of HMI and resistance mechanisms. In this case, the protective reactions of the whole organism aimed at reducing the toxic effect are separated from the cellular detoxification reaction. The diverse range of adaptation reactions that replace each other in the cell suggests that the cell has protective mechanisms to withstand the vast majority of stresses. The study of the effect of heavy metal ions is a new and promising area in the physiological and biochemical study of plants.

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МОДЕЛЮВАННЯ ПОСТТРАВМАТИЧНОГО СТРЕСОВОГО РОЗЛАДУ НА ЩУРАХ

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Важливість проблеми посттравматичного стресового розладу (ПТСР) у світі та в Україні полягає в її все більшому поширенні та впливі на фізичне та психічне здоров'я людей, а також на суспільство в цілому. Дослідження на тваринах в умовах моделювання стресових розладів дозволяють глибше зрозуміти механізми розвитку ПТСР, та надають перспективи для розробки нових підходів до терапії, спрямованих на відновлення функцій мозку та нормалізацію психічного стану. Метою нашої роботи було оптимізувати умови та підтвердити ефективність моделі ПТСР на щурах.

Для моделювання стресового розладу на щурах використовували одноразовий довготривалий стрес (ОДС). Ця модель вперше була запропонована Liberzon *et al.* (1997, 1999) [1, 2]. Щурів тримали іммобілізованими протягом 2 годин у спеціальному прозорому пластмасовому футлярі, після чого поміщували в круглий басейн з водою (24°C), де протягом 20 хвилин тварини вимушено плавали та надалі відпочивали протягом 15 хв. На останньому етапі щурів піддавали дії діетилового ефіру, до втрати ними свідомості, після чого залишали у спокійному стані протягом 7 днів.

Визначали рівень тривожності у щурів, які піддавалися ОДС у порівнянні з контрольною групою, оцінюючи результати поведінки тварин у припіднятому хрестоподібному лабіринті (ПХЛ). Аналізувалися кількість входів та час перебування щурів у відкритих та закритих рукавах лабіринту [3]. Було виявлено, що щури дослідної групи набагато рідше входили у відкриті рукави в порівнянні з контрольними тваринами. Відмінності в цьому показнику мали достовірну різницю. Також виявлено, що контрольні тварини перебували на відкритих рукавах лабіринту значно більше часу, ніж щури після стресу, які довше розташовувалися у закритих рукавах лабіринту. Результати ПХЛ тесту вказують на підвищений рівень тривожності у дослідних щурів.

Отже, ОДС викликає суттєві зміни у поведінці тварин, що підтверджує ефективність даного підходу для моделювання ПТСР на щурах, а дана модель може бути використана для подальших досліджень механізмів розвитку мозкових порушень при ПТСР та пошуку терапевтичних підходів до корекції відповідних патологічних станів.

Ключові слова: посттравматичний стресовий розлад, модель

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Session 4

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MECHANISMS OF NEUROPROTECTIVE ACTION OF VITAMIN D_3 IN GLUCOCORTICOID-INDUCED NEUROTOXICITY

Ihor Shymanskyi **Lecture 1**

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The therapeutic application of glucocorticoids (GCs) leads to the development of a number of side effects, among which the least studied are brain lesions, cognitive dysfunction and mental disorders. Nuclear transcription factor κB (NF-κB), a cellular sensor of oxidative stress and a key modulator of the inflammatory response, cell proliferation and apoptosis, may play a pivotal role at the intersection of various signaling pathways in the mechanism of GC-induced neurotoxicity. Vitamin D_3 (VD_3) is considered a potent para/autocrine regulator that can strongly influence nerve cell function and counteract the negative effects of GC therapy. The study was aimed at investigating the canonical pathway of NF-κB activation in association with the intensity of apoptotic and proliferative processes in different structural areas of the brain in GC-induced neurotoxicity and after vitamin D_3 treatment.

Female Wistar rats received prednisolone (5 mg/kg b.w.) with or without VD_3 (1000 IU/kg b.w., 30 days). VD_3 receptor, VD_3 binding protein, CYP24A1, CYP27B1, NF-κB/p65 and its phosphorylated forms, and molecular markers of apoptosis (AIF, Bax, Bcl-2, caspase-8, and p17 fragment of caspase-3) were determined by quantitative PCR and/or Western blotting. The number of apoptotic cells was studied by the TUNEL. Proliferative activity of cells in histological sections of the cerebral cortex and hippocampus was evaluated using double immunofluorescent labeling with 5-bromo-2'-deoxyuridine or Ki67 and marker proteins of oligodendrocytes, microglial cells and astrocytes (NG2+, Iba-1+, GFAP+ respectively).

GC supplementation led to 3.2-, 2.0-, and 2.2-fold depletion of 25OHD pool in serum, cerebrospinal fluid and brain tissue, elevated VDR and CYP27B1, while lowering CYP24A1 and VDBP protein levels. We demonstrated an increase in the transcriptional activity of NF-κB (assessed by the binding of the p65 subunit of NF-κB to the consensus DNA region of cell nuclei), as well as the expression of the p65 subunit of NF-κB and the intensification of its specific phosphorylation at Ser311, Ser536 and Thr435, indicating transcriptional activation of NF-κB by the canonical pathway. Prolonged administration of prednisolone did not affect the number of oligodendrocytes in the cerebral cortex of rats, but their number was significantly reduced in the hippocampus. There was a marked decrease in the number of microglial with an elevation of astrocytes in different brain sections. GC caused apoptotic cell death predominantly by intrinsic pathway. Elevated apoptosis was accompanied by inhibition of cell proliferation in different brain areas, mainly due to microglia. Vitamin D_3 partially attenuated most of the GC-induced abnormalities in the brain, but exacerbated the inhibition of cell proliferation.

In summary, VD_3 restored GC-induced changes in rat brain suggesting modulation of NF-κB-associated processes in the mechanism of antiapoptic, antiproliferative and neuroprotective actions of VD_3.

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LONG-COVID: THE ROLE OF NK CELLS AND HERPES VIRUS TYPE 6 ACTIVATION

Valentyna Chopyak **Lecture 2**

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Long-COVID often accompanied by the development of dysregulation of immune system and autoimmunity. They can be caused by "sluggish" herpesvirus infection HHV6 in COVID-19 patients [1,2,3]. Also involved in the development of autoimmunity a change in the regulatory and cytotoxic functions of NK cells. The aims of this work is to study the expression of receptor-ligand Fas-FasL, regulating marker CD38, and inhibitory receptor TIM-3 on NK cells [4, 5] in patients with Long-COVID who fell ill mild, moderate, and severe COVID-19 without and with HHV-6 and to identify risk factors for the formation of autoimmune disorders in these patients.

In this study was investigate 124 adults (73 (59%) female and 51 (41%) males) aged 18 to 65 years with Long-COVID after mild, moderate and severe forms COVID-19 without/with HHV6 and 20 healthy participants. Molecular genetic studies (PCR) were performed on all patients to detect the existence of HHV6 and define the study groups. Multiparametric flow cytometry was performed on EDTA peripheral blood samples collected from COVID-19 patients and 20 healthy participants.

As a result of our research, we have obtained data that in patients with Long COVID after mild, moderate, and severe COVID-19 without HHV-6 in the stage of replicative activity. They installed an imbalance of antiviral mechanisms. The presence of HHV-6 in groups with long-term COVID-19 is accompanied by higher expression of FasL and CD38, especially in patients who have suffered a more severe forms of COVID-19. The decrease in TIM-3 in patients with HHV-6 compared to patients without HHV-6 puts the preservation of immunological tolerance. In these patients with Long COVID, the degree of dysregulation of the immune system is more significant, and the risk of developing immunopathology and autoimmunity is higher. The presence of HHV-6 in groups with long COVID is accompanied by statistically significant higher expression of Fas ($p<0,01$) and CD38 ($p<0,01$) in comparison with control group, especially in patients who have suffered a moderate and severe forms of COVID-19. The decrease in TIM-3 in patients with HHV-6 compared to patients without HHV-6 ($p<0,01$) puts the preservation of immunological tolerance at risk of development of autoimmune disease by cell type.

A detailed study of changes in the expression of Fas/FasL and TIM-3 receptors and regulatory marker CD38 in Long-COVID patients without/with HHV-6 allows predicting the loss of immunoregulation in these patients and the formation of immunopathology. HHV-6 affects the functioning of NK cells in patients with moderate and severe forms Long COVID, its presence affects changes in the expression of Fas and CD38, which indicates increased hyperactivation of NK cells and subsequent exhaustion. NK cells of this patients lose the immunological tolerance, this creates prerequisites for the development of autoimmune processes, often in the nervous system.

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Application of liposomal form curcumin and microRNA in Alzheimer's disease therapy

Shulga Sergiy **Lecture 3**

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A lot of scientific research points to inflammation as a mechanism most often present in the development of Alzheimer's disease (AD). Curcumin targeted delivery may be a viable alternative to classical treatment protocols. The delivery system can be liposomes – universal systems that can be loaded with both lipophilic and hydrophilic compounds. Mixture of sunflower phospholipids in the form of powder (deoil dry lecithin) was used as a raw material for the liposomes formation..

Liposomes were prepared by "sonication" and extrusion methods. The most effective method for liposomal form of curcumin creating was determined. It was the extrusion through membranes method. Curcumin liposomal form obtaining method included dissolving a mixture of sunflower phospholipids, cholesterol, and polyethylene glycol and curcumin solution adding; evaporating of mixture until a lipid film was formed; hydration, and sequential extrusion through membranes with pore diameters of 200, 100, and 50 nm. The size of "empty" liposomes and liposomes with curcumin and microRNA was determined using: a) dynamic light scattering using a laser photocorrelation spectrometer and b) atomic force microscopy (AFM). The efficiency of curcumin encapsulation was 95.34 ± 3.76%. MicroRNAs (miRNAs) are short (22 nucleotides), non-coding RNAs that act to inhibit protein expression by interacting with specific recognition elements in the 30 -UTR of target transcripts. The miRNA guides a ribonucleoprotein complex termed RNA-in_duced silencing complex (RISC) to the target site in the 3'-UTR where RISC mediates either translation repression or mRNA destabilization.

The effect of curcumin on the expression of APP and Aβ in response to treatment with curcumin at different concentrations and the effect of curcumin on the expression of APP and Aβ from miRNAs were evaluated. Curcumin was shown to decrease mRNA, APP, Aβ40 and Aβ42 levels compared to untreated cells.

The obtained data found the sunflower phospholipids using as raw material for the nano-sized containers creation – liposomes for pharmacologically active ingredients transport and strategy for the Alzheimer's disease treatment, taking into account the key role of the liposomal form of flavonoids and micro RNA.

PPAR-γ AGONIST PIOGLITAZONE RESTORED MOUSE LIVER, KIDNEY MEDULLA AND LUNG MRNA EXPRESSION OF CLOCK GENES AND INFLAMMATION-RELATED GENES DISRUPTED BY REVERSED FEEDING

Igor Kaidashev **Lecture 4**

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The circadian rhythm system regulates lung function as well as local and systemic inflammations. The alteration of this rhythm might be induced by a change in the eating rhythm. Peroxisome proliferator-activated receptor gamma (PPARG) is a key molecule involved in circadian rhythm regulation, liver, kidney and lung functions, and metabolic processes.

We described the effect of the PPARG agonist pioglitazone (PZ) on the diurnal mRNA expression profile of core circadian clock genes (*Arntl*, *Clock*, *Nr1d1*, *Cry1*, *Cry2*, *Per1*, and *Per2*) and metabolism- and inflammation-related genes (*Nfe2l2*, *Pparg*, *Rela*, *Cxcl5 and* Scnn1g*)* in the male murine liver, kidney medulla and lung disrupted by reversed feeding (RF). Additionally, we measured liver expression of PPAR-*γ*, pNF-*κ*B, and IL-6 by Western blotting, urinary K^+, Na^+, urine volume, food, and H_2O intake, cellularity and interleukin concentration in BAL.

In mice, RF disrupted the diurnal expression pattern of core clock genes in liver, kidney medulla and lung.

Administration of PG at 7 AM to nighttime-feeding mice did not reveal any influence on the expression of the clock or inflammation-related genes either at midnight or at noon. In the daytime-feeding group, PG intake at 7 PM led to an increase in *Per2* and *Rev-erb alpha* mRNA at noon, an increase in *Ppar-γ* mRNA at midnight, and a decrease in *Nfκb* (*p65*) mRNA at noon. In general, PG administration at 7 PM slightly normalized the impaired expression of clock genes and increased anti-inflammatory potency impaired by reversed feeding. According to our data, PG intake at 7 PM exerts strong normalization of clock gene expression with a further increase in *Nrf2* and, especially, *Ppar-γ* and PPAR-*γ* expression with inhibition of *Nfκb* and pNF-*κ*B expression in daytime-feeding mice. These expression changes resulted in decreased hyperglycemia, hypercholesterolemia, ALT, and AST activities. RF uncoupled the peripheral clock gene rhythm in mouse kidney tissues. It was accompanied by a decreased expression of Nfe2l2 and Pparg as well as an increased expression of Rela and Scnn1g. These changes in gene expressions concurred with an increase in urinary Na^+, K^+ , water excretion, microcirculation disorders, and cell loss, especially in distal tubules. PG induced the restoration of diurnal core clock gene expression as well as Nfe2l2, Pparg, Scnn1g mRNA, and decreased Rela expressions, stimulating Na^+ reabsorption and inhibiting K^+ excretion. RF decreased *Nfe2l2* and *Pparg* and increased *Rela* and *Cxcl5* expression in lung tissue. There were elevated levels of IL-6, TNF- alpha, total cells, macrophages, and lymphocyte counts in bronchoalveolar lavage (BAL) with a significant increase in vascular congestion and cellular infiltrates in male mouse lung tissue. Administration of PZ regained the diurnal clock gene expression, increased *Nfe2l2* and *Pparg* expression, and reduced *Rela*, *Cxcl5* expression and IL-6, TNF- alpha, and cellularity in BAL. PZ administration at 7 p.m. was more efficient than at 7 a.m. Thus, PG had a potent chronopharmacological effect when administered at 7 PM to daytime-feeding mice.

CHANGES OF EXPRESSION OF REGULATORY AND INHIBITORY RECEPTORS ON CD8 T CELLS IN LONG-COVID-19 PATIENTS

Anna Havrylyuk **Lecture 5**

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$CD8^+$ T cells with cytotoxic properties contribute to protective immune responses against SARS-CoV-2 in patients with coronavirus disease 2019 (COVID-19). In patients with COVID-19, $CD8^+$ T cells exhibited activated phenotypes, although the absolute number of $CD8^+$ T cells was decreased. Long-COVID syndrome was often accompanied by the dysregulation of the immune system. This may result from changes in various immune checkpoint receptors, expressed on $CD8^+$ T-cell and $CD8^+$ T-cell exhaustion [1, 2, 3]. The aims of this work were to study the expression of inhibitory checkpoint receptors-ligand PD-1/PD-1L, regulating marker CD38, and inhibitory receptor TIM-3 on $CD8^+$ T cells in patients with mild, moderate, and severe COVID-19 to identify risk factors for the formation of immunopathology syndromes in these patients.

This study involved 64 adults (43 (67%) female and 21 (33%) males) aged 18 to 65 years with post-COVID and was distinguished into mild, moderate, and severe forms of disease and 20 healthy control participants. Multiparametric flow cytometry analysis was performed on EDTA peripheral blood samples collected from 60 cases of COVID-19 and 20 healthy controls.

As a result of our research, in patients with long COVID-19 after severe form COVID-19 the level of expression of PD-1($CD279^+$) was significantly lower (0,041) than in the mild, moderate COVID-19 and the control group. The expression of PD-1L($CD274^+$) was significantly higher than in the control group (0,003) and mild, and moderate COVID-19 (0,011). The expression of CD38 was significantly higher than in the control group (0,042). However, the expression of TIM-1($CD366^+$) was significantly lower in comparison with the control group (0,046) and mild form moderate COVID-19 (0,043).

So we found the increasing expression of CD38 [5] and the decreasing expression of TIM-3 [4] in patients with long COVID after severe COVID-19. We indicated that patients with a severe form of COVID-19 had the greatest risk of immunopathology because they have: 1) a decreased count of $CD8^+$ T cells; 2) a tendency to switch to Th1-dependent immune response; 3) PD-1/PD-1L system imbalance and upregulation of activatory/inhibitory receptors. $CD8^+$ T cells of these patients lose their immunoregulatory ability, what creates prerequisites for the development of immunopathology, probably autoimmune processes.

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MORPHOLOGICAL AND QUANTITATIVE ANALYSIS OF CONDUIT FOR 3D SCAFFOLDS TO REPAIR INJURED PERIPHERAL NERVES

Valeriia Ustymenko **Lecture 6**

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Peripheral nerve injury (PNI) represents a significant clinical challenge due to its complexity and the severe impact it can have on patients' quality of life. In the ongoing project, we have implemented a new type of 3D-printed microscaffolds to guide the axons and vessels. However, the material of scaffolds can not be directly sutured to the stumps of the damaged sciatic nerve. Therefore, to treat the PNI using microscaffolds, we initially used the silicon tube sutured to the stumps to study its regeneration potential alone to further use it as a conduit to host the scaffolds.

The 10 mm long silicon tube having 1 mm inner and 2 mm outer diameter was used to bridge a 6 mm gap in the sciatic nerve of ~7-week old Wistar rats. Silicon tube conduits and contralateral intact nerves were harvested after 20 weeks. Immunohistochemical staining was carried out to visualize axons (cytoskeleton protein neurofilament heavy chain, NfH), myelinating and non-myelinating Schwann cells (myelin basic protein, MBP, and cytoplasmic protein S100, respectively) and endothelial cells of vessels (rat endothelial cell antigen, RECA). The confocal imaging was performed on two types of samples: (i) whole-mount sciatic nerve to observe PNI-induced morphological changes and (ii) nerve cross-sections to analyze them quantitatively. Obtained images were processed and analysed using Python. Acquired data were statically processed using R.

We found that the diameter of the nerve regenerated in the silicon tube was significantly smaller compared to the one of the contralateral nerves and even the inner diameter of the silicon tube. The regenerating axons within the tube were mainly grown in parallel to rostrocaudal axes. Immunohistochemistry revealed a significantly higher density of axons within the regenerating sciatic nerve inside the tube compared to the contralateral nerve indicating a substantial axonal sprouting after PNI. The mean diameter of the regenerating axons was significantly smaller compared to that of contralateral nerves. The presence of Schwann cells was almost negligible in the nerve within the tube implying disrupted remyelination of nerves in the silicon tube. The behaviour testing results showed that there was no significant recovery of motor function during 20 weeks after nerve dissection as revealed by evaluating the sciatic functional index (SFI).

We have concluded that the silicon tube creates conditions for partial peripheral nerve regeneration after excision, but it does not lead to functional recovery. Thus, if the implantation of 3D-printed scaffolds in the silicone tube conduit results in significant structural and functional improvements after the PNI, it should be attributed to the scaffold usage.

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THE ROLE OF TRPV4 IN THE COLON OF RATS WITH 6-OHDA-INDUCED PARKINSONISM

Arsen Ishchuk **Lecture 7**

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There is substantial evidence supporting the close link between gastrointestinal (GI) dysfunction and the development of neurodegenerative diseases such as Parkinson's disease (PD) [1]. Specifically, disturbances in intestinal motility, constipation, bloating, nausea, and dysbiosis can occur even before the onset of motor symptoms in PD, indicating early involvement of the GI tract in the pathological process [2-3]. TRPV4 non-selective cation channels play a significant role in the pathogenesis of inflammation and disruption of GI barrier function [4]. Activation of these channels inhibits the contractile activity of the colon and delays defecation in mice [5]. However, the involvement of TRPV4 in the pathogenetic changes in GI motor function in PD remains unclear.

Therefore, our study aims to establish the role of TRPV4 ion channels in the mechanisms of secretory and motor functions of the colon under experimental PD.

PD was modeled in male non-linear white rats by stereotaxically injecting the selective neurotoxin 6-OHDA into *medial forebrain bundle* (AP=-5,3; ML=±2,0; DV=-7,2). The percentage of dopaminergic neuron degradation was assessed using the apomorphine test 1 and 2 weeks post-operation. The contractile activity and transmembrane potential difference of an isolated section of the colon were recorded under the influence of 0.3 μM GSK1016790A (a TRPV4 agonist, hereafter GSK) using the balloonography method and the Ussing chamber, respectively.

The study established that rats with 6-OHDA-induced parkinsonism exhibited an increased number of rotations following the apomorphine test, as well as reduced water content in feces and decreased water intake. Gastrointestinal motility in these rats was 26% slower compared to the control group. Additionally, administration of GSK resulted in a 42% reduction in contractile activity of the distal colon in 6-OHDA rats compared to control (carbachol stimulation). Investigation of the effects of GSK on intestinal secretion and epithelial permeability revealed that all experimental groups experienced a decrease in short-circuit current over time, indicating reduced ion secretion across the intestinal epithelium. Simultaneously, the transepithelial permeability of the colonic epithelium in Parkinsonian rats was lower under DMSO treatment but significantly higher under GSK treatment, suggesting increased epithelial permeability due to TRPV4 agonist action.

These results demonstrate a significant impact of TRPV4 channels on colonic contractile activity and epithelial permeability in 6-OHDA rats. This may indicate a potential role for these channels in the pathophysiology of Parkinson's disease.

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CORRECTIVE EFFECT OF GRAPE POMACE EXTRACT IN OXIDATIVE-NITRATIVE STRESS IN DIABETES MELLITUS

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In the study, we evaluate the effect of grape pomace extract, rich in natural polyphenol complex on oxidative and nitrative stress markers in the blood plasma and liver of rats with streptozotocin-induced diabetes mellitus (DM).

The activities of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) decreased in plasma and liver in DM. When the extract was entered to animals with DM, the activity of the antioxidant enzymes increased to control in plasma and liver. Excess amounts of reactive species cause oxidation of proteins, lipids, and nucleic acids, and highly toxic by-products formation. In DM, the content of proteins carbonyl derivates and TBA-reactive substances (TBARS) increased in plasma and liver compared to control. The extract caused a decrease in the content of oxidative stress markers to control in plasma in DM. However, the content of proteins carbonyl derivates and TBARS remained higher than the control in liver, when the extract was administered to animals with DM.

Hyperglycemia and oxidative stress lead to impaired production of nitric oxide (NO). NO is synthesized by enzymes belonging to the family of NO synthases (NOS). It was shown the increase of the activity of both constitutive and inducible NOS in plasma and liver in DM. The grape pomace extract caused the decreased of NOS activity in studied tissues under DM. To evaluate the level of the NO production, the concentration of NO stable metabolites (NOx) was studied. The levels of nitrites and nitrates increased in liver and to a greater extent in the plasma of rats with DM. The extract administration to diabetic animals reduced the content of NOx to normal range, except for the content of nitrates in the liver, which remained 20% higher than the control.

L-arginine is the substrate of the NOS reaction. The L-arginine level was higher than the control in plasma and liver in DM. When the extract was administered, the content of L-arginine decreased in plasma, and remained slightly elevated in the liver in DM. In normal condition, the major site of arginine metabolism in ureotelic animals is the liver. Here, arginine is synthesized from citrulline in the urea cycle. Arginine is then converted to urea and ornithine by arginase. To examine possible alterations of urea cycle in the NO pathway defects, we measured concentrations of urea and uric acid. We found an increased level of urea in plasma and liver in rats with DM. Under DM the content of uric acid in the liver decreased, while it did not change in the plasma. The extract caused the decrease of the urea in plasma, but not in the liver in DM. The extract also normalized the content of uric acid in the plasma, and further reduced its content in the liver.

The obtained results confirm that the grape pomace extract, rich in a natural complex of polyphenols, is capable of correcting systemic oxidative and nitrative stress in DM, in particular affecting the activity of antioxidant enzymes and L-arginine / NO-synthase system, and suppressing the pathological oxidation of proteins and lipids.

TOXIC EFFECT OF DRINKING WATER BY USING IN VITRO METHODS

Maya Vergolyas **Lecture 9**

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Recently, the study of the impact on the health of the population of various adverse environmental factors is the most urgent and difficult task. The danger of a negative impact on the health of the population and biota in general may arise as a result of anthropogenic pollution of the aquatic environment. The main causes of water pollution, according to sanitary-epidemiological and microbiological indicators, are the discharge of untreated domestic and industrial wastewater, unsatisfactory operation of treatment facilities, lack of sanitary zones, etc. In this regard, today one of the urgent problems is providing the population of Ukraine with high-quality and safe drinking water [1, 2].

Water may contain biologically active impurities that have a negative impact on human health. Physicochemical methods of water composition analysis do not provide an opportunity to comprehensively assess water quality and predict the impact of substances present in it on living objects. These methods determine only the presence and quantity of chemical elements in the studied water samples, but cannot determine the specifics of the formation of the quality of the studied water samples due to a very large number of possible combinations of chemical compounds in water solutions (there are more than 75 million of them). Thus, there is a need to develop and use new methods of comprehensive assessment of the safety and quality of drinking water with the possibility of predicting its impact on various living organisms [2].

The aim of the study was to evaluate the quality of drinking water of various origins by its cytotoxic effect on human and animal cell cultures in vitro.

Many studies have confirmed that in vitro methods are quite accurate, quick to set up, and economically viable. The prospects of research using in vitro methods are also strengthened by the growing attention to the role of ethical aspects when choosing the object of research, the increasing interest of scientists and the general public in the humane treatment of warm-blooded animals, and the reduction of their number in scientific experiments [3, 4]. The results of the conducted work confirm the received data of scientific research on the perspective of using human and animal cell cultures to evaluate the quality of drinking water.

The research was carried out within the framework of the project No. 0123U103297 "Hygienic substantiation of the use of a portable water disinfection system in the conditions of hostilities and emergency situations").

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FUNCTIONAL STATE OF ERYTHROCYTES IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS AND ITS' CORRECTION UNDER ADMINISTRATION OF FRUIT EXTRACT OF HYBRID *CORNUS MAS* × *CORNUS OFFICINALIS*

Anna Moroz **Lecture 10**

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Type 1 diabetes mellitus (DM) is the most common endocrine disease characterized by absolute insulin deficiency and increased blood glucose concentration. It should be noted that prolonged hyperglycemia alter the normal hallmarks of red blood cells (RBCs) biochemistry and physiology, such as metabolism properties, protein homeostasis, redox status, cytoskeletal dynamics, deformability and shape [1, 2]. Thus, it is not surprising that the functional state of RBCs is altered under diabetes, which underlies its clinical importance. Indeed, possibilities of the pharmacological therapy based on plant raw materials are focused on normalization of the general condition of patients with DM and lowering risk of diabetes-related complications. One of the promising medicinal plants is Cornaceae family. Cornelian cherry contains health-promoting compounds in its fruits. Generally, phytocompounds from fruits of cornelian cherry show a wide range of biological properties: antidiabetic, antioxidant, and immunomodulatory [3]. Since the versatile properties of erythrocytes allow them to reflect an organism's pathophysiological status, therefore our work aimed to investigate the effect of the fruit extract of hybrid *C. mas* × *C. officinalis* on the functional state of RBCs in rats with DM.

DM was induced by intraperitoneal injection of streptozotocin (STZ) [3]. On the 10th day from the moment of diabetes induction, an extract of the fruit of hybrid *C. mas* × *C. officinalis* was administered orally to rats for 14 days at a dose of 20 mg/kg of b. w. On the 25th day of the experiment, the animals were decapitated, and hematological biomarkers were determined.

We observed an increase in the number of reticulocytes, their daily production, aggregation ability of RBCs, and the concentration of sialic acids in the blood plasma against a decrease in the resistance of RBCs to acid hemolytic agents, in the duration of hemolysis, and a significant decrease in the content of sialic acids in RBCs under STZ-induced DM. Sialic acids provide the surface of RBCs with a high negative charge, thereby preventing aggregation. The findings taken above identify that RBCs have a wide array of functional alters in diabetic rats. The administration of the fruit extract of hybrid *C. mas* × *C. officinalis* to animals with diabetes caused reliable positive changes in the normalization of these blood parameters to the level of the control group of rats. It is noteworthy that the studied extract in rats with STZ model of diabetes increased sialic acids content in RBCs and their resistance to the acid hemolytic agent, suggesting a promising therapeutic approach. Since the functional state of RBCs has been restored, one may speculate that such changes are due to the properties of bioactive substances of the extract from fruit of hybrid *C. mas* × *C. officinalis.*

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ВПЛИВ НАБУТОЇ КОРОТКОЗОРОСТІ НА СИСТЕМНИЙ ІМУНІТЕТ ТА ПОКАЗНИКИ НЕЙРОДИНАМІЧНИХ ФУНКЦІЙ

Віталій Шейко **Lecture 11**

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Серед патологій зорової сенсорної системи перше місце посідає набута короткозорість, на поточний момент даною патологією страждають 30 % населення нашої планети. Набута короткозорість, розглядається як адаптаційна реакція на тривале розглядання дрібних предметів (літер, слів, символів, цифр) з короткої відстані [1-2].

Нашими дослідженнями встановлено, що набута короткозорість різного ступеня (слабкого, середнього, високо) супроводжується формуванням вторинних порушень в системному імунітеті: слабкий ступінь – порушення в неспецифічній ланці системного імунітету, середній ступінь – прояв дисфункції в клітинній та неспецифічній ланці системного імунітету, висока – формування порушень неспецифічної, клітинної та гуморальної ланок системного імунітету [1-2].

Про стан нейродинамічних функцій судили за показниками латентних періодів (ЛП) сенсомоторних реакцій різної складності (СМРРС) та функціональною рухливістю нервових процесів (ФРНП).

Нами було з’ясовано, що на тлі набутої короткозорості різного ступеня відбуваються різнонаправлені зміни величин ЛП СМРРС. ЛП СМРРС у людей, що страждають на набуту короткозорість різного ступеня знаходились в межах референтних значень. Саме ЛП мають високу генетичну детермінованість, як фізіологічна характеристика діяльності нервової системи. ФРНП на фоні слабкого та середнього ступеня набутої короткозорості характеризувалися достовірним зменшенням часу витраченого на проходження тесту, що вказує на високу працездатність центральної обробки інформації ЦНС. Високий ступень набутої короткозорості характеризувався достовірним погіршення ФРНП, збільшенням часу витраченого на проходження тесту, що вказує на значне погіршення центральної обробки інформації ЦНС.

Нами виявлена кореляційна залежність між показниками системного імунітету та ФРНП на тлі набутої короткозорості різного ступеня [1-2].

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HEMATOPOIESIS AND CHEMICAL SKIN BURNS: WHAT IS THE DIFFERENCE IN THE BLOOD MARKERS OF THE HEALING PROCESS?

Iryna Byelinska **Lecture 12**

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According to the Global Burden of Disease (GBD) 2019 study, 8,378,122 cases of burns are reported annually, resulting in 111,292 deaths [1]. Most cases were thermal burns. Treatment protocols are directed at pain management and inflammation prevention. Given chemical burns' relatively low prevalence, they have not been as extensively investigated as thermal burns. This underscores the urgent need to develop specific treatments which can potentially improve patient outcomes and reduce mortality rates. Blood cells are active players in the regenerative process. Nevertheless, the hematopoietic system involvement in tissue restoration after chemical injuries and blood cell characteristics have not been investigated. Moreover, the easy availability of blood parameters is a good marker of the pathological process course.

This study ***aimed*** to investigate hematopoiesis and blood cells' dynamic following acid or alkaline burn for reparation mechanisms depicting as a basis for treatment approaches development. The chemical burns were induced by a 10-minutes skin application of 10N HCL or 3M NaOH solutions after intramuscular xylazine hydrochloride (8 mg/kg) and intraperitoneal telazol (5 mg/kg) anesthesia. In both cases, a second-degree burn was developed [2].

After 7 days of both types of chemical burn, leukocytosis developed. But, in the alkaline burn group, leukocyte count was 20% higher than the acid-burn group and remained elevated on day 14 (acid burn: WBC tends to normalize due to hematopoiesis failure). In contrast to acid burn, which is associated with a three-fold elevating neutrophils count in blood, alkaline burn demonstrated relative neutropenia with lymphocytosis and monocytosis on the background of the left shift. An increase in the band blood count was specific for both burn types. Hematopoiesis demonstrates activation of all myelopoietic lineages with progenitor cell juvenilities. Opposite neutrophils and lymphocytes dynamic in blood followed by acid or alkaline burn can be associated with different skin injuries: coagulation or liquefactive necrosis, respectively.

Conclusion. Chemical burn regeneration is assisted by blood cells with different markers: lymphocytosis and monocytosis with shortening period regeneration in alkaline burns vs. neutrophilia on the background prolonged skin regeneration after acid burns.

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EFFECT OF TRANSLOCATION t(8;21) ON CYTOMORPHOLOGICAL CHARACTERISTICS OF BLASTS AND NEUTROPHILS OF PEDIATRIC ACUTE MYELOID LEUKEMIA

Maksym Hutsaliuk **Lecture 13**

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Introduction. The most common variant of AML in children is AML with signs of maturation (FAB variant M2) [1]. Numerous studies demonstrate a more favorable prognosis in the presence of the t(8;21) translocation in patients with M2 AML [2]. Even though polymerase chain reaction (PCR) is still the standard method for detecting t(8;21), there is data on its morphological characteristics in bone marrow [3].

Aims. To study the association between cytomorphological features of leukemic cells in the presence/absence of t(8;21) translocation in children with AML.

Materials and methods. The study was conducted on 20 bone marrow samples of patients aged 0 to 18 years (9 female and 11 male) with a laboratory-confirmed diagnosis of AML. Bone marrow smears were stained using Romanovsky-Giemsa method and myeloperoxidase staining. The following cytomorphological signs were evaluated: the presence of Auer's rods, myeloperoxidase activity, the intensity and nature of neutrophil cytoplasmic staining, the mature granulocyte with pseudo-Pelger-Huet anomaly of the nuclei.

Results. Light microscopy found that in 80% of samples with a confirmed t(8;21) translocation, homogeneous pink staining of the neutrophil cytoplasm with prominent azurophilic granulation was observed. At the same time, in samples without t(8;21), these features were found in only 20% of cells. There was a predominance of pale cytoplasm staining of mature granulocytes without pronounced granulation ($p<0.01$). Kramer's coefficient V (0.6) indicates a direct moderate-strong correlation between indicators.

In 70% of samples with t(8;21), high myeloperoxidase activity was detected in more than 90% of blast cells, in contrast to samples without t(8;21), in which low myeloperoxidase activity was specific for blasts and just 20% of them have moderate/high myeloperoxidase activity ($p<0.01$). Kramer's coefficient V (0.528) indicates a moderate correlation between the t(8;21) translocation and myeloperoxidase activity.

No statistically significant correlation was found between the presence of t(8;21) and the presence of Auer's rods (V=0.18) or presence of pseudo-Pelger-Huet anomaly (V=0) ($p>0.05$).

Conclusions. The study's results revealed the homogeneous eosinophilic staining of mature neutrophil cytoplasm with abounded azurophilic granularity in combination with high myeloperoxidase activity in bone marrow blasts are morphological predictors for the t(8;21) translocation in leukemic cells of children with AML (M2 variant).

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REDISTRIBUTION OF SIALYLATED MEMBRANE GLYCOCONJUGATES AND ERYTHROCYTE STABILIZATION WITH BIOLOGICALLY ACTIVE COMPOUNDS *G. OFFICINALIS* IN TYPE 1 DIABETIC RATS

Halyna Hachkova **Lecture 14**

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Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels, severity continues to rise worldwide. Chronic hyperglycemia affects the morphological and functional changes of erythrocytes: stimulates redox imbalances, disturbs normal cell membrane architecture, and alters the expression levels of various membrane transporters [1-2]. Erythrocytes dysfunction may lead to deranged tissue oxygenation through a variety of mechanisms contributing to changes in normal rheology and hemodynamics [3]. Collectively, these contribute to erythrocytes dysfunction, reducing their lifespan in circulation and leading to anemia and the development of various DM-associated complications.

Many medicinal plants are known to be used in alternative medicine in many countries around the world to treat of DM, but the list of official antidiabetic drugs based on them is insufficient. A promising raw material for the creation of antidiabetic drugs of natural origin is *Galega officinalis* L.

The study aimed to investigate the effects of the non-alkaloid fraction of *G. officinalis* extract on the structural and functional state of erythrocytes under the condition of DM by determining erythrocyte resistance to acid hemolysis and changes in the structure of erythrocytes membranes glycoconjugates carbohydrate determinants.

Type 1 diabetes was induced by streptozotocin (60 mg kg^{-1}) in Wistar male rats. The non-alkaloid fraction of *Galega officinalis* extract [4] was administered to rats *per os* using a tube at doses 600 mg/kg (one dose per day) daily for 14 days.

An increase in the resistance of erythrocyte to hemolysis, inhibition of lipid peroxidation and an increase in the content of membrane-bound sialic acids and the degree of their exposure in the terminal position of the oligosaccharide sequences of erythrocyte glycans during the administration of herbal preparation based on the non-alkaloid extract of *G. officinalis* under conditions of diabetes is a characteristic feature of the rejuvenation of the erythrocyte pool in bloodstream. Increase in sialylation of erythrocyte membrane glycoconjugates is a molecular mechanism of reducing the aggregation these cells under the influence of biologically active substances of *G. officinalis*. Corrective effect of the biologically active substances of *G. officinalis* on the morphofunctional state of erythrocyte can be an experimental confirmation of the need for the development of functional foods based on *G. officinalis*, which can be used in complex therapy of diabetes and prevent the harmful effects of hyperglycemia.

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FUNCTIONAL STATE OF ERYTHROCYTES AFTER THE ADMINISTRATION OF THE MEDICINAL MUSHROOM *GANODERMA LUCIDUM* (*AGARICOMYCETES*) TO ANIMALS WITH EXPERIMENTAL METABOLIC SYNDROME

Tetiana Petryn **Lecture 15**

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Metabolic syndrome (MetS) and its components are now actively studied worldwide. Still, pathological changes in the human blood system under this pathology, in particular in erythrocytes, remain insufficiently studied. The study of the biologically active components of *G. lucidum* and their effects on metabolism is a promising area of research that will help to find an effective means for the prevention and treatment of patients with MetS. Therefore, our work aimed to investigate the effect of *G. lucidum* extract on the functional state of red blood cells in experimental MetS.

The research was carried out on white outbred male Wistar rats. MetS was induced by a high-carbohydrate diet: for 42 days, the animals consumed a 10% fructose solution instead of drinking water. After the induction of MetS, a suspension of freeze-dried extract of the mycelium of the *G. lucidum* mushroom was administered *per os* in a dose of 1 g/kg of the animal's body weight for 14 days.

MetS is characterized by an increase in the number of erythrocytes by 14.7% on the background of a decrease in hemoglobin concentration by 28.2% compared to control animals. For a more detailed assessment of the level of hypoxia, we conducted a study of the affinity of hemoglobin for oxygen. The analysis of the graphs of the oxyhemoglobin dissociation curve showed that in MetS there is an increase in the affinity of hemoglobin for oxygen by 9% compared to the control. Under the same conditions, an increase in the content of glycated hemoglobin by 29.5% and fetal hemoglobin by 17.6% was established, which are characterized by an increased affinity for oxygen and explain the changes in the oxyhemoglobin dissociation curves recorded by us [1]. Since hypoxia causes increased secretion of erythropoietin, which leads to increased erythropoiesis [2], we decided to investigate the number and daily production of reticulocytes. We found that MetS is accompanied by a 2.6-fold increase in the number of reticulocytes and a 2.3-fold increase in the daily production of reticulocytes, compared to controls. Also, MetS was accompanied by a decrease in the resistance of erythrocytes to the action of an acid hemolytic: the number of hemolyzed erythrocytes at the peak of hemolysis was 30.8% higher compared to control animals, and the duration of hemolysis was reduced from 8.0 min to 6.5 min.

The use of the extract led to the normalization of the number of erythrocytes, the concentration of hemoglobin, the number and daily production of reticulocytes, led to an increase in the resistance of erythrocyte membranes to acid hemolytic and a decrease in the affinity of hemoglobin for oxygen, which may be associated with a decrease in the content of glycated and fetal hemoglobin.

The obtained results show that the G. lucidum mushroom extract has a corrective effect on the functional state of the erythron system. This suggests a high potential for using this mushroom to correct the pathological conditions that accompany the development of MetS.

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VITAMIN D_3 MODULATES HEPATOCELLULAR UNFOLDED PROTEIN RESPONSE IN NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH EXPERIMENTAL TYPE 2 DIABETES

Mykola Veliky **Poster 17**

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Vitamin D_3 (D_3) is a potent regulator of liver function, but its precise hepatoprotective mechanisms in nonalcoholic fatty liver disease (NAFLD) associated with type 2 diabetes mellitus (T2DM) are not fully understood. The aim of the study was to examine the effect of D_3 on the unfolded protein response (UPR) in the liver of diabetic rats with NAFLD.

Type 2 diabetes was induced in male Wistar rats (230.0±12.0 g) kept on a high-fat diet for 2 months followed by a low dose STZ injection (25 mg/kg b.w.). Diabetic rats were divided into 2 groups, treated with or without D_3 (1000 IU/kg b.w., 30 days). NAFLD was assessed histologically (Sudan III&Gill's Hematoxylin II and AFOG). Serum 25-hydroxyvitamin D_3 ($25D_3$) was measured by ELISA. We used Western blotting to determine target protein levels.

T2DM was accompanied by hyperlipidemia, hyperglycemia and an increase in HOMA-IR (2.5-fold vs. control). T2DM caused total lipids accumulation in the liver (42.1±2.3 vs. 34.0±1.2 mg/g of tissue in control, $p=0.04$) that was also confirmed histologically. We found an increase in the number/size of lipid droplets in hepatocytes without significant degenerative changes in cells. ApoB100 increased by 1.68 times, and PPARγ level decreased almost twice in the diabetic liver compared with control. As evidence of endoplasmic reticulum (ER) stress in liver associated with T2DM, we identified upregulation of key UPR players (GRP78 by 1.68-, ATF6 by 1.90-, PERK by 1.34-, and IRE1 by 2.45-fold vs. control, $p<0.05$). ER stress-inducible protein CHOP was also found to be elevated (by 1.65-fold) in T2DM vs. control. $25D_3$ circulatory pool decreased to 27.9±4.2 nmol/L in T2DM vs. 88.5±7.3 nmol/L in control ($p=0.02$). D_3 supplementation completely restored $25D_3$ to 85.6±7.5 nmol/L, reduced glucose levels by 16% and HOMA-IR by 36% compared with T2DM, without affecting HbA1c. In parallel, D_3 partially normalized lipid profile, reducing the levels of total lipids, total cholesterol, triacylglycerides and free fatty acids (by 38%, 26%, 230%, 34%, respectively) vs. T2DM. There was no effect of D_3 on ApoB100, while PPARγ increased 3-fold vs. T2DM. D_3 downregulated GRP78 by 2.2-, ATF6 by 1.23-, PERK by 1.63-, and CHOP by 1.2-fold vs. T2DM ($p<0.05$), with no effect on IRE1. XBP1 was decreased by 14% in diabetics and by 20% after D_3 vs. control. D_3 treatment reduced signs of NAFLD, but lipid droplets were still observed in individual hepatocytes.

T2DM-induced metabolic changes were associated with D_3 deficiency, moderate signs of steatosis and impaired UPR status in liver. The effectiveness of D_3 as hepatoprotective agent for NAFLD treatment was supported by its ability to reduce lipotoxicity and diminish UPR activation.

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ДИСФУНКЦІЯ ЕНДОТЕЛІЮ ТА КЛІТИННІ МАРКЕРИ СИСТЕМНОГО ЗАПАЛЕННЯ У ХВОРИХ НА ХРОНІЧНЕ ОБСТРУКТИВНЕ ЗАХВОРЮВАННЯ ЛЕГЕНЬ ТА У ПОЄДНАННІ З ІШЕМІЧНОЮ ХВОРОБОЮ СЕРЦЯ

Раїса Піскун **Poster 18**

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Хронічне обструктивне захворювання легень (ХОЗЛ) та ішемічна хвороба серця (ІХС) є актуальною проблемою XXI століття у зв'язку з високою летальністю та інвалідизацією населення різних країн світу, а також з багатофакторністю патологічних процесів, що лежать в їх основі. Незаперечним на сьогодні є факт багатокомпонентності патогенезу ХОЗЛ, де головним механізмом є запальний процес, який місцево вражає дихальні шляхи та має системні прояви. У світлі сучасних тенденцій ХОЗЛ розглядається не лише як захворювання легень, а як системне захворювання, при якому виникають зміни у різних органах та системах. При ХОЗЛ клітини запалення накопичуються не лише в паренхіми легень та стінках дихальних шляхів, а й у судинній стінці. Разом із зниженим поступленням кисню, запальний процес спричиняють ендотеліальну дисфункцію у судинах малого кола кровообігу. Ендотеліальна дисфункція судин малого кола кровообігу спостерігається як при ХОЗЛ важкого, так і легкого ступенів. Це зумовлює порушення регуляції судинного тонусу та проліферацію клітин у судинній стінці.

Клітинні показники запалення були вивчені в основній групі хворих з поєднанням ХОЗЛ та ІХС і в двох групах порівняння – хворих з ізольованими ХОЗЛ і ІХС.

Відомо, що в продукції медіаторів запалення приймає участь велика кількість клітин, провідну роль серед яких відіграють лейкоцити. Аналізуючи показники рутинного розгорнутого загального аналізу крові, у хворих з ІХС не виявлено суттєвого збільшення загальної кількості лейкоцитів порівняно з контрольною групою. Порівняно з контрольною групою кількість лейкоцитів була збільшена помірно у хворих з ХОЗЛ та у пацієнтів з коморбідною патологією. Потрібно зазначити, що спільний перебіг ХОЗЛ та ІХС має тенденцію до збільшення показників неспецифічного запалення порівняно з пацієнтами з ізольованим ІХС: за кількістю лейкоцитів – (7,64 ± 2,92) $x10^9$/л проти (6,84 ± 1,31) $x10^9$/л та показника швидкості осідання еритроцитів (13,5 ± 3,12) мм/год проти (9,26 ± 2,57) мм/год. Тобто розподіл результатів дослідження хворих за кількістю лейкоцитів дає змогу виявити активність системної запальної реакції, яка найбільш виражена при респіраторній патології. Вважається, що для ХОЗЛ притаманне переважно нейтрофільне запалення дихальних шляхів, яке асоціюється з хронічним бронхітом та верифікується підвищеним рівнем нейтрофілів у периферичній крові. Найбільший нейтрофільоз в крові виявлено у пацієнтів з ізольованим ХОЗЛ – (80,14 ± 4,02) %. Показник нейтрофілів в групах хворих з ізольованою стабільною ІХС (71,34 ± 3,85) % та при поєднаній патології (76,31 ± 2,77) % був значно вищий ніж в групі контролю.

Таким чином, контроль клітинних маркерів запалення є необхідним критерієм в персоніфікованому підході до діагностики, прогнозу та прийняті терапевтичної тактики для хворих на ХОЗЛ.

ARGININE DEPRIVATION - SEMIRESISTANT HUMAN SQUAMOUS CARCINOMA SAS-R9 SUB-LINE: CLONOGENIC POTENTIAL, MOTILITY AND ADHESIVE PROPERTIES

Olena Vovk **Poster 19**

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Despite global efforts to advance metabolic anticancer enzymotherapy based on arginine (Arg) deprivation, the development of spontaneous therapeutic resistance remains a major concern. To better understand this issue, a considerably more resistant to Arg deprivation sub-line of human oral squamous carcinoma, SAS-R9, was isolated. The corresponding selection involved multiple sequential transfers of Arg-sensitive SAS cells from Arg-free medium (AFM) treated with produced in yeast recombinant human arginase to Arg-containing complete medium (CM), followed by a period of cell growth restoration. The number of such selection cycles was nine, therefore the resulting sub-line was named SAS-R9. SAS-R9 exhibited an increased ability to restore proliferation after the onset of Arg deprivation compared to the parental SAS cell line. We evaluated differences in clonogenic potential, motility and adhesive properties between the parental SAS cell line and the derived SAS-R9 sub-line.

Cell clonogenic assay revealed that SAS-R9 cells are characterized by the significantly higher clonogenic potential capacity of a single cell to grow into a colony than parental SAS cells. It was observed that the migration rate in the “wound healing” assay was slightly higher in SAS-R9 cells under CM conditions. However, Arg deprivation offset this difference and similarly profoundly inhibited the ability of SAS and SAS-R9 cells to migrate into the scratch area.

It was also revealed that SAS-R9 cells cultivated in CM exhibited a dramatically higher ability to form aggregates in agarose-coated dishes as compared to parental SAS cells. Also, the percentage of adherent to collagen I SAS-R9 cells was twice as high. Thus, SAS-R9 sub-line exhibits elevated cell-cell aggregation (homotypic adhesion) and cell-matrix adhesion (heterotypic adhesion) relative to SAS line, which indicates its potentially increased invasive and metastatic properties.

It remains to be elucidated whether the observed differences stem from genetic or epigenetic alterations evoked by repetitive exposure to Arg starvation. These results also have clinical implications for a better understanding the behaviour of malignant cells that underwent and survived after such a metabolic therapeutic intervention.

ВІДТЕРМІНОВАНИЙ ВПЛИВ ГЕОХРОНОКЛІМАТИЧНИХ ФАКТОРІВ НА ПОКАЗНИКИ СИСТЕМНОГО ІМУНІТЕТУ ОРГАНІЗМУ ЛЮДИНИ

Наталія Козачук **Poster 20**

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Регуляція фізіологічних функцій організму реалізується трьома системами нервовою, ендокринною та імунною системами. Імунна система має як ефекторну функцію так і секреторну та рецепторну, що забезпечує її учасниками міжклітинних взаємодій. Імунна система – це одна із найскладніших систем нашого організму, яка чутливо реагує на дрібні зміни зовнішнього та внутрішнього середовища. Саме імунна система забезпечує імунологічний нагляд для підтримання внутрішнього гомеостазу. Унаслідок порушень функцій будь-якої ланки імунної системи можуть виникати різні патологічні стани й захворювання [1]. Порушення імунного статусу формуються під впливом бактеріальних, вірусних та інших інфекцій, а також різноманітних патологічних станів та екстремальних умов; до яких можна віднести геохронокліматичні фактори [2-3]. Вплив геохронокліматичних факторів зумовлений високим темпом життя та наявністю швидкісного транспорту (літаки, гелікоптери, швидкісні потяги). Саме можливість швидкого переміщення через часові, географічні та кліматичні поясів і викликає функціональні зміни в організмі людини, в тому числі і в імунної системи.

Метою нашого дослідження стало вивчення відтермінованого впливу геохронокліматичних факторів на показники системного імунітету у людей, що подолали понад 6500 км. та перетнули 6 часових поясів.

Дослідження проводилося в період листопад- грудень з 2017 по 2022 рік.

Учасники дослідної групи подолали 6500 км за 8 годин та 40 хвили, вилетівши літаком з міжнародного аеропорту «Бориспіль» Україна і прилетіли до міжнародного аеропорту «Шоуду» м. Пекін КНР. Тривалість польоту становила 14-15 годин. Пекін розташований в мусонно-субтропічному поясі та в 8-му часовому поясі. Київ розташований в помірно-континентальному кліматичному поясі та в 2-му часовому поясі.

На основі наших досліджень можна стверджувати, що відтермінований вплив (7 діб після перельоту) геохронокліматичних факторів на системний імунітет супроводжується зменшенням абсолютної кількості лейкоцитів, моноцитів, нейтрофілів, лімфоцитів за рахунок всіх субпопуляцій, концентрація Ig всіх класів не змінювалась. Таким чином відтермінований вплив геохронокліматичних факторів викликає функціональне навантаження на всі ланки системного імунітету, формуючи прояви імунної дисфункції та погіршення захисних функцій організму людини.

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ПОКАЗНИКИ ЦЕНТРАЛЬНОЇ ГЕМОДИНАМІКИ НА ФОНІ ВРОДЖЕНОЇ ПАТОЛОГІЇ ЗОРУ (ПОВНА ТА ЧАСТКОВА СЛІПОТА)

Данило Казначеєв **Poster 21**

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Сліпота та слабкозорість є основною причиною, яка формує очно-зорову інвалідності як у дорослого та і дитячого населення світу. Інвалідність, яка зумовлена морфофункціональними патологіями ока посідає четверте місце [1]. Патологія зорової сенсорної системи, як набута так і вроджена супроводжується порушення гемодинаміки ока, це зниженні кровообігу в циліарному м'язі, у зменшенні пульсового та хвилинного об’єму крові в судинній системі ока по мірі прогресування короткозорості [1-2].

Таким чином метою нашого дослідження стало вивчення показників що характеризують діяльність серцево-судинної системи організму юнаків, які страждають на вроджену патологію зорової сенсорної системи (повна або часткова сліпота).

В дослідженні взяла участь група волонтерів, 21 особа, які мають вроджені патології зорової сенсорної системи: повна або часткова сліпота, чоловічої статі, віком 22,8±1,2 років, які дали письмову згоду на участь в дослідженні.

Базою для проведення дослідження була спеціально загальноосвітня школа-інтернат для сліпих та слабозорих людей, м. Слов’янськ, Донецької області (евакуйована з 2022 року в місто Кам’янське, Дніпропетровської області). Координація досліджень здійснювалась кафедрою біології Ніжинського державного університету імені Миколи Гоголя та кафедрою фізичної терапії, фізичного виховання та біології Донбаського педагогічного університету (м. Дніпро).

Про стан серцево-судинної системи за такими показниками частота серцевих скорочень (ЧСС), артеріальний тиск систолічний та діастолічний (САр. тиск, ДАр. тиск), пульсовий тиск, систолічний об’єм крові (СОК), хвилинний об’єм крові (ХОК), методи статистичної обробки.

Результати наших досліджень вказують, що САр. тиску, СОК та ХОК були більші за референті величини на 14,4±0,28 мм.рт.ст. (12 %), 1,12±0,1 мл. (8 %), 811,4±1,61 мл/хв. (20 %) відповідно. Коефіцієнт економічності кровообігу на фоні вродженої патології зорової сенсорної системи був більший в порівнянні з референтними значеннями на 51,3 %, що вказує на збільшення енергозатрат, для забезпечення циркуляцію крові. Нами виявлено зростання індекса Кердо, що вказує на зменшення резервних можливостей регуляції кровообігу та зменшення аеробних можливостей організму на фоні посилення симпатичного тонусу (референтне значення 0,0 у.о. +7.33 на фоні вродженої патології зорової сенсорної системи).

На фоні вродженої патології зорової сенсорної системи (повна або часткова сліпота) спостерігається погіршення показників центральної гемодинаміки та індексів ССС. Такі функціональні зміни в показниках центральної гемодинаміки викликані стресовими процесами, які сформувалися за умов не повної соціалізації.

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LACTIC ACID IS A MARKER OF ENERGY METABOLISM AND A REGULATORY EFFECTOR IN THE HUMAN BODY

Yuriy Boretsky **Poster 22**

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Lactic acid is a hydroxycarboxylic acid formed in almost all human body cells. The lactate content in the blood at rest should not exceed 2.2 mmol/L. The lactate clearance rate after high-intensity exercise depends on both the type of sport being practiced and the level of athlete performance. The physiological lactate/pyruvate concentration ratio is 10:1. Disruption of the pyruvate-lactate balance is one of the primary markers of the development of myocardial hypertrophy and heart failure (Cluntun et al., 2021). In addition to physical exertion, an increase in lactate concentration can be caused by cancer, cardiovascular diseases, and septic shock. Under such conditions, severe lactacidaemia (> 10 mmol/L) correlates with high mortality, especially if lactate clearance does not change within 10–12 hours (Haas et al., 2016). Therefore, the determination of lactate concentration is used to estimate changes in a person's general state, adaptation to intensive physical loads, and speed of recovery after loading, as well as to determine the level of restoration of motor qualities in physical rehabilitation.

Lactate transporters MCT1, MCT2, MCT3, and MCT4 of the SLC16 solute carrier family (TCID 2.A.1.13) play a key role in distributing lactate between body tissues (Bosshart et al., 2021). Further studies are required to understand the relationship among *MCTs* genetic polymorphisms, endurance-trained athletes, and a high-intensity performance. The redistribution of lactate between the cells producing it and the cells that metabolize it is extremely important to maintain a stable pH and to hold lactate in the body since this compound is a huge energy source as well as an effector of important regulatory mechanisms. It has been established that lactate is involved in regulating angiogenesis, respiration, mitochondrial biogenesis, macrophage polarization, and the proliferation of muscle tissue stem cells (Zhang et al., 2020; Hashchyshyn et al., 2022). The mechanisms of lactate's involvement in the epigenetic regulation of metabolism require detailed investigation.

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EXPLORING RESISTANCE MECHANISMS TO ARGININE DEPRIVATION THERAPY OF SAS-R9 SUB-LINE: SIGNALING PATHWAYS, AUTOPHAGY, ER-STRESS, EMT-TRANSITION

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Arginine deprivation therapy (ADT) has demonstrated strong anticancer effects in different types of cancer *in vitro* and *in vivo*. The acquired spontaneous therapeutic resistance to ADT in cancer cells poses a significant challenge in treating solid tumors. Therefore, the aim of the study was to uncover the potential mechanisms that drive cancer cells to develop spontaneous resistance to ADT.

To achieve this, we isolated the ADT-resistant human oral squamous carcinoma subpopulation, SAS-R9. Cell subline SAS-R9 shows significantly greater growth, survival and metastatic potential relative to the original SAS cell line.

We demonstrated that the ADT resistance in SAS-R9 cells does not depend on reexpression of the *ASS1* gene through c-myc/HIF1α-mediated mechanism. Instead, SASR9 cells exhibit increased activity of pro-survival signaling pathways (PI3K/AKT/mTOR, JNK, and MAPK). In comparison to the original parental cell line, SAS-R9 exhibited increased autophagy and an altered response to ER stress induced by arginine deficiency.

Alterations in cell functions such as migration, adhesion, and aggregation may be linked to a heightened epithelial-mesenchymal transition (EMT) phenotype, as evidenced by the increased levels of *MMP2, Vimentin*, and the Snail transcription factor (*SNAI1)*. We also observed changes in the activation of Focal Adhesion Kinase (FAK), which is involved in regulating cell migration and adhesion through the Akt-FAK signaling pathway.

In conclusion, our findings indicate that ADT-semiresistant SAS-R9 cells exhibit increased metastatic activity and aggressiveness as compared to the parental SAS cell line, which may be linked to the activation of key pro-survival signaling pathways and a more pronounced EMT phenotype.

ADAPTOR PROTEIN CIN85 PROMOTES MIGRATION OF OSTEOSARCOMA CELLS *VIA* THE MMP2/COL3A1 PATHWAY

Iryna Horak **Poster 24**

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Adaptor protein CIN85 is a ubiquitous protein overexpressed in many cancers [1-4], and its elevated expression is associated with metastasis and poor prognosis [1-3]. This study aimed to investigate the role of CIN85 in the migration of osteosarcoma cells.

Two human osteosarcoma (OSA) cell lines, HOS and SAOS-2, were used. CIN85 overexpressing cells (upCIN85) were compared to mock-transfected control (Mock), and knocked down (siCIN85) cells were compared to negative siRNA control (siNeg) in all experiments. Motility was studied using xCELLigence Real-Time Cell Analyzer and by scratch assay. Gene expression was evaluated by RNA sequencing and validated by RT^2-qPCR. Knockdown of HCLS1, MMP2, and COL3A1 was performed using specific siRNA, and its effect on cell motility was studied by scratch assay.

It was demonstrated that CIN85 overexpression in OSA cells results in a significant increase in their motility in xCELLigence and scratch assays. In contrast, CIN85 knocked down HOS and SAOS-2 cells were characterized by reduced motility. RNA sequencing of HOS cells with different levels of CIN85 expression revealed differentially expressed genes (DEGs) involved in cell migration, adhesion, angiogenesis, differentiation, and cell death. Search for CIN85 binding partners and genes correlated with CIN85 in OSA was performed using publicly available databases. Finally, a network containing CIN85, HCLS1 (downregulated by CIN85), COL3A1, and MMP2 (both upregulated by CIN85) was identified. We knocked down these genes to investigate their effect on OSA cell motility. It was found that HCLS1 knockdown had no significant impact on the motility of OSA cells. In contrast, the knockdown of COL3A1 and MMP2 significantly suppressed the motility of HOS upCIN85 cells by 30% and 50%, respectively, and of SAOS-2 upCIN85 cells – by 18% and 43%.

Taken together, in this study we demonstrated that CIN85 modulates the motility of OSA cells, analyzed possible molecular mechanisms, and identified COL3A1 and MMP2 as possible mediators of CIN85-driven migration.

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APPLICATION OF MACHINE LEARNING FOR DETERMINING GLYCATED HEMOGLOBIN IN FTIR BLOOD SPECTRA

Tetiana Makhnii **Poster 25**

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Glycated hemoglobin (HbA1c) is a critical biomarker for the diagnosis and management of diabetes, providing a reliable indicator of long-term glycemic control. Traditional HbA1c measurement techniques, though effective, are invasive and often time-consuming. This study explores the application of Fourier-transform infrared (FTIR) spectroscopy combined with advanced machine learning techniques to develop a non-invasive and rapid method for predicting HbA1c levels from blood samples.

The study analyzed blood samples collected from a cohort of 294 participants, including healthy individuals, prediabetics, and diabetics. Two regression models were developed for predicting HbA1c levels: Partial Least Squares Regression (PLSR) and Polynomial Curve Fitting (CF) utilizing a pseudo-Voigt function. Machine learning models were trained using the H2O AutoML platform, specifically Generalized Linear Model (GLM) was used to determine the importance of the variables. and identifying significant variables contributing to HbA1c prediction. The PLSR model achieved a coefficient of determination $R^2 = 0.809$, $R = 0.899$, and RMSEP = 0.843%, where R^2 is the coefficient of determination, R is the Pearson correlation coefficient, and RMSEP is the Root Mean Square Error of Prediction. The CF model, incorporating the pseudo-Voigt function, also exhibited high accuracy with an $R^2 > 0.999$ and minimal residuals, effectively deconvoluting the FTIR spectra to reveal key molecular structures linked to HbA1c levels, such as α-helices, β-sheets, and polysaccharides. The GLM model, which demonstrated slightly lower accuracy compared to PLSR ($R^2 = 0.588$, $R = 0.767$, RMSEP = 1.242%), nonetheless identified peaks that were strongly associated with glycated hemoglobin levels.

This study demonstrates the feasibility and potency of using machine learning models in conjunction with FTIR spectroscopy for the prediction of HbA1c levels from blood spectra. Future research should focus on validating these findings across larger, more diverse cohorts and exploring the clinical applicability of this approach in routine healthcare settings.

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DECREASE OF SENSITIVITY TO ANTIMICROBIALS AND THE APPEARANCE OF ATYPICAL FORMS OF *CORYNEBACTERIUM DIPHTHERIAE* UNDER THE INFLUENCE OF *CANDIDA ALBICANS* EXOMETABOLITES

Olena Motyka **Poster 26**

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Corynebacterium diphtheriae long-term carriage facilitated by violation of human oropharyngeal normocenosis. An increase in the number of fungi, most commonly *Candida albicans*, is considered a sign of this violation. It is known that the coexistence of Candida in biofilms can protect gram-positive bacteria from antimicrobial drugs; the biofilm matrix is considered to be an important protective factor [1-2]. Synergism between fungal and bacterial pathogenicity factors has been reported [3]. The effect of *C. albicans* metabolites directly on the properties of *C. diphtheria* cells has not been sufficiently investigated.

The aim of the present study was to investigate the effect of *C. albicans* metabolites on the main biological properties of *C. diphtheriae*, including sensitivity to bactericidal antimicrobials.

Four typical, antibiotic-susceptible *C. diphtheriae* strains and clinical isolates (tree of which are toxigenic) and four *C. albicans* isolates (two in hyphal form) were used.

After interaction with Candida exometabolites, changes in the biological properties of all corynebacterial strains were observed. All corynebacterial colonies acquired an internal red colour of different shades. In more than half of the cultures tested, the red-pigmented cultures did not show the morphological and biochemical characteristics necessary for identification of *C. diphtheriae*. All toxigenic corynebacteria isolates retained the ability to produce diphtheria toxin. An increase in the percentage of persister cells in broth cultures of corynebacteria was observed. Changes in the biological properties of corynebacteria cultures were more pronounced after interaction of bacteria with hyphal-form Candida. Atypical bacterial properties were maintained for 3 to 6 passages without fungal metabolites.

After interaction with *C. albicans* exometabolites in *C. diphtheriae* decrease the susceptibility to bactericidal action of penicillin, cefotaxim, ceftriaxone, ciprofloxacin, pefloxacin. Minimum bactericidal concentrations of these microorganisms increase in 2 to 4 times. In 20,0 to 53,4 cases corynebacteria acquired tolerance to antimicrobials.

Therefore, exposure to exometabolites of *C. albicans* led to the appearance of atypical forms of *C. diphtheriae* that could not be typed according to the standard identification scheme and had reduced antimicrobial susceptibility but retained toxigenicity.

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INDUCTION OF IMMUNOGENIC CELL DEATH IN MURINE MELANOMA BY A NOVEL THIOSEMICARBAZONE DERIVATIVE

Mykola Klishch **Poster 27**

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Immunogenic cell death (ICD) is a novel mode of action of several anticancer drugs, which is associated with activation of tumor-specific immune responses by release of damage-associated molecular patterns, activation of antigen-presenting cells and cytotoxic T-lymphocytes (Kroemer et al., 2013). ICD is crucial for the long-term success of anticancer therapies. Apart from doxorubicin (Dox), oxaliplatin and multiple other ICD-inducing chemotherapeutics, recent evidence suggests the ICD-inducing capabilities of novel thiosemicarbazone derivatives, such as COTI-NMe_2, synthesized by the group of Prof. C. Kowol from the University of Vienna. The current study aimed to compare the ICD-inducing properties of COTI-NMe_2 in two lines of murine melanoma and investigate the possibility of cross-immunization using these two lines.

Methods: cell culture studies *in vitro*, trypan blue assay, tumor cell inoculation *in vivo*, morphophysiological analysis of tumor-bearing animals, haemocytometry. Murine melanoma cells of the B16F10 line (wild type) and the B16F10/ADR line (Dox-resistant) were treated with COTI-NMe_2 for 24 and 48 hours to determine cytotoxicity and LC_{50} using trypan blue assay. For animal immunization studies, C57BL/6 mice were inoculated with the COTI-NMe_2-treated cells. As a negative control, we used necrotic tumor cells subjected to multiple freeze-thaw cycles (-196 to +20°C, 3x) and thus killed without ICD induction. Dox-treated cells were used as a positive control. After 14 days, the mice were rechallenged with alive B16F10 and B16F10/ADR cells. Blood samples were collected from surviving animals 30 days after the immunization and analyzed on an automatic blood analyzer Dymind DF51 Vet.

B16F10/ADR melanoma tended to be more resistant to COTI-NMe_2 than B16F10 when treated for 48h. However, this difference was not observed after 24h treatment. Comparison of different time and dose combinations of COTI-NMe_2 treatment showed that the cell vaccine prepared from B16F10 cells treated with 500 nM of COTI-NMe_2 for 48 hours was the most effective in immunization against alive B16F10 cells in comparison with other treatment combinations (1μM/48h, 5μM/24h, 10μM/24h). However, the rechallenge with alive B16F10/ADR cells has not demonstrated any signs of immunization under the current experimental setup.

Overall, we demonstrated that treatment with lover doses of COTI-NMe_2 for longer time periods is more effective in preparing cell vaccines from B16F10 cells. Although no signs of cross-immunisation between B16F10 and B16F10/ADR have been detected so far, it cannot be excluded entirely yet, considering the similarities between these two lines of melanoma. More studies are needed to investigate the possibility of cross-immunization and the conditions under which it can or cannot occur.

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SIGNIFICANCE OF LYMPHOCYTIC INFILTRATION AND LYMPHOVASCULAR INVASION IN THE PROGRESSION OF ENDOMETRIAL CANCER

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Currently, the main prognostic factors of the course of endometrial cancer (EC) are clinicopathological characteristics (stage of the disease, histological type, degree of differentiation, level of tumor invasion into the myometrium and lymphovascular space invasion - LVSI). Considering the clinical polymorphism of EC, which is a significant factor in both tumor progression and low efficiency of therapy, it is not enough to objectively predict the course of the tumor process based only on these features [1, 2]. According to the literature, components of the tumor microenvironment, in particular lymphocytes infiltrating the tumor (lymphocytic infiltration - LI), play an important role in the formation of biological features of a number of malignant neoplasms. However, the heterogeneity of LI in terms of population composition determines the complexity of the forecast [3-5].

Aim: to evaluate lymphocytic infiltration and lymphovascular invasion in endometrial cancer depending on the clinical and pathological features of the patients.

Object: samples of operative material of 247 patients with EC. **Methods:** clinical, morphological, immunohistochemical, statistical, bioinformatics.

Results: in the retrospective analysis of EC, it was found that in 44.1% of cases there was a slight LI, in 55.9% - a significant LI. It was found that the majority of low-differentiated EC with deep invasion into the myometrium are characterized by insignificant LI, which is associated with a low content of $CD8^+$-lymphocytes and a high content of $FOXP3^+$- lymphocytes. In 63.3% of such tumors, LVSI was observed, which is associated with a decrease in CXCL12 expression and an increase in CXCR4 in tumor cells, a high content of $CXCL12^+$-fibroblasts and higher proliferative potential. Bioinformatics analysis (GEPIA db) found that *CXCR4* mRNA level is associated with unfavorable 5-year recurrence-free survival of patients with EC.

Conclusion: it is shown that the progression of EC is determined by the integral influence of microenvironmental factors and changes in the expression of chemokine and its receptor in tumor cells. The obtained data can be used to create a predictive model of the course of EC and correct the treatment algorithm for patients with this form of cancer.

The work was carried out within the framework of the project "Hormonal-receptor status of cells of the tumor microenvironment as a factor in the modulation of oncogenesis in the endometrium and mammary gland" (0123U100100).

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BIOSAFETY ASSESSMENT OF BISPHOSPHONATE-MODIFIED POLYMER-COATED NAYF4:YB,ER,PR UPCONVERTING NANOPARTICLES: *IN VITRO* AND *IN VIVO* STUDIES

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Lanthanide-doped upconversion nanoparticles (UCNPs) have been subjected to a variety of biomedical applications, including drug delivery, photodynamic and gene therapy, cancer diagnosis, biosensors, and bioimaging. That is due to their unique ability to convert low energy of the near infrared (NIR) light into higher energy UV/visible photons. However, due to safety concerns, the *in vivo* applications of the UCNPs stay limited to laboratory use. Before any clinical application, the potential adverse effects of the nanoparticles (NPs) need to be evaluated in real biological environments. Typically, the preparation of a biocompatible surface architecture is required to achieve a surface composition suitable for the biomedical purposes. Currently, data on the cytotoxicity of UCNPs *in vivo* are scarce, but essential for their further utilization.

The aim of this work was to evaluate the toxicity (in *in vitro* and *in vivo* experiments) of NaYF4:Yb,Er,Pr-based neat and bisphosphonate-modified poly(isobutylene-alt-maleic acid)-graft-poly(N,N-dimethylacrylamide)-coated UCNPs (UCNP@PIMAPDMA). The neat UCNPs were found to be cytotoxic towards human embryonic kidney HEK293 cells as confirmed by the MTT assay, while the PIMAPDMA-coated particles proved to be biocompatible. To determine a systemic toxicity *in vivo*, male BALB/c mice aged 6-8 weeks were intravenously injected with UCNP@PIMAPDMA particles at a dose of 5 mg/kg, while the control group received the phosphate buffered solution (PBS). Mice were weighed twice a week for two weeks. There were no statistically significant changes in the body mass of the treated animals.

Two weeks after administration of the NPs, blood samples were collected from the orbital sinus of anesthetized mice. The samples were analyzed using a DYMIND DF-51 automated hematology analyzer (5-Part; Dhaka, Bangladesh). No significant adverse effects were observed in the blood cell profile. In addition, blood serum was investigated for enzymatic and metabolic markers of hepatotoxicity (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) and nephrotoxicity (urea and creatinine). All parameters were within normal limits except for a slight but statistically significant decrease in urea level which did not correlate with malnutrition or renal failure. It was also confirmed that extracellular serum proteins present in the blood were adsorbed on the surface of UCNP@PIMAPDMA particles, forming a protein corona, further enhancing the biosafety of the NPs.

Good biocompatibility of the newly developed UCNP@PIMAPDMA makes them promising candidates for use in tissue bioimaging and drug delivery, as various bioactive substances, including drugs, can be immobilized due to the available amino and carboxyl functional groups.

All animal studies were performed in accordance with the guidelines of the Bioethics Committee of the Institute of Cell Biology, NAS of Ukraine, Protocol No. 1 dated by July 1, 2023.

SAFETY AND SIDE EFFECTS OF MUSHROOM PREPARATIONS

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A large number of mushroom bioactive components called secondary metabolites, possess many therapeutic properties such as antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, cholesterol-lowering, antiviral, antibacterial, anti-parasitic, antifungal, detoxicative, hepatoprotective, anti-diabetic, anti-obesity, neuroprotective, neuroregenerative [1,2]. The ever-growing mushroom products market includes dietary food (with dietary supplements) and a new class of drugs called "Mushroom Pharmaceuticals". About 80% of mushroom production is obtained from fruiting bodies, both wild and artificially grown. In both cases, the resulting products differ significantly in composition and properties. Growing fruiting bodies in artificial conditions is a lengthy process, requiring from one to several months, and is often energy-intensive. While growing pure cultures of mushrooms in submerged conditions in a liquid nutrient medium allows to reduce its duration to several days, significantly reduce energy costs, regulate the metabolism of mushrooms to obtain a high yield of biomass and a large amount of specific substances of constant composition [3].

First of all, researchers note the beneficial effect of mushroom preparations on improving the quality of life of patients and compensating for the side effects of traditional therapy, primarily radiotherapy and chemotherapy for oncological diseases [2]. Most researchers report the safety of using mushroom preparations at a dose of 2000 mg/kg. Animal studies have shown the safety of long-term use of mushroom preparations, even at high doses of 2625 mg/kg [4]. Minor side effects are recorded: gastrointestinal disorders, diarrhea, allergy, a decrease in platelet cell count and skin reactions [2,4,5,6], difficult of breathing [4]. However, the lack of experimental data does not allow us to obtain an objective picture. Oral administration of suspensions and mycelium extracts of various fungi to experimental animals resulted in both a reduction in tumor size during the period of active growth and, in some cases, accelerated the death of the experimental animals. Long-term administration of a preparation from some fungi resulted in tumor growth. The reason for this may be the effect that a preparation from the same fungus can have an opposite effect at different stages of cancer [7].

There is an urgent need to investigate the safety and possible interactions of medicinal mushrooms. Further studies should be aimed at determining the qualitative and quantitative composition of fungi mycelium and its aqueous extract, isolating biologically active compounds with therapeutic activity and clarifying the exact mechanisms of this activity. The correct dosage and possible drug interactions also need to be further clarified in future clinical studies. More research is also needed to determine the effectiveness of short-term and long-term therapy.

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Session 4: Medical problems of cell biology/ Медичні проблеми клітинної біології

ЕФЕКТИВНІСТЬ АНТИБАКТЕРІАЛЬНОЇ ТЕРАПІЇ НА ОСНОВІ СИНЕРГІЗМУ В СЕПТИЧНИХ ПАЦІЄНТІВ В УМОВАХ ВІДДІЛЕННЯ ІНТЕНСИВНОЇ ТЕРАПІЇ

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На разі актуальною є проблема стійкості до антибіотиків (АБ) і поява штамів бактерій із множинною стійкістю, які поширені в лікарнях. Метою було дослідити ефективність використання синергічної дії АБ у рандомізованої вибірки пацієнтів із сепсисом, обумовленим мультирезистентною флорою у відділенні інтенсивної терапії НДСЛ «Охматдит». До вибірки увійшло 5 пацієнтів віком від 1 місяця до 4 років, пацієнти хірургічного та соматичного профілю, у критичному стані з підтвердженим септичним процесом, спричиненим мультирезистентною флорою, – перед призначенням антибактеріальної терапії на основі синергічної дії та на її фоні.

Пацієнт 1. За результатами бактеріологічного дослідження мокроти – K. pneumonia MBL. Лейкоцити (Л) $21.8 \cdot 10^9$/л, С-реактивний білок (СРБ) 98.7 мг/л (норма < 6 мг/л), прокальцитонін (ПКТ) 0.106 нг/мл (норма 0.020–0.046 нг/мл). Призначено колістин. Враховуючи важкий стан пацієнта та можливе погіршення стану, для прийняття рішення про посилення антибактеріальної терапії – методом перехресного тестування виявлена синергічна дія АБ: тігециклін+колістин, фосфоміцин+колістин, іміпенем+фосфоміцин, азтреонам+цефтазидим/авібактам. На монотерапії колістином відмічається позитивна динаміка. Л $13.3 \cdot 10^9$/л, СРБ 6.4 нг/мл. *Пацієнт 2.* За результатами бактеріологічного дослідження мокроти – K. pneumonia MBL. Л $8.45 \cdot 10^9$/л, СРБ 21 мг/л, ПКТ 0.148 нг/мл. Призначено меронем+ципрофлоксацин. Методом перехресного тестування виявлена синергічна дія АБ: іміпенем+колістин, іміпенем+тігециклін, тігециклін+колістин, фосфоміцин+колістин. Методом співвідношень виявлена синергічна дія: азтреонам+цефтазидим/авібактам. Враховуючи стрімке погіршення стану призначено: азтреонам+цефтазидим/авібактам. Л $13.3 \cdot 10^9$/л, СРБ 42.11 мг/л, ПКТ 0.17 нг/мл. Стан пацієнта погіршився, використано іншу схему антибактеріальної терапії. *Пацієнт 3.* За результатами бактеріологічного дослідження матеріалу з рани – A. baumanii, P. aureginosa. Л $8.5 \cdot 10^9$/л, СРБ 251.8 мг/л, ПКТ 89.19 нг/мл, інтерлейкін 6 (ІЛ6) 848 пг/мл (норма < 7.0). Кількісним методом виявлена синергічна дія АБ: меропенем+тігециклін. Призначено: меропенем+тігециклін. Л $16.1 \cdot 10^9$/л, СРБ 138.7 мг/л, ПКТ 0.43 нг/мл, ІЛ6 69.08 пг/мл. *Пацієнт 4.* За результатами бактеріологічного дослідження мокроти – K. pneumonia. Л $12.2 \cdot 10^9$/л, СРБ 182.2 мг/л, ПКТ 15.3 нг/мл. Методом перехресного тестування виявлена синергічна дія АБ: колістин+тігециклін. Призначено: колістин+тігециклін. Л $8.5 \cdot 10^9$/л, СРБ 7.58 мг/л, ПКТ 0.5 нг/мл. *Пацієнт 5.* За результатами бактеріологічного дослідження бронхо-альвеолярного лаважу – K. pneumonia. Л $13.2 \cdot 10^9$/л, СРБ 13.2 мг/л. Методом перехресного тестування виявлена синергічна дія АБ: колістин+тігециклін. Призначено: колістин+тігециклін. Л $10.4 \cdot 10^9$/л, СРБ негативний. Превалюючою мультирезистентною флорою у відділенні інтенсивної терапії є штами K. pneumonia. Згідно принципів призначення антибактеріальної терапії: спочатку емпірична терапія; перегляд через 24–48 год. відповідно до результатів бак. посівів і стану пацієнта; в разі погіршення стану пацієнта за умови інфікування мультирезистентною флорою необхідно мати резервний варіант антибактеріальної терапії, згідно синергічної дії. Методика синергічної дії є перспективною та може запобігати підвищенню летальності. Отримані результати безумовно потребують продовження дослідження ефективності антибактеріальної терапії на основі синергізму.

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ОСОБЛИВОСТІ МІКРОКРИСТАЛІЙНОЇ КАРТИНИ НА ФОНІ ПАТОФІЗІОЛОГІЧНИХ ПРОЦЕСІВ ВИКЛИКАНИХ ВІЛ-ІНФЕКЦІЄЮ ТА ГЕПАТИТОМ С

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Біологічна рідина ротової порожнини має високий потенціал для нагляду за загальним станом організму та захворювань, містить різноманітні сигнальні біомаркери, є неінвазивною, зручною, швидкою та легкою для діагностики та психоемоційної складової досліджуваної особи [4].

Мікрокристалізація слини – неінвазивне дослідження, в основі якого лежить дегідратація краплі слини [2]. Зневоднення краплі білково-сольових розчинів, у тому числі слини, під час сушіння призводить до поступового збільшення концентрації солей, що стимулює процес фазового поділу білка і формування структур у білковому золі [3].

Слина – це біологічна рідина, яка містить широкий спектр мікробіоти, дезоксирибонуклеїнової кислоти (ДНК), рибонуклеїнової кислоти (РНК), білків, метаболітів та іншої біологічної інформації, що робить її багатообіцяючим сурогатним індикатором оральних і системних фізіологічних і патофізіологічних станів [5].

Метою дослідження є вивчення особливостей мікрокристалізації слини у людей, що мають ВІЛ-інфекцію та гепатит С.

Наше дослідження носило векторний характер. В дослідженні взяла участь група волонтерів, загальною кількістю 20 осіб - контрольна/перша група (практично здорові), 10 осіб - друга група (волонтери, які мають гепатит С), 10 осіб - третя група волонтери, які мають ВІЛ-інфекцію та гепатит С. Всі волонтери були чоловічої статі.

Мікрокристалізацію слини досліджували по-методиці Леуса П. А. [1; 6].

Робота виконувалась у відповідності до біоетичних норм.

На основі отриманих результатів можна стверджувати, що для групи волонтерів, які страждають на ВІЛ-інфекцію та гепатит С характерно наявність мікрокристалів V типу, сама мікрокристалізаційна картина оптично характеризується: зменшенням центральної зони (для якої характерно наявність кристалів зірчато-неправильної форми) за рахунок розширення крайової (в якій наявна велика кількість органічних складових та невеликих кристалів округлої або неправильної форми).

Мікрокристалічна картина для другої групи волонтерів (які страждають на гепатит С) характеризується мікрокристалізацією IV типу. Мікрокристалізаційна архітектоніка в данному випадку характеризується наявністю мікрокристалів гілчатоподібної форми по периферії спотвореної форми або наявністю додаткових мікрокристалізаційних ниток між мікрокристалами першого порядку.

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VITAMIN D LEVELS AND HEMATOLOGICAL INDICES IN WOMEN IN THE FIRST TRIMESTER OF PREGNANCY ACROSS DIFFERENT AGE GROUPS

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Vitamin D plays a crucial role in maintaining health, and its deficiency is linked to various diseases, including musculoskeletal, metabolic, cardiovascular, malignant, autoimmune, and infectious disorders. The International Society of Endocrinology emphasizes the global prevalence of Vitamin D deficiency, especially among pregnant women. Recent studies indicate a possible connection between Vitamin D deficiency and decreased hematological indices in pregnant women, potentially leading to the development of anemia [1-5].

The aim of our study is to explore the potential impact of Vitamin D deficiency on hematological indices in pregnant women of different age groups during the first trimester, registered at the specialized women's consultation of the municipal non-commercial enterprise "Perinatal Center of Kyiv." Data from 142 women in the first trimester of pregnancy were analyzed, divided by levels of 25(OH)D (<10 ng/ml, 10-20 ng/ml, 20-30 ng/ml, 30-50 ng/ml) and age (18-30 years and 31-41 years). Measurements included leukocytes, hemoglobin, red blood cells, hematocrit, mean corpuscular volume (MCV), and platelets using the Abacus 3CT hematological analyzer (Hungary), and concentrations of 25(OH)D were assessed using an immunoassay method (reagents from Monobind, USA, and reader from Sinowa ER 500).

The study results demonstrate a correlation between Vitamin D levels and hematological indices in women during the first trimester of pregnancy across different age groups. It was found that low levels of Vitamin D (<10 ng/ml) are associated with lower levels of hemoglobin, red blood cells, hematocrit, MCV, and platelet counts compared to normal Vitamin D levels (>30 ng/ml). However, further research is needed to confirm whether Vitamin D deficiency indicates a risk of developing anemia in later stages of pregnancy. No significant fluctuations in hematological parameters were observed at Vitamin D concentrations of 10 to 30 ng/ml. Additionally, a decrease in leukocyte counts was noted with increasing Vitamin D levels, suggesting its potential anti-inflammatory effect. Overall, the data underscore the importance of maintaining adequate Vitamin D levels, specifically above 30 ng/ml, among women in the first trimester of pregnancy, to improve hematological parameters.

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Session 5

Biotechnology and metabolic engineering

11-13 September 2024
Lviv, Ukraine

STRUCTURE-FUNCTION INTERRELATIONS IN DRUG DESIGN AND ACTIVITY: NOVEL HYBRID BENZOISOTHIAZOLE-1,2,3-TRIAZOLE-4-CARBOXAMIDES FOR TREATMENT OF HUMAN BREAST CARCINOMA

Rostyslav Stoika **Lecture 1**

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Molecular hybridization is an efficient approach in drug design based on a combination of two different bioactive molecules (or pharmacophoric parts of them) to produce a new hybrid compound with an improved affinity and efficacy [1]. Here this strategy was implemented to combine biologically active scaffolds for design and synthesis of benzoisothiazole-1,2,3-triazole-4-carboxamide conjugates via piperazine linker [2]. Two lead compounds - 5h and 5j – were identified as potent anticancer agents, with para-chlorine- or fluorine- substitution in the phenyl ring, and isopropyl or cyclopropyl substituents in position 5 of 1,2,3-triazole cycle. These compounds demonstrated toxicity (MTT assay) towards human breast adenocarcinoma cells of the MCF-7 line in sub-micromolar concentrations with IC50 = 0.76 ± 0.04 μM and 0.90 ± 0.02 μM, respectively, comparing to doxorubicin (positive control) effect at 0.46 ± 0.06, and showing no toxicity to pseudonormal and normal cells (IC50 > 100 μM). Colony formation and proliferation of carcinoma MCF-7 cells were effectively inhibited by the derivatives 5h and 5j [2].

These derivatives were shown to cause DNA fragmentation of the MCF-7 cells. We suggest that the DNA-damaging action of the compounds 5h and 5j may be related to high capability of intercalating into DNA molecule that is comparable to such ability of a known anticancer agent – the doxorubicin. High affinity of the compounds 5h and 5j for the DNA was confirmed using computational molecular docking *in silico* [2].

Recently, we combined molecular hybridization with the bioisosteric replacement of 1*H*-1,2,3-triazole with 1*H*-tetrazole ring that enhanced significantly the anti-leukemic activity of the generated compounds [3,4]. Thus, molecular hybridization and bioisosteric replacement are effective approaches for discovery and synthesis of novel anticancer agents of the addressed action.

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Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

CANDIDA FAMATA CELL FACTORY FOR PRODUCTION OF VITAMIN B2

Dariya Fedorovych **Lecture 2**

Dariya Fedorovych [1], Andriy Tsyrulnyk[1], Ljubov Dzanajeva[1], Wen Liu[1], Justyna Ruchala[2], Dominik Wojdyla[2], Kostyantyn Dmytruk[1], Andriy Sibirny[1,2]

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Riboflavin is a precursor of flavin mononucleotide and flavin adenine dinucleotide, which play a key role as cofactors in energy metabolism and are required in numerous oxidation and reduction reactions in all aerobic forms of life. Riboflavin is synthesized by many bacteria and by all fungi and plants, but humans and other animals need to acquire it with their diet or through supplements to maintain optimal health. Riboflavin deficiency raises concerns in both developing and developed countries. The annual market for this vitamin is estimated at USD 451.57 million in 2024. Currently chemical riboflavin production has been replaced by microbial synthesis because the last one is single step fermentation, diminishes production costs, reduces waste, energy requirements also is favorable relative to the chemical synthesis from the ecologic point of view.

Riboflavin is produced biotechnologically using engineered strains of the bacterium *Bacillus subtilis* and the filamentous fungus *Ashbya gossypii*. Some mutants of the flavinogenic yeast *Candida famata* also belong to the most effective riboflavin overproducers but their great flavinogenic potential remains unrealized. To enhance riboflavin production, strains were improved through random selection and metabolic engineering. A stable riboflavin overproducing strains has been constructed by co-overexpression of the gene *SEF1* encoding positive regulator of riboflavin biosynthesis and *RIB1*, *RIB7* and *RIB6* structural genes in riboflavin producing *C. famata* AF-4 strain obtained by classical selection. It was shown that enhancement of riboflavin production by *C. famata* can be achieved by increasing the riboflavin excretion and improving the supply of GTP and ribulose-5-phosphate. All these studies were carried out using glucose as a carbon source. The price of riboflavin significantly depends on the cost of the used carbon substrate. *C. famata* can efficiently utilize not only glucose but also other sugars. We showed that the overexpression of *SEF1* gene under control of promoter of the *LAC4* gene encoding β-galactosidase in *C. famata,* led to increase in riboflavin production on media with lactose and cheese whey. Our data indicate that the waste whey from milk industry, can be a promising substrate for riboflavin production by *C. famata*

Engineered strains of the yeast *C. famata* have successfully overproduced riboflavin on lignocellulosic hydrolysate. Riboflavin accumulation increased in the recombinant strains *C. famata* overexpressing own *XYL1* and *XYL2* genes, which code for xylose reductase and xylitol dehydrogenase, respectively. The possibility of using other industrial waste and semi-products of food industry for production vitamin B_2, is discussed.

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Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

DEVELOPMENT OF *OGATAEA POLYMORPHA* STRAINS CAPABLE OF HIGH-TEMPERATURE CELLOBIOSE ALCOHOL FERMENTATION

Roksolana Vasylyshyn **Lecture 3**

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Plant cell walls are a promising source of sugars for biofuel production. The main complex sugars included in their composition are cellulose (a polymer of glucose) and hemicellulose (a heterogeneous polymer of pentoses, hexoses, and sugar acids). Successful conversion of cellulosic biomass into biofuel requires organisms that can efficiently utilize xylose, cellodextrins, and glucose. *Ogataea polymorpha* is a thermotolerant yeast that naturally metabolizes xylose, making it a good candidate for biofuel production. However, wild-type *O. polymorpha* NCYC495 cannot naturally ferment cellodextrins like cellobiose, which is a disadvantage for producing cellulosic biofuels.

Cellobiose-fermenting strains, derivatives of an improved ethanol producer from xylose, *O. polymorpha* BEP/cat8Δ, were constructed by the introduction of heterologous genes *gh1-1* of β-glucosidase, *CDT-1m* and *CDT-2m* of cellodextrin transporters from *Neurospora crassa*, and the *CBP* gene coding for cellobiose phosphorylase from *Saccharophagus degradans*. Through metabolic engineering, laboratory evolution, and mutagenesis of the obtained recombinant strains, mutants were developed, showing improved parameters for high-temperature alcoholic fermentation of cellobiose and simultaneous consumption of all quantitatively significant sugars in lignocellulose hydrolysates (Vasylyshyn et al. 2024).

Vasylyshyn R, Dmytruk O, Sybirnyy A, Ruchała J, Engineering of *Ogataea polymorpha* strains with ability for high-temperature alcoholic fermentation of cellobiose, *FEMS Yeast Research*, 2024, 24.

NON-TRADITIONAL YEAST IN THE PURSUIT OF SUSTAINABLE DEVELOPMENT - MICROBIAL PRODUCTION OF RIBOFLAVIN FROM RENEWABLE RESOURCES

Justyna Ruchala **Lecture 4**

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Riboflavin is a valuable commodity with an annual market value of approximately 9 billion US dollars. It is predominantly used in agriculture as a feed additive (80%) and in the food industry as a beverage colorant (10%), as well as in the medicine. Currently, riboflavin is produced through microbial fermentation from glucose by *Bacillus subtilis* or from fats by *Ashbya gossypii*. However, it can also be synthesized using the flavinogenic yeast *Candida famata* from various carbon sources, offering several advantages. By applying targeted mutagenesis techniques, such as the expression of the *XYL1*, *XYL2*, *SEF1*, *RIB6*, and *GND1* genes, recombinant strains have been developed that actively produce riboflavin on substrates like glucose, cheese whey, or lignocellulosic hydrolysates. Additionally, overexpressing the *FMN1* gene, which codes for riboflavin kinase, has led to the creation of FMN overproducing strains. Furthermore, the cloning and overexpression of the *Streptomyces davaonensis rosB*, *rosC*, and *rosA* genes in *Komagataella phaffii* and *C. famata* yeast enabled the construction of the first yeast strains capable of accumulating flavin antibiotics like roseoflavin and 8-aminoriboflavin. Ongoing research is needed to enhance the efficiency of producing riboflavin, FMN, and flavin antibiotics, and strategies to achieve this will be discussed.

METABOLOMIC ANALYSIS OF THE *CLOSTRIDIUM* SP. UCM B-7570 DELETION MUTANT MODEL

Olena Tigunova **Lecture 5**

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Microbiological conversion of renewable resources of the biosphere in order to obtain useful products, in particular biofuel, is currently one of the urgent problems of biotechnology. To create a cost-effective butyl fermentation, high-performance strains with a change in the genetic structure of the organism are needed to increase the accumulation of the product. To increase the accumulation of butanol, it is necessary to change the way of formation of propanediol.

The aim of the work was to create a model of a deletion mutant of *Clostridium* sp. UCM B-7570 using whole-genome sequencing of the strain followed by *in silico* metabolomic analysis. In this study, the large subunit of glycerol dehydratase encoded by the dhaB gene was chosen as the target for gene deletion. Primer-BLAST, NEBcutter V2.0 programs were used to establish the necessary primers, Addgene, GeneBank databases were used to select the plasmid. The glycerol dehydrogenase deletion vector pNickclos1.0-cas9n-dhaB, which contained the cas9 nickase, the dhaB guide RNA, and the H1 and H2 editing matrix, was generated using BLAST and SnapGene programs. Linear programming algorithms were used to analyze metabolic flows. To build the model, a model of 20 metabolites for C. pasteurianum DSM 525 and 28 main reactions was taken as a basis [1]. Metabolic flux analysis was performed using the MATLAB toolbox CellNetAnalyzer. Metabolic flux analysis revealed an important role of a newly identified electron bifurcation pathway for the conversion of crotonyl-CoA to butyryl-CoA in the regulation of redox balance. Compared with the parent strain, the flux of dhaB mutant electron bifurcation pathway increased (from crotonyl-CoA to butyryl-CoA and butanol), indicating a novel 1,3-propanediol-independent fermentation scheme in *Clostridium pasteurianum*.

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RECOMBINANT CREATININE DEIMINASE FROM *CORYNEBACTERIUM GLUTAMICUM*: OVEREXPRESSION, ISOLATION, CHARACTERIZATION, AND BIOANALYTICAL APPLICATION

Andriy Zakalskiy **Lecture 6**

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Creatinine, the waste product of creatine, is produced in human muscles, transported *via* bloodstream into kidney and then excreted with urine. Serum creatinine level is an important indicator of the renal function and valuable biomarker to control the hemodialysis procedure. Currently available chemical methods of creatinine detection possess several limitations due to insufficient sensitivity and a low specificity, while enzymatic approaches require exploiting several enzymes and co-factors and are expensive (Zakalskiy *et al.*, 2019). Usage of microbial creatinine deiminase (EC 3.5.4.21, CDI), catalyzing hydrolysis of creatinine to N-methylhydantoin and ammonia, could overcome these obstacles.

In current report, we describe the construction of microbial recombinant strains overproducing $(His)_6$-tagged CDI of *Corynebacterium glutamicum*, isolation and purification of the enzyme, and its physicochemical and kinetic characterization. The *codA* gene of *C. glutamicum* PCM 1945 coding for CDI has been amplified and cloned in both *Escherichia coli* and *C. glutamicum* parental cells. The recombinant strain of *E. coli* that overproduces the inactive CDI as inclusion bodies has been constructed while the recombinant strain of *C. glutamicum* is capable of constitutive oversynthesis of soluble $(His)_6$-tagged enzyme. After solubilization of inclusion bodies from *E. coli* in the presence of 0.3% N-lauroylsarcosine, the enzyme was renaturated and purified by a single-step procedure using metal-affinity chromatography. The yield of the $(His)_6$-tagged CDI is ~30 mg from 1 l culture. The soluble $(His)_6$-tagged enzyme was purified from *C. glutamicum* cells to a homogeneous state using chromatography on Ni-NTA-agarose. The yield of the CDI is ~20 mg from 1 l culture. The enzyme preparations, purified from *E. coli* and *C. glutamicum*, possess an activity of 20 and 150 U/mg, respectively.

The most prominent advantages of the obtained CDI preparations are a high yield of the enzyme, simple isolation and a possibility to apply the one-step procedure for purification of the enzyme, and its high stability under condition designed. These findings can be used to develop a cost efficient large-scale procedure for production of a highly specific tagged CDI necessary for construction of enzymatic kits and biosensors for assay of creatinine (Stasyuk *et al.*, 2023).

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THREE-DIMENSIONAL CONSTRUCTS BASED ON MSCs WITH DIFFERENT CELL ORGANIZATION: BIOENGINEERING AND SAFE STORAGE

Oleksandr Petrenko **Lecture 7**

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Mesenchymal stromal/stem cells (MSCs) have unique properties: self-renewal, ability to differentiate, secretory activity. Standard cultivation do not reproduce the natural microenvironment of MSCs, which leads to a loss of their potential. The generation of three-dimensional (3D) MSC-based constructs with different microenvironments is essential for the development of relevant model systems and effective therapeutics. In addition, the peculiarities of the metabolism of MSCs in the various bioengineered constructs can affect the cell sensitivity to external adverse factors, including damaging during storage at ambient temperatures.

Here we aimed to investigate the properties of MSCs in the multicellular spheroids, alginate microspheres (AMS) and macroporous scaffolds from blood plasma before and after storage at ambient temperature.

Methods: Human adipose tissue MSCs (obtained with the informed consent of adult donors) were used. Spheroids were formed by the "hanging drop" method, AMS were obtained by electrospraying, scaffolds were obtained by cryogelation of modified human plasma and seeded with cells. The constructs were cultured in alpha-MEM with 10% fetal bovine serum, 50 μg/ml penicillin and 50 μg/ml streptomycin (37 °C, 5% CO2, 95% humidity) for 3 days, after which they were stored in culture medium in sealed cryotubes (Nunc) at 22°C. Viability/apoptosis (6-CFDA/ annexin V-Cy3), metabolic activity (resazurin), capacity for induced differentiation were studied.

Results: MSCs had a spindle-like morphology in scaffolds, a round shape in spheroids and AMS, and demonstrated high viability and ability for multilineage differentiation in all the mentioned 3D constructs. Storage of MSCs in suspension led to a significant viability decrease on the 3d day. The viability of MSCs stored in 3D constructs remained high for 7 days of storage. Metabolic activity in AMS and spheroids was reduced compared to suspension and scaffolds before storage, but was better maintained during subsequent storage. Incorporation in AMS ensured the most considerable resistance of cells to apoptosis development during the storage.

Conclusions: The possibility of effective storage of MSCs for 7 days at 22°C in the composition of spheroids, alginate microspheres and macroporous scaffolds from blood plasma is shown. The obtained results demonstrate the importance of MSC cultivation in three-dimensional structures for the different cell organization and development of resistance to short-term storage at ambient temperature.

The research was carried out with the support of the National Research Foundation of Ukraine (project No. 2021.01/0276).

SACCHAROMYCES CEREVISIAE BAKER'S YEAST STRAIN FOR THE PRODUCTION OF BREAD WITH AN INCREASED CONTENT OF VITAMIN B_2

Lubov Fayura **Poster 28**

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Riboflavin is an essential vitamin in the human diet and animal feed. The human body cannot produce riboflavin, and its deficiency can lead to various health issues, including migraines, cardiac and skin disorders, and alterations in sugar metabolism. The necessary dose of riboflavin can be obtained through a balanced diet. In many countries, certain vitamins and minerals are added to flour, including the riboflavin. Yeast – overproducers of the riboflavin are able to provide stable enrichment of bread with this vitamin. Genetic engineering methods make it possible to construct baker's yeast strains capable of oversynthesis of riboflavin. However, in a number of countries there are restrictions on the use of genetically modified yeast for the production of bread.

In order to select the producer of the riboflavin, the method of adaptive laboratory evolution was applied. This method is based on the long-term cultivation of yeast in a media supplemented with increasing concentrations (from 30 to 250 mg/L) of a selective agent - a natural riboflavin analogue - roseoflavin (7-methyl-8-dimethylamine-(1'-D-ribityl)-isoalloxazine). 12 rounds of transfer of the yeast strain *Saccharomyces cerevisiae* IMB Y-5058 on media with increased concentrations of roseoflavin (the concentration step was 20 mg/L)

After the analysis of about a hundred colonies, the F57 strain was selected, which produces 3 times more (0.6 mg/l) riboflavin in the culture medium and accumulates 37% more intracellular flavins compared to the parent strain. The activity of GTP cyclohydrolase II which catalase the initial step of riboflavin biosynthesis, increased 2.7 times compared with the initial strain.

When cultivated on an industrial medium with the addition of molasses, the selected strain F57 does not differ from the original strain in terms of kinetic parameters of growth and lifting force (speed of rising dough balls). Test bread made on the basis of the F57 strain contained 1.4-1.6 times more riboflavin than when using the industrial strain.

The presented results are thus an important step in the development of fermented foods, for which the traditional starter can be replaced by a riboflavin-producing equivalent, resulting in the vitamin being produced *in situ*, thereby contributing to the required intake of the vitamin. The selected strain can be also used as a food additive for farm and domestic animals.

Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

DISSIMILATORY SULFATE, NITRATE, NITRITE AND Cr(VI) REDUCTION BY BACTERIA *DESULFOVIBRIO* SP.

Mariana Hembara **Poster 29**

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Sulfate reducing bacteria use sulfates and other electron acceptors (nitrates, nitrites, oxidized forms of heavy metals, in particular, hexavalent chromium) in the process of anaerobic respiration [1-2]. The intensity of anaerobic respiration of microorganisms in contaminated ecotopes is determined by the level of their adaptation to unfavourable conditions of environment [3]. The Cr(VI) is highly toxic to living organisms [4]. To persist in Cr(VI)- contaminated environments, microorganisms must have efficient systems to neutralize its negative effects (including biosorption and bioaccumulation, exopolymeric substance production, Cr(VI) efflux pumps, extracellular reduction of Cr(VI) to Cr(III), activation of enzymes involved in the detoxification of active oxygen forms, repair of DNA and protein damages) [3-5]. Selection of isolated from technogenically altered ecotopes resistant to pollutions strains of bacteria, capable to transformation of various pollutants, is especially actual task for the creation of biotechnologies for purification [1, 5].

The aim of the work was to determine the efficiency of SO_4^{2-}, NO_3^- or NO_2^-, and Cr(VI) reduction after their simultaneous addition into the medium at equal concentrations by strains of sulfate reducing bacteria *Desulfovibrio desulfuricans* IMV K-6, *Desulfovibrio* sp. Yav-6 and *Desulfovibrio* sp. Yav-8, isolated from Yavorivske Lake. The efficiency of sulfate, nitrate, and hexavalent chromium reduction by bacteria in the medium with 3.5 mM SO_4^{2-}, NO_3^-, and Cr(VI) decreased 3.3–3.4, 1.5, and 1.3 times, respectively, compared to its reduction in media only with SO_4^{2-}, NO_3^- or Cr(VI). In the medium with SO_4^{2-}, NO_3^-, and Cr(VI) bacteria reduced NO_3^- 2.2−2.3 times more than SO_4^{2-}, and 1.1−1.2 times more than Cr(VI). The efficiency of sulfate, nitrite, and hexavalent chromium reduction by bacteria in the medium with 3.5 mM SO_4^{2-}, NO_2^-, and Cr(VI) decreased 3.5–3.7, 1.5–1.6, and 1.4 times, respectively, compared to its reduction in media only with SO_4^{2-}, NO_2^-, or Cr(VI). In the medium with SO_4^{2-}, NO_2^-, and Cr(VI) bacteria reduced NO_2^- 2.3–2.4 times more than SO_4^{2-}, and 1.2 times more than Cr(VI). The processes of nitrate and nitrite reduction carried out by *Desulfovibrio* sp. appeared to be less sensitive to the negative influence of $K_2Cr_2O_7$ than the process of sulfate reduction. Due to the ability to carry out reductive transformation of chromium, sulfur and nitrogen compounds, investigated strains are perspective for application in technologies of complex bioremediation of the environment.

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THE MECHANISM OF REGULATION FOR THE RIBOFLAVIN BIOSYNTHESIS IN THE YEAST *CANDIDA FAMATA* BY THE GENE *SEF1*

Serhii Romanov **Poster 30**

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Riboflavin (RF, also known as vitamin B_2) is a yellow water-soluble compound, one of the most important vitamins for human and animals. It is a precursor for flavin coenzymes FAD and FMN, which participate in multiple redox reactions in living cells. For a long time, it has been produced in large-scale industry exclusively with the use of microorganisms, such as bacteria *Bacillus subtilis*, fungi *Ashbya sp.*, and yeast *Candida famata* [1].

Although the metabolic pathway for RF biosynthesis in different yeast species is well established, little is known about its regulation. Sef1, the transcriptional factor of the zinc cluster family $Zn(II)_2Cys_6$, encoded by the respective gene *SEF1*, plays a central role in the regulatory circuit of RF biosynthesis in *Candida famata*. It positively regulates RF biosynthesis and is induced under iron deficiency in the growth medium [2]. It is known that the deletion of the *SEF1* gene leads to the loss of the ability of yeast to overproduce riboflavin under conditions of iron deficiency [3]. Another interesting finding is that *SEF1* promoters from other flavinogenic yeast species, when fused to the native *SEF1* ORF of *C. famata*, are capable to restore riboflavin overproduction in *SEF1*-deletion mutants [4]. However, the mechanism by which Sef1 activates RF synthesis remains unclear. We hypothesized that Sef1 activates the transcription of RF synthesis structural genes by interacting with their corresponding promoters (*RIB*-promoters). We developed a Yeast-One-Hybrid system based on the yeast *Saccharomyces cerevisiae* to confirm this hypothesis. The yeast was transformed with two expression constructs: the first contained the reporter gene *LAC4* from *Kluyveromyces lactis*, encoding β-galactosidase, under the control of *RIB*-promoters; the second carried *SEF1* ORF under the galactose-inducible *GAL1* promoter. *S. cerevisiae* strains containing both plasmids were grown on media supplemented with galactose to induce Sef1, or with glucose as a control. Activity of β-galactosidase indicated the interaction of Sef1 with *RIB*-promoters.

The obtained results demonstrate that Sef1 interacts with and activates the promoter of the *RIB1* gene. Furthermore, a Sef1 binding sequence (TAAAAATCCGAACCCCGG) has been identified in the *RIB1* gene promoter, as evidenced by modified versions of this promoter. Substitution or deletion of this site leads to the loss of β-galactosidase activity. Sef1 also activates the promoters of *RIB3*, *RIB6*, and *RIB7* genes; however, the β-galactosidase activity observed under their activation is 50%, 73%, and 22% of that observed under *RIB1* promoter activation, respectively. Sef1 does not interact with *RIB2* and *RIB5* promoters.

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LACTIC ACID PRODUCTION BY ENGINEERED *OGATAEA POLYMORPHA* YEAST STRAINS WITH HETEROLOGOUS EXPRESSION OF LACTATE DEHYDROGENASE

Aksyniia Tsaruk **Poster 31**

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The increasing global demand for lactic acid necessitates more focused research on microbial fermentation processes. While yeast generally have limited natural capacity to produce lactic acid, metabolic engineering enables these microorganisms to become effective lactic acid producers through the heterologous expression of lactate dehydrogenase genes. *Ogataea polymorpha*, a thermotolerant yeast species capable of growing on renewable and cost-effective substrates, presents a promising opportunity for developing engineered yeast strains that can efficiently produce lactic acid.

The lactate dehydrogenase (LDH) gene from *Rhizopus oryzae*, *Bos taurus*, and *Plasmodium falciparum* were introduced into the wild type strain of *O. polymorpha* under the control of the strong constitutive promotor of glyceraldehyde-3-phosphate dehydrogenase gene. LDH gene codes an enzyme that catalyses the conversion of pyruvate into lactic acid. The initial selection of transformant yeast strains was performed on a YPD medium with the addition of colourful pH indicator bromophenol blue. The best performing transformant strains each with heterologous expression of the LDH gene from a different source were further studied for their ability to produce lactic acid and ethanol during high temperature flask fermentation on a minimal YNB medium. For these experiments various fermentation conditions were tested including different sources of carbon (glucose, xylose, and methanol), agitation rates, and pH buffering with calcium carbonate as a neutralizing agent. Each of the transformant strains showed distinct kinetics of lactic acid and ethanol productions depending on the fermentation conditions.

Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

DEVELOPEMENT OF OINTMENTS BASIS BY USE OF CHITOSAN-AMPICILLIN HYDROGEL COMPLEX AND STUDIES OF ITS ANTIMICROBIAL ACTIVITY

Maxim Lootsik **Poster 32**

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In our research on wound healing it was developed the method of synthesis of a novel ointment basis by use of chitosan hydrogel conjugated with ampicillin, attached via ionic bonds (further signet as chitosan-ampicillin hydrogel complex). The process consists of three stages: 1). Obtaining of chitosan hydrogel by treatment of commercial chitosan solutions in diluted acetic, acid, neutralization of solution with water ammonia to pH 8,5, the sediment was collected by centrifugation and washed with a distilled water. The sediment was dispersed using Potter-Elvehjem homogenizer and finally was produced as a compact mass by centrifugation at 3500-4000 g 30 min. 2) Obtaining of chitosan-ampicillin hydrogel complex by mixing the sediment of chitosan with acid form of ampicillin. The last was prepared by treatment of ampicillin medicinal powder by small volume of 2,5 M HCl. The ratio of chitosan to ampicillin was 8:1 (calculated on dry weight), the viscose mixture was left for 12 hrs at room temperature. Thereafter it was treated with small doses of 0,5 M acetic acid and stepwise adjusted pH to 5,6 during of 7-10 days (due to a slow diffusion process in a viscose medium). The mixture becomed semitransparent at pH 5,5-5,7 due hydratation of chitosan particles. 3). Formation of ointment basis composite by mixing chitosan-ampicillin hydrogel with 40% (w/w) solution of Povidon in 1,2-propylen glycol. The obtained product is of ointment-like consistency and possess amphiphylic properties. It is readily mixed with water soluble substances and also accepts distinct quantities of hydrophobic components, like plant oils. This permits to introduce into ointments water insoluble substances after its dissolution in oils.

The antimicrobial activity of ointment bases with pure chitosan hydrogel and with chitosan-ampicillin hydrogel complex was investigated by use of MTT test [1] on 4 strains of bacteria: Staph. aureus (ATCC 25923), E. coli (dH5α), Ps. aeruginosa (ATCC 9027), B. subtilis (ATCC 31324). It was found that both types of ointment bases showed a distinct inhibition of metaboilic activity of St. aureus ($IC_{50\%}$ 1,6±0,1 mg/ml, equal in both samples) and E. coli ($IC_{50\%}$ 1,8±0,1 mg/ml for pure chitosan and 2,0±0,1mg/ml for chitosan-ampicillin complex; diff. not significant). The effect towards Ps. aeruginosa was weak (25% inhibition at agent concentration 10 mg/ml) and was provided by chitosan. Towards Bac. subtilis effect was also weak (30% inhibion at agent concentration 10 mg/ml) and was provided by ampicillin, as a pure chitosan showed no inhibitory effect.

The results showed that chitosan-ampicillin hydrogel complex composition proposed as ointment basis for production of wound healing ointments exhibits moderate yet distinct antimicrobial activity which is desirable in wounds management and proposed formulation of ointment basis can be used as a platform in obtaining ointments with wound healing properties.

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ENGINEERING OF CANDIDA FAMATA YEAST FOR RIBOFLAVIN OVERPRODUCTION ON LIGNOCELLULOSE HYDROLYSATE

Ljubov Dzanaeva **Poster 33**

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Riboflavin (vitamin B_2) is the metabolic precursor of flavin nucleotides, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) involved as coenzymes in numerous enzymatic reactions. Vitamin B_2 has many areas of application: food industry, agriculture and medical field. It is expected that the volume of the riboflavin market in 2029 will increase by 27.5% compared to 2024 and will reach 623.06 million USD.

During the last decade, riboflavin is produced using recombinant strains of bacterium *Bacillus subtilis* and the filamentous fungus *Ashbya (Eremothecium) gossypii*. The construction of the competitive riboflavin yeast producers has great scientific and practical value. Before, the *Candida famata* strains with high level of riboflavin productivity were constructed, due to using a combination of classical selection methods and metabolic engineering. Moreover, it is known that *C. famata* characterized by the ability to grow on unconventional substrates, in particular on xylose, which is the second (after glucose) component of lignocellulosic hydrolysates. Plant biomass has the largest share among other renewable energy sources and is a key factor in achieving the European renewable energy target by 2030.

In the BRPI *C. famata* strain, riboflavin production was the highest in the medium with glucose (812.5 mg L^{-1}) or galactose (757.5 mg L^{-1}), whereas the lowest - when xylose was used (337.5 mg L^{-1}). To increase riboflavin production from xylose and lignocellulose hydrolysate, the genes *XYL1* and *XYL2* coding for xylose reductase and xylitol dehydrogenase were overexpressed. The strain BRPI/XYL1 produced 450 mg L^{-1} of riboflavin in xylose medium, showing 1.36-fold increase of vitamin B2 production as compared to that of BRPI. Strain BRPI/XYL1/XYL2 demonstrated 1.44-fold increase in riboflavin production that amounted to 474 mg L^{-1} in xylose medium when compared to the BRPI. Riboflavin production by *C. famata* yeast strain BRPI/XYL1 on YNB medium with the addition of of 30% bagasse hydrolysate as the carbon source, reached 1.5 g L^{-1}.

Lignocellulose hydrolysates contain inhibitors, that can potentially inhibit *C. famata*, such as furfural, phenols and carboxylic acids. It is expected that increased resistance to inhibitors will correlate with increased production of the desired product - riboflavin.

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CONSTRUCTION OF RIBOFLAVIN OVERPRODUCERS BY INTRODUCTION OF GENES *RFE1*, *RIB6*, AND *GND1* INTO THE YEAST *CANDIDA FAMATA*

Wen Liu **Poster 34**

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Riboflavin (vitamin B_2) is an essential compound for the nutrition of living organisms, serving as a precursor of coenzymes flavin mononucleotide and flavin adenine dinucleotide, which are involved in numerous enzymatic reactions of oxidative metabolism. The yeast *Candida famata* is a natural riboflavin-producing species, which are able to oversynthesis riboflavin under conditions of iron starvation.

Previously, studies demonstrated that increased expression of the *RFE1* gene, which encodes riboflavin excretase, in the riboflavin overproducer strain *C. famata* BRP (Best Riboflavin Producer), resulted in 1.4-1.8-fold enhanced riboflavin production [1-3]. Overexpression of the *GND1* gene, which encodes 6-phosphogluconate dehydrogenase, led to 1.3-fold increased riboflavin production in strain *C. famata* BRP [4]. Recently, overexpression of the riboflavin biosynthesis structural *RIB6* gene, which encodes 3,4-dihydroxy-2-butanone-4-phosphate synthase, resulted in 1.13-fold higher accumulation of riboflavin in comparison to the parental strain *C. famata* BRPI (Best Riboflavin Producer Improved) [5].

The aim of this study was to obtain recombinant strains with increased synthesis of riboflavin due to the simultaneous overexpression of the mentioned genes *RFE1*, *RIB6*, and *GND1* in the *C. famata* yeast genome. The plasmids with combinations of these genes were successfully constructed based on the vector pTTb [3]. The fidelity of the plasmids was confirmed by restriction digest and PCR. Furthermore, the linearized plasmids were introduced into the wild-type *C. famata* VKM Y-9 and *C. famata* BRPI strains by electroporation. The presence of gene expression cassettes in the yeast genome was confirmed by PCR.

Obtained recombinant strains *C. famata*, with overexpression of genes *RFE1*, *RIB6*, and *GND1* in different combinations demonstrate up to 2.3-fold increased riboflavin yield on the 5th day of cultivation, compared to the parental strains. Our results suggest that the overexpression of genes *RFE1*, *RIB6*, and *GND1* led to increased riboflavin production in the yeast *C. famata*.

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FUNCTIONAL RESPONSE OF L929 CELLS IN SPHEROIDS TO COLD STRESS IN THE PRESENCE OF SYNTHETIC NEUROPEPTIDES

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The modern use of 3D cultures, particularly spheroids (SP), as test systems requires the search for effective methods of their low-temperature preservation [1-3]. Therefore, understanding the response of cells within SP to cold and searching for protective compounds to prevent its negative impact is an urgent task in cell engineering. Our previous work on the functional response of isolated cells (leukocytes and fibroblasts) under cold conditions demonstrated that synthetic analogs of natural neuropeptides (dalarin and kyotorphin) are among such protective compounds [4-6]. Given the fact that the structure of three-dimensional objects significantly differs from isolated cells, the aim of this study was to investigate the effect of synthetic neuropeptides (dalarin and kyotorphin) on the functional potential of L929 cell line within spheroids under cold stress conditions.

The conducted studies established that cold stress (which includes cooling SP to 0°C, followed by a return to normothermic conditions) reduces the number of adhered SPs by 2 times and the migratory activity (by the area of monolayer s formed by cells, which migrated from the spheroids) by 2 and 2.5 times after 24 and 48 hours of cultivation, respectively, compared to intact indicators. A similar trend was observed when determining the bioenergetic state of cells within SP, assessed using the MTT-test.

Pre-incubation of SP in a medium with the addition of dalarin or kyotorphin at a concentration of 100 µg/L helped normalize the above indicators of fibroblasts under cold stress conditions, which may indicate the probable protective effect of these drugs. The nature and mechanisms of such effects of synthetic neuropeptides remain open and require further study.

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Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

СТІЙКІСТЬ ЕРИТРОЦИТІВ СОБАКИ ДО ГІПЕРТОНІЧНОГО ШОКУ ПІСЛЯ ВИСНАЖЕННЯ ВНУТРІШНЬОКЛІТИННОЇ АТФ

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Біохімічні та структурні зміни в еритроцитах, що зумовлені зміною енергетичного метаболізму, включають порушення деформованості, везикуляцію, конформаційні модифікації фосфоліпідів та зміну градієнта іонів [1]. Ці та інші зміни можуть далі транслюватися в потенційно незворотні зміни в структурі та функції цитоскелета, плазматичної мембрани та ферментів. Часто метаболічне виснаження відбувається під час зберігання клітин і може бути відповідальним за посттрансфузійні побічні реакції у реципієнта [2-3]. Швидким тестуванням змін, що відбуваються в клітинах внаслідок можуть бути прості стресові впливи, такі як, наприклад, гіпертонічний шок.

Метою даної роботи є дослідження рівня гіпертонічного гемолізу еритроцитів собаки після виснаження внутрішньоклітинної АТФ.

Для дослідження використовували еритроцити, отримані з крові собаки. Роботу з тваринами проводили відповідно до «Загальних принципів експериментів на тваринах» (V Національний конгрес з біоетики, Київ, 2016) Виснаження АТФ проводили методом інкубування з 2-дезоксіглюкозою (10 ммоль/л) протягом 2 год при 37°С. Гіпертонічний шок здійснювали перенесенням еритроцитів у 4,0 моль/л NaCl при 22°С. Вміст гемоглобіну в супернатанті визначали спектрофотометрично. Статистичну обробку отриманих експериментальних результатів проводили за допомогою програми «Statistica 6.0» («StatSoft Inc.», США).

Контрольні клітини, що інкубувалися без дезоксиглюкози, показали зниження рівня гемолізу зі збільшенням часу інкубування. Так, початковий рівень складав 39±5%, через 30 хв – 26±4%, 60 хв – 13±2%, 90 хв – 13±3%, 120 хв – 11±2%. Для клітин, що виснажувалися за АТФ – 42±2%, 31±3%, 26±3%, 27±4%, 24±3%, відповідно. Отримані дані демонструють збільшення чутливості метаболічно виснажених еритроцитів собаки до гіпертонічного шоку починаючи з 60 хв інкубування, що відображається у зростанні рівня гемолізу щонайменше у 2 рази.

Отже, виснаження внутрішньоклітинної АТФ у еритроцитів собаки призводить до зниження стресостійкості клітин, зокрема до гіпертонічного шоку. Таким чином, метаболічне виснаження є значущим явищем у крові собак, що зберігається, і підтримка пулу АТФ є важливою для посттрансфузійного функціонування еритроцитів.

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Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

USE OF MICROBIAL BIOSTIMULANTS FOR *IN VITRO* OBTAINING NEW POTATO LINES WITH RNAi-MEDIATED RESISTANCE TO THE NEMATODE *H. SCHACHTII*

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An urgent objective of modern agricultural biotechnology is to increase the resistance of agricultural crops to diseases caused by plant-parasitic nematodes. Annual global crop losses from plant damage by parasitic nematodes range from 70 to 90 %, which is estimated at 100 - 157 billion dollars [1]. In this study, we obtained *in vitro* new lines of potato (*Solanum tuberosum* L.) cultivar Vernissage with RNAi-mediated resistance to the parasitic nematode *H. schachtii* on nutrient MS media containing biostimulants developed on the basis of secondary metabolites of soil strains *Streptomyces avermitilis* (Avercom, Avercom nova-2), *S. netropsis* (Phytovit) and *S.violaceus* (Violar) [1]. The experimental nutrient MS media contained 25 µl/l of Avercom, 75 µl/l of Avercom nova-2, 75 µl/l of Violar and 100 µl/l of Phytovit in combination with 2 mg/l BAP and 0.1 mg/l NAA. The control nutrient MS medium contained only 2 mg/l BAP and 0.1 mg/l NAA. The study of molecular genetic indicators of the resistance of potato plants to *H. schachtii* showed an increase in the index of dot blot hybridization between cytoplasmic mRNA isolated from larvae of *H. schachtii* and si/miRNA isolated from potato plants grown on artificial invasive background on MS media supplemented with biostimulants: Avercom - up to 38 %, Avercom nova-2 - up to 31 %, Phytovit - up to 25 %, Violar – up to 19 %, respectively, compared to the index of dot blot hybridization between cytoplasmic mRNA isolated from larvae of *H. schachtii* and si/miRNA isolated from control plants. The study of the silencing activity of plant cytoplasmic si/miRNA on the template of nematode mRNA, carried out in the wheat embryo cell-free protein synthesis system, showed a significant increase in the silencing activity of si/miRNA isolated from experimental potato plants grown on artificial invasive background on MS media supplemented with biostimulants: Avercom nova-2 – up to 37 %, Avercom – up to 33 %, Phytovit – up to 28 %, Violar – up to 25 %, respectively, compared to the silencing activity of cytoplasmic si/miRNA isolated from control plants on the template of nematode mRNA. Our research has proven that microbial biostimulants significantly increase the potato resistance to parasitic nematodes by inducing RNAi processes in plant cells, i.e. by stimulating the synthesis of endogenous si/miRNAs with immune-protective properties against nematode *H. schachtii*.

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Session 5: Biotechnology and metabolic engineering/Біотехнологія та метаболічна інженерія

GENOME-WIDE IDENTIFICATION OF *CAMELINA SATIVA* AND *BRASSICA CARINATA* TAG-LIPASES AND THEIR COMPARISON WITH INDUSTRIAL FUNGAL LIPASES

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The use of liquid biofuels, particularly biodiesel, derived from vegetable oils could significantly reduce the rate of greenhouse gases and other pollutants emissions [1]. Unfortunately, the low efficiency of biofuel production and, therefore, their high cost significantly limit the large-scale implementation of alternative fuels [1]. To the date, number of species from Cruciferous family are viewed as promising oilseed crops, suitable for biofuel production, in particular, false flax (*Camelina sativa*) and carinata (*Brassica carinata*) [2,3]. Complete transesterification of vegetable oils may be achieved only if respective lipases are used [4].

In the present study we analyzed the sequence features of the key functional domains of the endogenous lipases of *C. sativa* and *B. carinata*, which are evolutionarily adapted for cleavage of seed TAGs, and compared them with commercial lipases used for the production of biodiesel.

Based on the results of a genome-wide search, 13 genes of *B. carinata* TAG-lipases and 15 orthologous genes of *C. sativa* were identified. The reconstruction of TAG-lipases phylogeny revealed the presence of two large groups of TAG-lipases, which include canonical TAG-lipases, or patatin-like proteins, and sugar-dependent TAG-lipases. The domain structure of the identified TAG-lipases was analyzed, and the rate of sequence divergence of their functional regions was revealed, which made it possible to uncover a rather low level of sequence conservancy of the functional domains of TAG-lipases in *C. sativa* and *B. carinata*, especially if compared lipases of *Candida antarctica*, *Thermomyces lanuginosa*, *Rhizomucor miehei* and *Asperigillus oryzae*.

Based on the results of the analysis, it was established that the lipases of *A. oryzae* and *C. antarctica* may be the most suitable candidate candidates for effective conversion of *C. sativa* and *B. carinata* TAGs. However, a direct relationship between sequence similarity and high efficiency of lipid conversion has not been confirmed so far, therefore, more studies are needed in the future to clarify this issue.

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Session 6

Bioanalytics

MICROBIAL ENZYMES AND RECOMBINANT CELLS COUPLED WITH NANOZYMES AS SENSING ELEMENTS OF AMPEROMETRIC BIOSENSORS

Mykhailo Gonchar **Lecture 1**

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Analytical Biotechnology plays a crucial role in modern biotechnology, focusing on the use of biological objects, submolecular complexes, biomolecules and principles of functioning of living organisms for analytical purposes. The main advantage of bioanalytical systems is their exceptional specificity which can be explained by extreme selectivity of biorecognition at the molecular level. Besides enzymatic kits, biosensors are the most used analytical devices based on using enzymes of microbial origin. We have developed different amperometric biosensors for assaying practically important analytes which serve as biomarkers for prevalent diseases and indicators of food quality and environmental pollution.

In recent years, nanomaterials with enzyme-mimetic properties (nanozymes) have attracted considerable attention. Nanozymes (NZs) as artificial catalysts are promising alternatives to the natural enzymes [Stasyuk *et al.*, 2020]. NZs have essential advantages such as low preparation costs, high stability, an advanced surface area, self-assembling capability, size- and composition-dependent activities, broad possibility for modification, and biocompatibility. They have wide potential practical applications as catalysts in biosensors, fuel-cell technology, environmental biotechnology, and medicine.

We have synthesized a lot of catalytic nanomaterials that mimic the activity of peroxidase, catalase, and even reductase. In the case of catalase-like nanopartices, we have reported the first, to the best to our knowledge, example of successful substitution of natural enzyme by catalase-like nanoparticles transferred to the cells of the yeast *Ogataea polymorpha* [Prokopiv *et al*., 2023].

On the model of the yeast *Saccharomyces cerevisiae*, we have developed a new approach to increase the sensitivity of cell-based biosensors. The yeast cells were enriched with the enzyme and nanozyme by combining three ways of genetic and nanotechnological engineering: on the genetic level – by overexpression of the methylamine oxidase (MAO) gene coding for MAO in recombinant cells; under nanotechnological approaches – by the additional enrichment of the cells with the purified enzyme MAO, co-immobilized with peroxidase mimetics. The proposed approaches for increasing catalytic efficiency of sensing cells can be used for elaboration of other microbial sensors based on their enzymatic activity.

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BIOANODE BASED ON GENETICALLY ENGINEERED BACTERIAL CELLS ENRICHED WITH CREATININE DEIMINASE AND N-METHYLHYDANTOIN-SENSITIVE BIONANOCOMPOSITE IN CONSTRUCTION OF SELF-POWERED BIOSENSORS

Nataliya Stasyuk **Lecture 2**

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The search for biomarkers for prevalent diseases and indicators for evaluating disease progression is a priority area of world science in the field of Analytical Biotechnology. Metabolites, in particular, creatinine (Crn), play an important role among such biomarkers [1]. Determination of Crn, which is an important indicator of the kidney function, in biological fluids (blood serum and urine) is widely used in medical practice. The content of Crn in blood serum and urine is an important diagnostic biomarker for the detection of kidney diseases of various genesis, including acute chronic interstitial nephritis, nephrosis, renal failure, and myocardial infarction. Given that the Crn assay is widely used to evaluate a patient's condition, ensuring the accuracy of this assay in cases of the impaired renal function is of paramount importance.

Biofuel cell-powered sensors for creatinine detection as miniaturized devices are crucial for clinical application and personal usage. Microbial biosensors based on whole cells, especially in combination with nanomaterials, are efficient and cost-effective tools for a variety of analytical applications. In the current study, the enrichment of recombinant bacterial cells by creatinine deiminase (CDI) was accomplished through the combination of gene engineering and nanotechnology approaches: (*1*) by overexpression of the corresponding CDI gene in recombinant bacterial cells *Corynebacterium glutamium*, and (*2*) *via* the nanotechnological strategies, such as loading the cells with CDI co-immobilized with N-methylhydantoin-sensitive nanoparticles (nCu) [2]. The enriched bacterial cells (CDI-nCu) were successfully used for the construction of a bioanode utilized in the biosensor and biofuel cell construction (MFC). The fabricated amperometric biosensor possessed sufficient sensitivity (172 $A \cdot M^{-1} \cdot m^{-2}$), a high limit of detection (0.2 μM) and good selectivity towards the target analyte. The developed MFC generated an open circuit potential of 520 mV with a maximum power density of 3 $\mu W \cdot cm^{-2}$ at an optimum of 0.8 mM creatinine. The constructed biofuel cell was tested on the real samples of human serum and urine.

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SCREENING OF MUSHROOMS AS PRODUCERS OF EXTRACELLULAR LACCASE, ENZYME'S ISOLATION AND BIOANALYTICAL APPLICATION

Olha Demkiv **Poster 35**

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Laccase is multicopper oxidase that exhibits polyphenol oxidase activity. This enzyme can oxidize a wide range of substrates by catalyzing the four-electron oxidation in the presence of O_2 as a co-substrate to form H_2O [1]. Laccase is used in food and paper processing, dye discoloration, production of beverages, cosmetics, drugs and other valuable compounds [2]. The industrial use of laccase in biotechnology and bioremediation requires the search for new producer strains and the selection of optimal cultivation conditions to increase the synthesis of the enzyme [3].

In this study, we have screened 38 fungi strains for the ability to produce extracellular laccase. *Trametes zonata* 1525 and *Trametes hirsuta* 338 strains were identified as the best potential enzyme producers. We have done detailed investigation of optimal cultivation conditions for *T. zonata* 1525, focusing on the impact of different carbon and nitrogen sources, as well as various possible inducers on the synthesis of laccase. Our findings demonstrate that using 1 mM Cu^{2+} as an inducer resulted in an 18-fold increase in activity. We have revealed inducing effect of the two other metals ions (Mg^{2+} and Mn^{2+}) and several organic compounds on laccase-producing activity of the fungus. The laccase from the mushroom *T. zonata* was purified from the extracellular medium using initial precipitation with ammonium sulfate, followed by subsequent purification via column chromatography. The enzyme consists of identical subunits with a molecular weight of 62 kDa. It exhibits an optimal pH of 4.5 and an optimal temperature of 35 °C. The enzyme shows higher stability within the pH range of 3.5-6.0 and at temperatures between 30°C and 55°C. We proposed simple amperometric biosensor for the determination of 5-hydroxyindole acetic acid (5-HIAA) in urine using *T. zonata* laccase as a sensing element. The developed laccase-based biosensor can detect 5-HIAA with a high sensitivity (1900 ± 9 $A \cdot M^{-1} \cdot m^{-2}$) and has a broad linear range (2 – 50 μM). This sensor can be applied to the 5-HIAA assay in urine samples and shows promise for diagnosing certain non-invasive carcinoid tumors.

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GREEN-SYNTHESIZED HEXACYANOFERRATES OF TRANSITION METALS: OBTAINING, CHARACTERIZATION AND APPLICATION

Galina Gayda **Poster 36**

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Nanozymes have shown remarkable potential as alternatives to natural enzymes, particularly in industrial, medical, and environmental applications. Their advantages, including enhanced stability, lower production costs, and the ability to operate under harsh conditions, make them highly suitable for a wide range of biotechnological uses, being explored for pollutant degradation, biosensing, drug delivery, and as catalysts in various chemical processes.

The "green" synthesis of hexacyanoferrates (gHCF), using the oxido-reductase as biocatalysts, offers several advantages. In this approach, nanoparticles (NPs) were synthesized using yeast flavocytochrome b_2 (Fcb_2) in the presence of its substrate L-lactate and $K_3Fe(CN)_6$.

The structure, size, composition, catalytic properties, and electron-mediator activity of the obtained NPs were characterized. A more detailed study was specifically conducted for copper gHCF (gCuHCF) [1-2]. According to the SEM study, gCuHCF exhibits a flower-like structure, making it suitable for concentrating and stabilizing Fcb_2. gCuHCF has been identified as an effective mimetic of peroxidase (PO). Consequently, it has been utilized for H_2O_2 analysis in both optical and amperometric sensors, as well as in oxidase-based biosensors [2-5]. Additionally, gCuHCF demonstrates the ability to amperometrically sense ammonium ions NH_4^+, making it a potential component in biosensors that rely on enzymes producing NH_4^+ as a byproduct of the enzymatic reaction [5-7].

Thus, gHCF of transition metals, synthesized *via* oxidoreductase, may serve as promising platforms for developing amperometric biosensors, bioreactors, biofuel cells and other devices. The originality of gCuHCF lies in its multifunctionality, acting as a PO-like NZ, a chemo-sensor for ammonium ions, and a biosensor for L-lactate. The proposed approach of using biocatalysts for NPs synthesis represents a promising and innovative direction in materials science and biotechnology.

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