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

ISCACMB

International Scientific Conference
"Advances in Cell Biology, Microbiology, and Biotechnology"

ABSTRACT BOOK

To 25th Anniversary of Institute of Cell Biology,
NAS of Ukraine

12 – 13 June 2025
Lviv, Ukraine

Abstract Book of the International Scientific Conference "Advances in Cell Biology, Microbiology, and Biotechnology" (ISCACMB 2025)

Edited by:
Prof., D.Sc. Sibirny A.
D.Sc. Stasyuk N.
Ph.D. Barska M.
Ph.D. Manko N.

The authors are solely responsible for the content of the abstracts.

http://DOI:10.5281/zenodo.15624723

Organizing committee of the International Scientific Conference “Advances in Cell Biology, Microbiology, and Biotechnology” (ISCACMB 2025)

1. Head of the organizing committee – Andriy Sibirny, D.Sc., Prof., Academician of the National Academy of Sciences of Ukraine, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
2. Co-chairman of the organizing committee – Roman Gladyshevskii, D.Sc., Prof., Academician of the National Academy of Sciences of Ukraine, Ivan Franko National University of Lviv, Lviv.

Members of the organizing committee:

1. Yaroslav Blume, D.Sc., Prof., Academician of the National Academy of Sciences of Ukraine, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv.
2. Svitlana Hnatush, Ph.D., Prof., Ivan Franko National University of Lviv, Lviv.
3. Valentyna Chopyak, D.Sc., Prof., Danylo Halytsky Lviv National Medical University, Lviv.
4. Nataliia Sybirna, D.Sc., Prof., Ivan Franko National University of Lviv, Lviv.
5. Kostyantyn Dmytruk, D.Sc., Corresponding Member of the National Academy of Sciences of Ukraine, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
6. Rostyslav Stoika, D.Sc., Prof., Corresponding Member of the National Academy of Sciences of Ukraine, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
7. Mykhailo Gonchar, D.Sc., Prof., Corresponding Member of the National Academy of Sciences of Ukraine, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
8. Oleh Stasyk, D.Sc., Senior Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
9. Nataliya Stasyuk, D.Sc., Deputy Director for Science, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.

Secretariat:

1. Head of Secretariat: Maryna Barska, Executive Secretary of Ukrainian Society of Cell Biology, Ph.D., Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
2. Deputy Director for scientific and technical work: Kostiantyn Kapustiak, Ph.D., Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
3. Director of Scientific Library of Ivan Franko National University of Lviv: Oleksandr Sedlyar, PhD.
4. Head of the Branch of the Ukrainian Society of Cell Biology in Lviv: Olena Vovk, Ph.D., Junior Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
5. Chairman of the Council of Young Scientists of the Institute of Cell Biology, National Academy of Sciences of Ukraine: Manko Nazar, Doctor of Philosophy, Junior Research Scientist.
6. Olha Demkiv, PhD, Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
7. Nataliya Finiuk, PhD, Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
8. Iryna Ivasechko, PhD, Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
9. Ljubov Dzanaeva, PhD, Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.
10. Demash Dmytro PhD, Research Scientist, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv.

International Scientific Conference "Advances in Cell Biology, Microbiology, and Biotechnology" (ISCACMB 2025)

CONTENTS

CONFERENCE PROGRAM

PROGRAM
of the International Scientific Conference "Advances in Cell Biology, Microbiology, and Biotechnology"
(ISCACMB 2025)
Lviv, June 12-13th 2025
https://www.cellbiol.lviv.ua/2025

June 12th (Thursday)

10:00 – 10:30 Registration of participants, Conference hall of the Scientific Library of Ivan Franko National University of Lviv

11:00 – 11:30 Conference opening, press release for the media.

Speakers: ***Andriy Sibirny*** *(Chair of the Organizing Committee, Director of the Institute of Cell Biology NAS of Ukraine);* **Лекція «Історія створення, становлення, сучасний стан та перспективи розвитку Інституту біології клітини НАН України»**

Roman Gladyshevskii *(Rector of Ivan Franko National University of Lviv);*

Valentyn Pidgorskiy *(Honorary Director of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine);*

Mykola Spivak *(Director of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine);*

Zynoviy Nazarchuk *(Head of the Western Scientific Center of the NAS of Ukraine and the Ministry of Education and Science of Ukraine);*

Oleksandr Sedlyar *(Director of Scientific Library of Ivan Franko National University of Lviv);*

Greetings from Lviv city council and Lviv regional administration.

11:45 – 18:35 Session "**Cell Biology / Клітинна біологія**" ***Chairs: Kostyantyn Dmytruk, Nataliya Stasyuk***	
11:45 – 12:00	Lecture 1. Yaroslav Blume (Kyiv). Development of autophagy as an adaptive response of plants to microgravity.
12:00 – 12:15	Lecture 2. Kostyantyn Dmytruk (Lviv). Vacuolar degradation of cytosolic enzymes in *Komagataella phaffii* upon carbon source shift.
12:15 – 12:30	Lecture 3. Sergiy Kosterin (Kyiv). Calixarenes as modulators of intracellular calcium homeostasis and energy of contraction-relaxation of smooth muscles.
12:30 – 12:45	Lecture 4. Alla Yemets (Kyiv). Cytoskeleton as an intracellular regulator and target for biologically active compounds.

12:45 – 13:00	Lecture 5. Rostyslav Stoika (Lviv). Ways for improvement of characteristics and anticancer action of novel synthetic heterocyclic compounds
13:00 – 14:00	**Lunch** (see the nearest possible locations at the end of the Program, marked*).
14:00 – 14:15	Lecture 6. Ярослав Шуба (Kyiv). Рецептор жару і капсаїцину TRPV1: від сенсорного відчуття до м'язового скорочення.
14:15 – 14:30	Lecture 7. Oleksandr Petrenko (Kharkiv). Mechanisms of resistance to ambient storage of mesenchymal stem cells after incapsulation in alginate hydrogel.
14:30 – 14:45	Lecture 8. Oksana Stoliar (Ternopil). Zinc-related effects of rare earth elements in bivalve mollusk under single and complex exposures.
14:45 – 15:00	Lecture 9. Elena Kashuba (Kyiv). Stability of mRNA, polyubiquitination and proteasomal degradation of MRPS18 family genes *in vitro*.
15:00 – 15:15	Lecture 10. Liudmyla Kozeko (Kyiv). Exogenous proline and gaba regulate stomata and cause ultrastructural changes in arabidopsis leaf cells under drought.
15:15 – 15:45	**Coffee break**
15:45 – 16:00	Lecture 11. Anna Havrylyuk (Lviv). Research of the molecular mechanisms for the pathologic impact of varicocele on fertility potential.
16:00 – 16:15	Lecture 12. Mykola Borysyuk (Kyiv). The role of leaf surface in plant abiotic stress tolerance.
16:15 – 16:30	Lecture 13. Олена Канюка (Lviv). Взаємозв'язок стресу ендоплазматичного ретикулюму та дисфункції мітохондрій у β-клітинах підшлункової залози при експериментальному цукровому діабеті.
16:30 – 16:45	Lecture 14. Марія Рудницька (Kyiv). Дослідження акумуляції іонів калія мітохондріями гладенького м'язу матки з використанням модуляторів K^+-каналів різних підтипів.
16:45 – 17:00	Lecture 15. Boris Negrutskii (Kyiv). Conformational Divergence of EEF1A paralogues as a key to their differential disease associations.
17:00 – 17:20	*Презентація Премії L'Oreal - UNESCO "Для жінок у науці-2025"*: • Ємець Алла Іванівна, чл.-кор. НАН України, д.б.н., проф., зав. відділом клітинної біології і біотехнології Інституту харчової біотехнології та геноміки НАН України; • Христина Гнатенко, член.-кор. НАН, проф. кафедри теоретичної фізики ім. проф. Івана Вакарчука ЛНУ імені Івана

	Франка, переможниця Премії 2023; • Наталія Стасюк, д.б.н., ст.н.с. відділу аналітичної біотехнології Інституту біології клітини Національної академії наук України, переможниця Премії 2023.
17:20 – 18:15	**Poster sessions** (sections 1, 2, 3: "*Cell Biology", "Molecular oncology and medicine*, *Microorganisms and their biotechnological applications*).
18:30 – 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 "Steak House", 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;
- Business Hub "Black Honey", Shota Rustaveli Str., 12, Lviv.

June 13th (Friday)

10:00 – 12:00	**Session 2: «Molecular Oncology and Medicine / Молекулярна онкологія та медицина»** ***Chairs: Rostyslav Stoika, Sergiy Shulga***
10:00 – 10:15	Lecture 1. Mykola Korpan (Austria). Cytological determinants in biocryoimmunological medicine.
10:15 – 10:30	Lecture 2. Oleh Stasyk (Lviv). Anticancer metabolic therapy: problems and developments.
10:30 – 10:45	Lecture 3. Natalya Finiuk (Lviv). Suppression of NK/LY lymphoma by novel pyrrolidinedione-thiazolidinone hybrids *via* DNA damage, PARP1 inhibition, and cell cycle arrest in G1.
10:45 – 11:00	Lecture 4. Larysa Kovalevska (Kyiv). Expression profile of transcription factors in B-lymphocytes at chronic lymphocytic leukemia.
11:00 – 11:15	Lecture 5. Sergiy Shulga (Kyiv). Therapy of chronic inflammation at the early stage of Alzheimer's disease using liposomal form of curcumin and microRNA aerosol.
11:15 – 11:30	Lecture 6. Halyna Hachkova (Lviv). Antidiabetic potential of biologically active compounds on the alkaloid-free extract of *Galega officinalis*.
11:30 – 11:45	Lecture 7. Daria Krasnytska (Kyiv). Inhibition of ERN1 affects the hypoxic regulation of homeobox transcription factor genes expression in ERN1-dependent manner.
11:45 – 12:00	Lecture 8. Rostyslav Panchuk (Lviv). Mechanisms of cell death induction by novel thiosemicarbazones in colon cancer cells.
12:00 – 12:30	**Coffee break**
12:30 – 16:30	**Session 3: Microorganisms and Their Biotechnological Applications / Мікроорганізми та їхнє біотехнологічне застосування** ***Chairs: Mykhailo Gonchar, Svitlana Hnatush***
12:30 – 12:45	Lecture 1. Sergiy Dzyadevych (Kyiv). Electrochemical enzyme biosensors for agricultural application.
12:45 – 13:00	Lecture 2. Mykhailo Gonchar (Lviv). Diaphorase nanomimetics and their application in construction of biosensors, bioreactors, and biofuel cells.
13:00 – 13:15	Lecture 3. Bohdan Ostash (Lviv). Ribosome as a regulatory device: a case of antibiotic-producing streptomycetes.
13:15 – 14:15	**Lunch** (see the nearest possible locations at the end of the

	Program, marked *).
14:15 – 14:30	Lecture 4. Dariya Fedorovych (Lviv). Production of riboflavin and its derivatives by metabolically engineered yeast *Candida famata*.
14:30 – 14:45	Lecture 5. Justyna Ruchala (Rzeszow, Poland). Metabolic engineering of *Candida famata* for efficient riboflavin production on xylose-based media.
14:45 – 15:00	Lecture 6. Svitlana Hnatush (Lviv). Metal-resistant bacteria and their plant growth-promoting potential.
15:00 – 15:30	**Coffee break**
15:30 – 15:45	Lecture 7. Maya Vergolyas (Kyiv). Efficacy of a portable UV-LED water disinfection system in combat zones.
15:45 – 16:00	Lecture 8. Roksolana Vasylyshyn (Lviv). Identification of new genes involved in the regulation of xylose alcoholic fermentation in the thermotolerant yeast *Ogataea polymorpha*.
16:00 – 16:15	Lecture 9. Olha Demkiv (Lviv). Natural laccase and its mimetics in amperometric sensors for determination of serotonin and catecholamine biomarkers of neuroendocrine disorders.
16:15 – 16:30	Lecture 10. Nataliya Stasyuk (Lviv). Amperometric biosensors and biofuel cells for detection and bioremediation of explosives in explosive-contaminated environments.
16:30 – 17:30	Lviv tour or walking excursion in Library
18:00 – 22:00	**Banquet** (Vienna Coffee House Lviv, 12 Svobody Ave., Lviv)

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

- Restaurant “Puzata Khata”, Shevchenka Ave., 10, Lviv;
- Restaurant “Steak House”, 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;
- “Pasta Cafe” – Dudayeva Str. 3, Lviv;
- Cafe “Lviv Croissants”, Shota Rustaveli St, 6, Lviv,
- McDonald's, Shevchenka Ave., 7, Lviv;
- Business Hub “Black Honey”, Shota Rustaveli Str., 12, Lviv.

Session 1

Cell Biology

**12-13 June 2025
Lviv, Ukraine**

ІСТОРІЯ СТВОРЕННЯ, СТАНОВЛЕННЯ, СУЧАСНИЙ СТАН ТА ПЕРСПЕКТИВИ РОЗВИТКУ ІНСТИТУТУ БІОЛОГІЇ КЛІТИНИ НАН УКРАЇНИ

Андрій А. Сибірний
Інститут біології клітини НАН України, вул. Драгоманова, 14/16, Львів 79005
Email address: *sibirny@yahoo.com*

Інститут біології клітини НАН України був створений на базі Львівського відділення Інституту біохімії ім. О.В. Палладіна, що існував з 1969 р. Інститут повстав згідно Постанови Кабінету міністрів України №1123 від 20 липня 2000 р. за підписом прем'єр-міністра Віктора Ющенка та Розпорядження Президії НАН України №824 від 22 липня 2000 р. За минулі чверть сторіччя Інститут пройшов складний шлях становлення та розвитку та досяг чималих успіхів в галузях клітинної біології та біотехнології, публікуючи роботи головним чином в міжнародних часописах 1-го та 2-го квартилів.

Співробітники Інституту також є авторами чималої кількості вітчизняних та іноземних патентів. Інститут складається з 4-х відділів, два з яких (молекулярної генетики і біотехнології та аналітичної біотехнології) працюють в галузі молекулярної мікробіології та біотехнології, а інші два (регуляції проліферації клітин і апоптозу та сигнальних механізмів клітини) – в галузі молекулярної онкології. Загальна кількість дослідників в Інституті становить 37 чол., з них 12 докторів та 23 кандидатів наук або докторів філософії. Авторитет працівників Інституту ілюструє той факт, що 4 з них обрані членами НАН України (Р.С. Стойка, М.В. Гончар, К.В. Дмитрук обрані членами-кореспондентами, а А.А. Сибірний - академіком НАН України). Р.С. Стойка є іноземним членом Польської академії наук і мистецтв, а А.А. Сибірний – членом Європейської академії мікробіології та іноземним членом Академії наук Латвії. В 2004 р. при Інституті зареєстровано Українське товариство клітинної біології (Президент – А.А. Сибірний).

Інститут активно проводить вітчизняні та міжнародні наукові конференції, зокрема, Р.С. Стойка організував 1-шу та 2-гу Парнасівські конференції, а А.А. Сибірний три (Львів, 2001 та 2011, Жешув, 2018) спеціалізованих міжнародних симпозіуми по дріжджах та 12-й Міжнародний конгрес по дріжджах, декілька міжнародних Вейглівських конференцій та сім з'їздів Всеукраїнської громадської організації "Українське товариство клітинної біології" з міжнародним представництвом. При Інституті діє аспірантура та Спеціалізована Вчена рада по захистах докторських дисертації.

Про наукову діяльність Інституту протягом останніх п'яти років свідчать такі цифри: загальна кількість публікацій в 2020-2025 рр. становила 206, з них 187 індексуються в Web of Science Core Collection і Scopus; 142- у закордонних виданнях, з них – 113 в журналах 1-го та 2-го квартилів. Сумарний Імпакт фактор журналів, в яких співробітники опублікували свої праці за останні п'ять років, становить 680,188.

DEVELOPMENT OF AUTOPHAGY AS AN ADAPTIVE RESPONSE OF PLANTS TO MICROGRAVITY

Yaroslav Blume **Lecture 1**

Yaroslav Blume, Alla Yemets
Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv, Ukraine
Email address: *cellbio@cellbio.freenet.viaduk.net*

Microgravity, characteristic of outer space, represents a significant stress factor for terrestrial plants, as it deprives them of the usual gravitational stimulus. The absence of gravity alters the expression of genes responsible for cell division, cytoskeletal organization, metabolism, hormone balance, and cell polarity. To study these processes, experimental systems such as clinostats are widely used to simulate microgravity conditions on Earth.

One of the key mechanisms enabling plants to adapt to stressful conditions is autophagy - a process through which cellular components are degraded and recycled. Autophagy is triggered by various stresses, including starvation, drought, salinity, infection, and aging. During autophagy, cellular structures are engulfed by specialized vesicles – autophagosomes - which transport the material to the vacuole for degradation. This mechanism maintains cellular homeostasis and promotes survival under unfavorable conditions.

Studies conducted on *Arabidopsis thaliana* under clinorotation have shown active induction of autophagy as early as day 6–7 of seedling growth, indicated by the appearance of autophagosomes in root cells [1]. At later stages (up to day 19), although some root cells show decreased viability, autophagy remains active. Concurrent analysis revealed significant changes in the expression of key autophagy genes (*atg8*) and α-/β-tubulin genes (*tua*, *tub*), highlighting the involvement of microtubules in autophagy regulation. Specific post-translational modifications of tubulin were shown to influence its interaction with ATG8 protein, which is crucial for autophagosome formation.

Special attention was given to the role of nitric oxide (NO) as a signaling molecule [2]. Treatment with its donor (SNP) in various concentrations affected seedling growth and slowed down autophagy development under clinorotation. At the same time, elevated levels of endogenous NO were observed during early stages of plant growth, suggesting its role in the initial adaptation to microgravity.

In conclusion, autophagy is a vital adaptive mechanism that allows plants to respond to altered gravitational conditions. This process is closely linked to microtubule function and signaling molecules such as NO and may serve as a biomarker for space-induced stress or as a basis for developing stress-resistant crop plants for future space missions.

Acknowledgment:
This research was partly funded by the project “Development of the concept of regulation of development and stress resistance of plants for their adaptation to space flight conditions by attracting cellular and biological resources” of the targeted comprehensive program of the National Academy of Sciences of Ukraine for scientific space research for 2018–2022 (state registration number 01118U003742).

References:
1 Yemets, R. Shadrina, R.Y. Blume, S. Plokhovska, Ya. Blume. npj Microgravity, **2024**, 10: 31. https://doi.org/10.1038/s41526-024-00381-9
2 S.H. Plokhovska, R.Yu. Shadrina, O.A. Kravets, A.I. Yemets, Ya.B. Blume Cytol. Genetics, **2022**, 56(3): 244-252. DOI: 10.3103/S0095452722030100

Session 1: Cell Biology / Клітинна біологія

VACUOLAR DEGRADATION OF CYTOSOLIC ENZYMES IN *KOMAGATAELLA PHAFFII* UPON CARBON SOURCE SHIFT

Kostyantyn Dmytruk **Lecture 2**

Kostyantyn Dmytruk[1], Olena Dmytruk[1], Ulyana Pelypyshyn[1], Andriy Sibirny[1,2]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]University of Rzeszow, Rzeszow, Poland
Email address: dmytruk77@gmail.com

Investigating the mechanisms of cytosolic protein degradation is crucial for advancing both fundamental biological knowledge and 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, Atg6, and Atg15 - genes involved in autophagy initiation, autophagosome formation, and lipase synthesis required for breaking down autophagic body membranes in the vacuole - affected the degradation of Fld1 and Fdh1, as reflected by their specific activity levels, which were similar to those observed in SMD1163. These results suggest that Fld1 and Fdh1 are degraded via the vacuolar microautophagy pathway.

CALIXARENES AS MODULATORS OF INTRACELLULAR CALCIUM HOMEOSTASIS AND ENERGY OF CONTRACTION-RELAXATION OF SMOOTH MUSCLES

Sergiy Kosterin **Lecture 3**

Sergiy Kosterin
Palladin Institute of Biochemistry of NAS of Ukraine, Kyiv, Ukraine

Calcium ions (Ca^{2+}) play a fundamental role in the functional activity of nearly all tissues, including muscles. Our research focuses on biochemical mechanisms for regulating the concentration of Ca^{2+} in the cells of smooth (unstriated) muscles. Smooth muscles ensure the functioning of internal organs and their systems: vascular, respiratory, lymphatic, genitourinary systems, gastrointestinal tract, ducts of glands of external and internal secretion, sphincter of the eye pupil, and others. These muscles are innervated by the autonomic nervous system; that is, their contractions occur spontaneously and cannot be regulated consciously. We interpret a smooth muscle cell - a myocyte as an open receptor strain electrochemical almost isothermal system capable of Ca^{2+}-dependent contraction.

The concentration of Ca ions in the extracellular space $[Ca^{2+}]_e$ is 10^{-3} M, and in an unexcited cell, the concentration of these ions $[Ca^{2+}]_i \sim 10^{-7}$ M, which, taking into account the magnitude of the electrical potential $\Delta\Psi$ on the plasma membrane, corresponds to the value of the Gibbs energy ΔG_{PM} for cell-directed transmembrane calcium gradient $\Delta G_{PM} = RTln\{[Ca^{2+}]_e/[Ca^{2+}]_i\} + 2F\Delta\Psi =$ 40 kJ/mol (R is the universal gas constant, T is the absolute temperature, ^{0}K, F is the Faraday constant). Upon excitation, Ca ions enter the cytoplasm through the calcium channels of the plasma membrane, as a result, the intracellular Ca^{2+} concentration increases approximately to 10^{-6} M. This increase in the concentration of Ca ions in a muscle cell from 10^{-7} M to $^{-6}$ M induces a number of complex biochemical processes that trigger smooth muscle contraction. On the other hand, for muscle relaxation, nature has provided for the presence of very important energy-dependent systems localized in the plasma membrane — Mg^{2+},ATP-dependent calcium pump (electroenzyme Ca^{2+}, Mg^{2+}-ATPase) and Na^+-Ca^{2+}-exchanger, which, on the contrary, remove Ca ions from the cell against the aforementioned electrochemical calcium gradient and control the process of muscle relaxation. Additionally, there are kinds of calcium depots in the muscle cell where calcium accumulates and is reversibly released. The role of such Ca^{2+} stores is played by intracellular organelles - the sarcoplasmic reticulum, which contains its own Mg^{2+},ATP-dependent calcium pump, which differs in its properties from the plasma membrane calcium pump, and mitochondria, which possess a Ca^{2+}-uniporter that enables electrophoretic accumulation of Ca ions in the mitochondrial matrix.

The fundamental problems of studying the molecular and membrane mechanisms regulating the concentration of Ca ions in myocytes also have an important practical aspect. After all, some severe pathologies of the smooth muscle contractile function are often associated with a disruption in the dynamics of the Ca ion intracellular concentration. In particular, this applies to hypo- and hypertension; atony of the intestinal tract and other pathologies of its motility; asthma; diseases of the genitourinary system (for example, hypo- and hypertonicity of the uterus, miscarriages, spontaneous abortions), among others. Therefore, the search for novel non-toxic (or low-toxic) reversible effectors (inhibitors, activators) — selective and high-affinity regulators of membrane-bound Ca^{2+}-transporting ATP-hydrolases and ATP-hydrolases of contractile proteins, is highly relevant. Such effectors can potentially serve as "molecular platforms" for the development of new-generation drugs that normalize the smooth muscle contractility under pathological conditions. In this aspect, the results of our previous research, conducted in cooperation with Academician V.I. Kalchenko and his colleagues from the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine since 2023, suggest that cyclic oligomers of phenols – calixarenes - are interesting and promising both from a fundamental and practical point of view.

The report will demonstrate, how transdisciplinary experimental and theoretical approaches can be employed to investigate the biochemical mechanisms of Ca^{2+}-dependent regulation of contractility and energy of smooth muscles, (using the smooth muscle of the uterus (myometrium) as a model), as well as the regularities of its directional modulation using macrocyclic compounds – calixarenes, mainly on the example of calixarene effectors Mg^{2+},ATP-dependent calcium pump of the plasma membrane.

CYTOSKELETON AS AN INTRACELLULAR REGULATOR AND TARGET FOR BIOLOGICALLY ACTIVE COMPOUNDS

Alla Yemets **Lecture 4**

Alla Yemets, Yaroslav Blume
Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

The cytoskeleton plays a central role in controlling the movement, shape, growth, division, and development of eukaryotic cells. It determines the localization of proteins and organelles throughout the cell and mediates their transport. It is composed of microtubules, actin filaments (microfilaments), and intermediate filaments. The cytoskeletal network also plays an important role in cellular signaling cascades, as it participates in transmitting signals from the cell membrane to the nucleus.

Although cytoskeletal proteins are fairly conserved among eukaryotes, plant cells possess unique features in the organization and function of the cytoskeleton due to the sessile nature of plant organisms. Thus, the cytoskeleton represents a potential target for a number of drugs (or even toxic agents) that can disrupt cellular function through direct or indirect interaction with microtubules, microfilaments, or intermediate filaments.

A number of compounds have already been developed, particularly those targeting microtubules, which are used as anticancer agents due to their ability to block cell proliferation and induce programed cell death. Among the substances that specifically bind to plant microtubules, some have been identified and used for their herbicidal activity or as inducers of cell polyploidy.

Currently, *in silico* methods and genetic engineering approaches are being used to search for more effective compounds with fungicidal, herbicidal, and nematicidal activity to enhance plant resistance to biotic stress and to control their growth and development.

WAYS FOR IMPROVEMENT OF CHARACTERISTICS AND ANTICANCER ACTION OF NOVEL SYNTHETIC HETEROCYCLIC COMPOUNDS

Rostyslav Stoika **Lecture 5**

Rostyslav Stoika
Institute of Cell Biology, NAS of Ukraine, Drahomanov Str. 14/16, 79005, Lviv, Ukraine
E-mail : stoika.rostyslav@gmail.com

Background. 2% of existing medicines are based on scaffolds of various heterocyclic compounds. 18% of known medicines were designed on Thiazoles platform, 80% - on 1,2,3 triazoles, 80% - on Thiazolidinones, and 80% - on Pyrazolines. Hybrid molecules created via structural condensation of pyrazolines with other heterocycles are of special clinical significance, for example, Thiazolyl-pyrazoline hybrid compounds gave rise to 38% medicines. However, despite of such outstanding characteristics of medicinally attractive heterocycles, these compounds have several drawbacks that hinder their quick application in clinics.
Specific aims. Anticancer potential of novel synthetic derivatives of thiazoles and 4-thiazolidinones was addressed and various approaches for improvement of their physico-chemical and biological characteristics were considered.
Results. An attachment of specific side chemical groups to the heterocyclic molecule, bio-isosteric replacement in five-membered multi-heterocyclic rings of heterocycles, and covalent conjugation of various pharmacophores and structural elements were used for design and synthesis of novel perspective drug-like compounds. The negative side effects of drugs in the body are mainly caused by their off-target action that is another adverse effect of the heterocyclic drug-like compounds. We found that condensation of thiopyrano[2,3-d]thiazoles with Juglone (5-hydroxy-1,4-naphthoquinone that is product of *Juglandaceae* plants) via hetero-Diels-Alder reaction, not only elevated considerably anticancer activity of resulting hybrid molecule *in vitro*, but also decreased its toxic action towards normal human blood lymphocytes and adverse effects towards normal tissues of laboratory mice, comparing to such effects of doxorubicin [**1**]. 5-ene-4-thiazolidinone hybrid molecules were found to be promising pro-apoptotic antitumor agents targeting PPAR gamma, topoisomerase II, Bcl-2, and tubulin [**2**]. *In silico* modeling was applied for searching these biological targets. It was detected that bio-isosteric replacement of 1H-1,2,3-triazole ring with 1H-tetrazole ring significantly enhanced anti-leukemic activity of (5-benzylthiazol-2-yl) benzamides reaching IC50 of 56.4 nM (sulforhodamine B test) and Selectivity Index of 101.0 [**3**]. We found that the Landomycin E (Streptomyces antibiotic with anticancer potential) can attach covalently the reduced glutathione and cysteine, exhausting tumor cells of these important antioxidants [**4**]. It is not so easy to circumvent another significant drawback of the heterocyclic compounds such as poor water solubility, since chemical modification of these compounds usually leads to a loss of their biological activity. However, this goal was achieved via non-covalent structural modifications of heterocycles through complexation with amphiphilic nano-scale poly(VEP-co-GMA)-graft-PEG carriers. In such a way, water soluble forms of heterocyclic inducers of apoptosis in tumor cells and suppressors of tumor growth in mice were prepared and their high bio-tolerance in animal body was achieved [5].

References. 1. Ivasechko et.al. Eur J Med Chem. 2023; **2**. Finiuk et al. Eur J Med Chem. 2022; **3**. Pokhodylo et al. Eur J Med Chem. 2023; **4**. Terenzi et al. Commun. Chemistry. 2021; **5**. Kobylinska et al. Cancer Nanotechnol. 2018.
Acknowledgements: Roman Lesyk (Danylo Halytsky National Medical University of Lviv), Nazariy Pokhodylo (Ivan Franko National University of Lviv), Alexander Zaichenko (Lviv Polytechnic National University).

РЕЦЕПТОР ЖАРУ І КАПСАЇЦИНУ TRPV1: ВІД СЕНСОРНОГО ВІДЧУТТЯ ДО М'ЯЗОВОГО СКОРОЧЕННЯ

Ярослав Шуба **Lecture 6**

Ярослав Шуба
Інститут фізіології ім. О.О. Богомольця Національної Академії Наук України, Київ, Україна
Електронна пошта: *yshuba@biph.kiev.ua*

Transient Receptor Potential Vanilloid 1 (TRPV1) – це проникний для кальцію іонний канал, найбільш відомий завдяки своїй здатності бути активованим пекучою складовою червоного перцю чилі, капсаїцином, та спорідненими хімічними сполуками із групи ванілоїдів, а також термічним жаром. Як такий, він переважно присутній в сенсорних нейронах, де відповідає за сприйняття больових подразників, викликаних різними хімічними речовинами та високими температурами. Активація TRPV1 сенсибілізується низкою ендогенних про-запальних медіаторів та деякими вторинними месенджерами, що робить його важливим детермінантом ноцицептивної (больової) сигналізації. TRPV1 також бере участь у реалізації еферентної (тобто рухової, або секреторної) функції чутливих нервових закінчень аферентних нейронів (тобто тих, що сприймають та передають збудження). Це відбувається завдяки вивільненню з цих закінчень у відповідь на активацію TRPV1 біоактивних нейропептидів, що у свою чергу діють на клітин органів, які ці закінчення інервують. Однак присутність TRPV1 була також виявлена у багатьох тканинах і клітинах за межами сенсорної нервової системи, де його активація та функція залишаються не зовсім зрозумілими. В доповіді будуть представлені результати власних досліджень на окремих клітинах і багатоклітинних препаратах м'язового шару сечового міхура (детрузора) щурів, які показують, що TRPV1 функціонально експресується у гладком'язових клітинах, у яких він діє не як плазмалемальний канал входу кальцію, а як активований капсаїцином канал вивільнення Ca^{2+} з ендоплазматичного ретикулума, що відповідає за підвищення внутрішньоклітинної концентрації кальцію. Скороченнях багатоклітинних препаратів детрузора, гладком'язові волокна яких переважно розташовувалися у поздовжньому, але не у циркулярному напрямках, викликані м-холіноміметиком карбахолом, містили TRPV1-залежний компонент. Активація TRPV1 у гладких м'язах детрузора під час мускаринової холінергічної стимуляції забезпечувалася каталізованим фосфоліпазою А2 утворенням арахідонової кислоти та її перетворенням ліпоксигеназами в ейкозаноїди, які діють як ендогенні агоністи TRPV1. Зроблено висновок, що TRPV1 є суттєвим фактором холінергічного скорочення поздовжніх гладком'язових волокон сечового міхура, що може бути важливим для створення просторової та/або часової анізотропії деформації стінки сечового міхура в різних ділянках під час парасимпатичної стимуляції.

Session 1: Cell Biology / Клітинна біологія

MECHANISMS OF RESISTANCE TO AMBIENT STORAGE OF MESENCHYMAL STEM CELLS AFTER INCAPSULTION IN ALGINATE HYDROGEL

Oleksandr Petrenko **Lecture 7**

Oleksandr Petrenko[1,2], Nataliya Trufanova[1], Oleksandra Hubenia[1,3], Oleg Trufanov[1], Olena Revenko[1], Yuriy Kot [2], Daria Cherkashina[1]
[1]Institute for Problems of Cryobiology and Cryomedicine of National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2]V.N. Karazin Kharkiv National University, Kharkiv, Ukraine
[3]Institute for Multiphase Processes, Leibniz University Hannover, Garbsen, Germany
Email address: petrenko@cryonas.org.ua

The application of mesenchymal stromal/stem cells (MSCs) in medical and research practice requires effective methods of storage and transportation. Storage under conditions of moderate hypothermia at ambient temperatures attracts increasing attention as a safe, simple, and effective method. Therefore, the issue of determining the optimal storage times for MSCs at ambient temperatures, the optimal three-dimensional organization of cells (monolayer culture, suspension, three-dimensional microenvironment of alginate microspheres (AMSs), etc.), is relevant. For ambient storage of various cell types, the protective effect of alginate hydrogel has been demonstrated, but the mechanisms of resistance development in encapsulated cells require investigation.

Aim: To determine the parameters of viability, metabolic status, cell cycle, and resistance to oxidative stress of MSCs in monolayer, suspension, and encapsulated in alginate microspheres, during cultivation and storage at a temperature of 22°C.

Methods: Human adipose-derived MSCs were used (obtained with informed consent from adult donors in compliance with the principles of biomedical ethics according to the requirements of the Institutional Committee on Bioethics). AMSs were obtained by electrospinning. MSCs in the form of monolayer, suspension, and in AMSs were stored in closed cryovials at a temperature of 22°C in complete culture medium for up to 14 days. Viability (staining with fluorescein diacetate (FDA) / ethidium bromide (EB)), and metabolic activity (Alamar Blue) were assessed before and after storage. Mitochondrial potential (JC-1), and cell cycle analysis (Premo™ FUCCI) were assessed during culture. Oxidative stress was induced by hydrogen peroxide in various concentrations. The level of reactive oxygen species (ROS) cultured in monolayer and AMSs cells was measured using the Abcam Cellular ROS Kit.

Results and discussion: Encapsulation of MSCs in AMSs maintained the viability of MSCs during storage, while in monolayer and suspension, a sharp decrease in this parameter was observed. Encapsulation of MSCs caused a decrease in metabolic and mitochondrial activity, reversible cell cycle arrest in the G1 phase. Encapsulated cells were also characterized by a lower basal level of ROS and increased resistance to the action of hydrogen peroxide compared to the monolayer.

Conclusions: Encapsulation of MSCs in alginate microspheres induces a reversible transition of cells to a quiescent state with a decrease in metabolic activity, mitochondrial membrane potential, and ROS production, cell cycle arrest, as well as an increase in the resistance of human MSCs to oxidative stress, which supports the viability of cells in the alginate hydrogel for up to 7 days during ambient storage.

Acknowledgment: The research was funded by the National Research Foundation of Ukraine (project No. 2021.01/0276).

Session 1: Cell Biology / Клітинна біологія

ZINC-RELATED EFFECTS OF RARE EARTH ELEMENTS IN BIVALVE MOLLUSK UNDER SINGLE AND COMPLEX EXPOSURES

Oksana Stoliar **Lecture 8**

Oksana Stoliar[1], Vira Khoma[2], Kateryna Yunko[1], Viktoriya Martyniuk[1,3], Brigita Gylyte[4], Rolandas Karitonas[4], Levonas Manusadžianas[4], Lesya Gnatyshyna[5]
[1]Ternopil Volodymyr Hnatiuk National Pedagogical University, Ternopil, Ukraine
[2]Ternopil Scientific Research Forensic Center of MIA of Ukraine, Ternopil, Ukraine [3]Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine
[4]Nature Research Centre, Vilnius, Lithuania
[5]I. Ya. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
Email address: Oksana.Stolyar@tnpu.edu.ua

Environmental concentrations of rare earth elements (REEs) are rising abruptly due to their unique properties and corresponding implementation in many modern technologies, including military industry and medical equipment [1]. However, the biological effects of REEs are unclear [2]. Since REEs can interact with the targets of essential metals, the goal of this study was to study the effect of two REEs of high demand, Gadolinium (Gd) and Yttrium (Y), on the Zn-dependent activities in the tissues of bivalve mollusc *Unio tumidus*. The combine exposure with the nifedipine (Nfd) was also applied owing to its impact on the metals biological activities [3]. The specimens of *U. tumidus* were distributed to four groups: untreated mussels and treated with $GdCl_3$ (Gd, 30 nM), YCl_3 (Y, 30 nM), or their mixture (Mix) with Nfd (10 μM) during 14 days. Digestive gland was utilized for the analyzis.

In all treated groups, Zn concentration in the tissue, in its buffering protein metallothionein (MT) and the rate of non-bound to MT Zn increased. In the Gd- and Y-groups the part of non-metalated MT increased also, promoting elevated redox properties of SH-group. In opposite, in the Mix-group hypermetalation of MTs was indicated. All exposures caused the multiple elevation of the NADH&NAD^+ and GSH&GSSG concentrtations with increased NADH/NAD^+ ratio and cholinesterase oppression. Dynamin-related GTP-ase activity increased in all exposures. However, I and II phase biotransformation manifestations (Cytochrome P450-related (EROD) and GST activities) were dependent on the exposure indicating the similarity with single Nfd effect [3]. The highest impact of Gd was confirmed by decrease of lysosomal membrane integrity. The caspase-3 activity reduced in the Gd-group but increased by Mix.

Summarizing, these results confirm the interaction between REEs and essential metals functionality and cumulative effect in the multiple exposure with Ca-channel blocker.

Acknowledgement: The work was supported by bilateral Ukraine-Lithuania scientific project of Ministry of Education and Science of Ukraine (M/48-2024, M/48-2025) and Project No S-LU-24-13 under the Lithuanian-Ukrainian Cooperation Program.

References

1. N. Malhotra, H.S. Hsu, S.T et al. Animals (Basel). 2020. 10, 1663.
2. N. Lachaux, A Otero-Fariña, L.B Sohm et al. Sci. Total Environ. 2023. 905, 167302
3. V. Khoma, L. Gnatyshyna, V. Martinyuk, T. Mackiv, L. Mishchenk, L. Manusadžianas, O. Stoliar. Ecotoxicol. Environ. Saf. 2021. 208, 111695.

STABILITY OF mRNA, POLYUBIQUITINATION AND PROTEASOMAL DEGRADATION OF MRPS18 FAMILY GENES *IN VITRO*

Elena Kashuba **Lecture 9**

Larysa Kovalevska, Vladislav Chreshchenyuk, Elena Kashuba
RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine, Kyiv
E-mail: lenakash@yahoo.com, Kashuba@nas.gov.ua

The MRPS18 family genes (MRPS18-1-3) encode proteins that are part of the small subunit of the mitochondrial ribosome that exhibit extra-ribosomal functions, related to cell cycle control, apoptosis, epithelial-mesenchymal transition, and maintenance of pluripotency. Thus, the MRPS18-2 protein interacts with the retinoblastoma protein (RB), freeing transcription factor E2F1 and promoting G_1-S transition. The transcription factor KLF4, known as a stem cell marker and as oncogen, is involved in the activation of MRPS18-2 gene transcription.

Earlier we found that MRPS18-2 is overexpressed in tumor cells of endometrial, prostate, liver, and breast cancer, in lymphomas and gliomas as well. Other family members, MRPS18-1 and MRPS18-3, remain much less characterized, except breast cancer, lymphomas and gliomas.

There are no data on protein and mRNA stability for the MRPS18 family genes. It is very important for understanding of molecular mechanisms of malignant cell transformation. We aimed to analyze the stability of mRNA of MRPS18 family genes (MRPS18-1-3) and their protein products *in vitro*. The kinetics of mRNA degradation was monitored upon treatment with actinomycin D (1 μg/ml) and quantitative PCR (qPCR). Ubiquitination and proteasomal degradation of proteins were studied, using pGEM constructs and T7/SP6 Coupled Wheat Germ Extract System.

We found that the mRNA of the *MRPS18-2* gene was characterized by the lowest stability among the analyzed transcripts (T½ ≈ 15–16 hours), while MRPS18-1 and MRPS18-3 demonstrate higher stability (≈20 and 22–23 hours, respectively).

The MRPS18-2 protein showed a high stability (T½>4 hours). At the same time, the T½ of the MRPS18-3 protein is approximately 45 min, and the MRPS18-1 protein is most likely rapidly destroyed by proteases (T½ no more than 5 min). More should be done to elucidate how mRNA and protein stability influence protein functions.

The work was supported by the grant No. 0123U100099 of the NAS of Ukraine “Study of molecular mechanisms of inhibition of embryonic tumor growth by inactivation of MRPS18-2 protein”.

EXOGENOUS PROLINE AND GABA REGULATE STOMATA AND CAUSE ULTRASTRUCTURAL CHANGES IN ARABIDOPSIS LEAF CELLS UNDER DROUGHT

Liudmyla Kozeko **Lecture 10**

Liudmyla Kozeko[1,2], Dmytro Klimchuk[1], Yuri Akimov[1], Yulia Ovcharenko1, Sigita Jurkonienė[2]
[1]M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Nature Research Centre, Akademijos str. 2, 08412, Vilnius, Lithuania
Email address: *liudmyla.kozeko@gmail.com*

The proteinogenic amino acid proline and the non-protein amino acid gamma-aminobutyric acid (GABA) accumulate in plant cells under stressful conditions and play different roles in cellular defence. GABA regulates stomatal closure (Xu et al., 2021). Treatment of plants with these bioactive molecules improves drought tolerance (Jurkonienė et al., 2023).

In this study, we investigated effects of foliar application of proline and GABA on stomata behaviour and leaf ultrastructure under drought in the model species *Arabidopsis thaliana* (Col-0). Long-day plants grown under normal conditions were sprayed with 0.1 mM proline and 0.1 mM GABA, and then half of the plants in each treatment group were subjected to soil drying for 7 days. Control plants were well-watered. Using scanning electron microscopy, it was found that not only exogenous GABA, but also proline reduced stomatal aperture width on both the adaxial (15% and 23%, respectively) and abaxial (46% and 40%) surfaces of leaves under drought, which may diminish stomatal conductance. Transmission electron microscopy revealed changes in the ultrastructure of palisade parenchyma cells under drought after application of amino acids. The length/width ratio of chloroplasts increased by 26% in the variant with proline and 66% in the variant with GABA that associated with a decrease in the partial volume of grana thylakoids (20% and 16%, respectively) and size of plastoglobules (37% and 18%). At the same time, a specific effect of GABA was an increase in the partial volume of stroma thylakoids (28%) and a significant decrease in the partial volume of starch grains per chloroplast (65%), suggesting that exogenous GABA affects starch and sucrose metabolism under water deficit. In addition, treatment with proline and GABA led to a decrease in peroxisome diameter (14% and 24%), which may indicate an improvement in the oxidative status of plants.

Thus, the increase in drought tolerance of plants under the influence of these bioactive molecules may be due to the reduction of stomatal aperture and specific structural changes in leaf cells, mainly in chloroplasts.

Acknowledgment:
The research was funded by the Joint Ukrainian-Lithuanian R&D Program of the Ministry of Education and Science of Ukraine (projects M/49-2023) and the Research Council of Lithuania (projects S-LU-22-4).

References:

1 B. Xu, Y. Long, X. Feng, X. Zhu, N. Sai, L. Chirkova, A. Betts, J. Herrmann, E. Edwards, M. Okamoto, R. Hedrich, M. Gilliham, *Nat. Commun*. **2021**, 12(1), 1952.

2 S. Jurkonienė, R. Mockevičiūtė, V. Gavelienė, V. Šveikauskas, M. Zareyan, E. Jankovska-Bortkevič, J. Jankauskiene, T. Žalnierius, L. Kozeko, *Plants*, **2023**, 12, 2718.

RESEARCH OF THE MOLECULAR MECHANISMS FOR THE PATHOLOGIC IMPACT OF VARICOCELE ON FERTILITY POTENTIAL

Anna Havrylyuk **Lecture 11**

Nakonechnyi Y.[1], Nakonechnyi A.[1], Fraczek M.[2], Havrylyuk A.[1], Kamieniczna M.[2], Chopyak V.[1], Kurpisz M.[2]

1 – Department of Clinical Immunology and Allergology, Urology of Medical University named Danylo Halytzkyy, Lviv, Ukraine

2 – Department of Biology of Reproduction and Stem Cells of Institute of Human Genetic of Polish Akademy of Science, Poznan, Poland

Varicocele is a pathologic dilatation of the testicular pampiniform venous plexus and considered to be one of the main causes of the male infertility. Varicocele is associated with decreasing in fertility potential, because with this disease is happening: 1) decreased DNA repair potential; 3) heat stress; 4) local ishemia – dilution of paracrine hormones; 2) testicular hypoperfusion/stasis. Present with varicocele oxidative stress: 1) decreased sperm motility; 2) decreased spermatogenesis; 3) increased DNA fragmentation; 4) decreased sperm ATP production; 5) causes mitochondrial dysfunction, increased autophagy, apoptosis and endoplasmic reticulum stress [1, 2, 3].

We were examined 57 infertility males with a primary, left-side varicocele, grade II-III from 19 to 33 years of age and 25 healthy individuals. Catalase, superoxide dismutase, malondialdehyde, total antioxidant capacity, alpha-glucosidase, fructose, citric acid were measured in seminal plasma, sperm DNA fragmentation – in spermatozoa.

In men with infertility and varicocele the level of malondialdehyde was statistically significant highest. The levels of catalase, superoxide dismutase and total antioxidant capacity in patients with varicocele was statistically significant lower. The level of sperm DNA fragmentation was statistically significant and 7.6 fold higher than in healthy males ($p<0,001$). In seminal plasma of patients with varicocele was found a statistically significant (3,0 – fold) increased concentration ($p<0,013$) of α-Glu in comparison to control group.

We evaluated that changes, causing infertility in patients with varicocele, are the imbalance between oxidants/antioxadants and lowered sperm DNA intergrity. We propose five sequential steps in the examination of these patients: 1) the definition of sperm parameters (volume of semen, total number of spermatozoa, motility a+b, sperm viability and morphology); 2) the study of oxidative stress parameters and determination of some substances with pro- and antioxidant effects; 3) the study of DNA damage in spermatozoa; 4) the status of carriage-systems Na^+K^+ATPase and $Ca^{2+}Mg^{2+}$ATPase; 5) the levels of major biochemical parameters in seminal plasma. The results of the research will allow you to predict the degree of risk of infertility.

References:

1. N. Kumara, N. K. Singh European Journal of Obstetrics & Gynecology and Reproductive Biology, **2020**, 253, 170–179.
2. M. Camargo, E. Ibrahim, P. Intasqui et al. Human Fertility, **2021**, 275, 1 - 9.
3. R. Finelli, F. Pallotti, F. Cargnelutti et al. Andrologia, **2021**, 53, 1 - 6.

THE ROLE OF LEAF SURFACE IN PLANT ABIOTIC STRESS TOLERANCE

Mykola Borysyuk **Lecture 12**

Mykola Borysyuk

Institute of Cell Biology and Genetic Engineering, NAS of Ukraine, Kyiv, Ukraine

Understanding the physiological, biochemical, and genetic mechanisms which allow plants to cope with environmental challenges is of vital importance for breeding crops with improved stress tolerance and performance. Plant leaves, as well as other areal organs are covered by cuticle, a "plant's skin" represented by continuous hydrophobic sheet formed as an exterior extension of epidermal cell walls [1]. As an interface in plants' interactions with environment, cuticle is a crucial barrier which in concert with stomata, controls plant water status and helps plants to survive under challenging environmental conditions. Our decade-long investigation of leaf surface in terrestrial wheat plants and aquatic duckweeds uncovered a number of basic biochemical and molecular features relating cuticle to plants' responses to drought and UV radiation stresses. In the course of our earlier studies, we *characterized biochemical features of cuticle in a range of Australian wheat cultivars [2], and revealed gene networks controlling leaf surface features and drought tolerance. O*verexpression of wheat transcription factor *TaSHN1* affected composition of cuticle waxes, frequency of stomata and drought tolerance of the transgenic wheat lines [3]. More recently, *we complemented these studies with the* data on leaf surface analysis in Ukrainian wheat varieties with contrasting drought tolerance, focusing on stomata physiology and key molecular factors controlling stomata frequency. In particular, we characterized cultivar-specific expression and promoter structure of genes encoding EPF polypeptides and transcription factor *MUTE*, the key components controlling stomata biogenesis.

In contrast to terrestrial plants, with the main function of cuticle in protecting areal tissues from water lost, aquatic plants, like duckweeds, have an unlimited water supply. Consequently, the cuticle should function more as a protector against harmful UV-radiation and pathogens. Our analysis showed that compared to the cuticle in wheat, composed by various *derivatives of the long fatty acids, the* cuticle in duckweed species contained mostly free fatty acids and primary alcohols [4]. Moreover, a large portion of the duckweed wax fraction was represented by phytostrerols (campesterol, stigmasterol, sitosterol and their common precursor squalene). The cryo-SEM microscopy uncovered significant differences between the surface structures of the top, air-facing and bottom, water-facing sides of the duckweed fronds. The top side of the fronds, containing multiple stomata complexes, is represented by a flat waxy film sporadically covered with wax crystals. On the bottom side of the fronds, the large epidermal cells were covered by the well-structured net. These structural differences between the abaxial and adaxial sides of the fronds evidently relate to their distinct physiological roles in interacting with the contrasting environments of sunlight/air and nutrients/water. The revealed unique structural and biochemical features of terrestrial (wheat) and aquatic (duckweed) plants pave the way for discovering specific biochemical/molecular components specifically contributing to plants protection against water limitation and UV-related stresses.

1. Borisjuk N, Hrmova M, Lopato S. *Biotechnol Advances,* 2014, 32: 526-540, doi:10.1016/j.biotechadv.2014.01.005
2. Bi H, Kovalchuk N, Langridge P, Tricker P, Lopato S, Borisjuk N. *BMC Plant Biology*, 2017, 17: 85, doi:10.1186/s12870-017-1033-3.
3. Bi H, Shi J, Kovalchuk N, Luang S, Bazanova N, Chirkova L, Zhang D, Shavrukov Y, Stepanenko A, Tricker P, Langridge P, Hrmova M, Lopato S, Borisjuk N. *Plant Cell and Environment*, 2018, 41:2549–2566. doi: 10.1111/pce.13339
4. Borisjuk N, Peterson A, Lv J, Qu G, Luo Q, Chen G, Kishchenko O, Zhou Y, Shi J. *Frontiers in Chemistry*, 2018, 6:317, doi: 10.3389/fchem.2018.00317

ВЗАЄМОЗВ'ЯЗОК СТРЕСУ ЕНДОПЛАЗМАТИЧНОГО РЕТИКУЛЮМУ ТА ДИСФУНКЦІЇ МІТОХОНДРІЙ У В-КЛІТИНАХ ПІДШЛУНКОВОЇ ЗАЛОЗИ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТІ

Олена Канюка **Lecture 13**

Канюка О., Сибірна Н.
Львівський національний університет імені Івана Франка
вул. Грушевського, 4, м. Львів, 79005, Україна
e-mail: kanukaolena@gmail.com

Цукровий діабет 1-го типу (ЦД1) – це аутоімунне захворювання, при якому відбувається руйнування бета-клітин підшлункової залози, що призводить до зниження продукції інсуліну. Гіперглікемія, яка при цьому виникає, може призводити до надмірного накопичення неправильно згорнутих білків і як наслідок розвитку стресу ендоплазматичного ретикулюму (ЕР). Одночасно надлишок вільних радикалів може також призводити до пошкодження мітохондрії. Накопичення пошкоджених мітохондрій має серйозні наслідки, а саме: зменшення утворення АТФ та додаткове збільшення кількості активних форм оксигену , вивільнення проапоптотичних факторів, такі як цитохром С, порушення транспорту кальцію з подальшою загибеллю клітини. Тому важливим критерієм функціонального стану клітини є оцінка ступеня стресу ЕР та мітофагії. Важливу роль у зв'язку між мітохондріями та ЕР, а також у регуляції мітофагії відіграють мембрани ендоплазматичного ретикулуму, асоційовані з мітохондріями (MAMs). MAMs слугують платформою для взаємодії між мітохондріями та аутофагосомами та містять ряд білків, які відповідають за процес активації мітофагії. За результатами дослідження, у щурів із стрептозотоцин-індукованим діабетом спостерігалося зниження поглинання флуоресцентного аналога глюкози 2-NBDG у 18,58 разів порівняно контрольною групою, а також показано зниження рівня АТФ у клітинах підшлункової залози на 20%. При пошкодженні мітохондрій або при зниженому постачанні кисню клітини змушені переходити на анаеробний метаболізм, при якому глюкоза метаболізується до лактату без участі кисню. Нами було виявлено збільшення вмісту лактату в лізатах підшлункової залози у 1,9 раза, а також активності лактатдегідрогенази у 1,3 раза у щурів із стрептозотоцин-індукованим діабетом. Таким чином, при цукровому діабеті 1-го типу, особливо на фоні хронічного оксидативного стресу, порушується нормальне функціонування мітохондрій, що призводить до переходу клітин на анаеробний метаболізм і збільшеного утворення лактату. Одночасно підвищена активність лактатдегідрогенази допомагає компенсувати енергетичний дефіцит клітин в умовах недостатнього постачання кисню та порушеної функції мітохондрій. Методом флуоресцентної мікроскопії було встановлено, що в умовах експериментального діабету рівень кальцію в ізольованих острівцях Лангерганса знижується в 2,2 раза, а за даними спектрофлуориметрії — у 2,7 раза, що свідчить про виснаження кальцієвого пулу.

Таким чином, при ЦД1 спостерігається тісно пов’язане порушення як енергетичного, так і кальцієвого метаболізму, що спричинене мітохондріальною дисфункцією, оксидативним стресом та стресом ЕР.

ДОСЛІДЖЕННЯ АКУМУЛЯЦІЇ ІОНІВ КАЛІЯ МІТОХОНДРІЯМИ ГЛАДЕНЬКОГО М'ЯЗУ МАТКИ З ВИКОРИСТАННЯМ МОДУЛЯТОРІВ K^+-КАНАЛІВ РІЗНИХ ПІДТИПІВ

Марія Рудницька (*молода вчена*) **Lecture 14**

Марія Рудницька, Максим Павлюк, Ганна Данилович, Юрій Данилович
Інститут біохімії ім. О.В. Палладіна НАН України, Київ, Україна
E-mail: *maria.rudnytska.v@gmail.com*

Суттєву роль у функціонуванні мітохондрій відіграють іони Са, активуючи мітохондрійні дегідрогенази, синтез активних форм азоту та кисню тощо. Водночас перевантаження Ca^{2+} матриксу призводить до відкриття пори перехідної провідності та вивільнення у цитозоль проапоптичних факторів. Іони К забезпечують контроль внутрішньоклітинного Ca^{2+}-гомеостазу, беруть участь у формуванні електричного потенціалу внутрішньої мітохондрійної мембрани, впливають на перебіг метаболічних процесів та осморегуляцію [1, 2]. Надходження K^+ в матрикс мітохондрій забезпечується K^+-каналами різних підтипів, що локалізовані у їхній внутрішній мембрані. Якщо наявність АТР-чутливого K^+-каналу в мітохондріях міометрія доведена [3], то можливість функціонування інших підтипів K^+-каналів в цих субклітинних структурах гладенького м'яза матки наразі не з'ясована. Тому метою наших досліджень було ідентифікувати різні підтипи K^+-каналів в мітохондріях міометрія щурів, використовуючи специфічні інгібітори/активатори.

Ізольовані мітохондрії одержували з міометрія невагітних щурів лінії Вістар методом диференційного центрифугування. Акумуляцію K^+ вивчали методом спектрофлуориметрії за допомогою K^+-чутливого флуоресцентного зонду PBFI-AM (10 мкМ, $\lambda_{зб}$ = 340/380 нм, $\lambda_{фл}$ = 480 нм).

Було продемонстровано, що мітохондрії міометрія ефективно акумулюють K^+ в діапазоні концентрацій 25-150 мМ. У разі заміни K^+ на холіну хлорид в еквімолярних концентраціях зростання флуоресценції зонда PBFI не спостерігали.

У присутності 5 мМ тетраетиламонія або 4-амінопіридина (інгібіторів потенціал-керованих K^+-каналів), 20 нМ харібдотоксина або 10 мкМ паксиліна (блокаторів Ca^{2+}-залежних K^+-каналів), 20 мкМ глібенкламіда або 200 мкМ 5-гідроксидеканоєвої кислоти, а також 200 мкМ АТР (інгібіторів мітоК$_{АТР}$) спостерігали суттєве зниження флуоресцентного сигналу від PBFI.

У той же час використання специфічних активаторів Ca^{2+}-залежних K^+-каналів 5 мкМ NS11021 та 50 мкМ NS1619, а також специфічного щодо мітоК$_{АТР}$ 10 мкМ кромакаліма супроводжувалося суттєвим зростанням акумуляції K^+ в мітохондрії.

Ефективність акумуляції K^+ зростала за додавання 25-100 мкМ Ca^{2+} (у присутності 4-амінопіридина та АТР).

Одержані результати свідчать на користь наявності в мітохондріях міометрія, крім мітоК$_{АТР}$, потенціал-керованих та Ca^{2+}-залежних підтипів K^+-каналів.

Література:

1. M. Laskowski, B. Augustynek, B. Kulawiak, P. Koprowski, P. Bednarczyk, W. Jarmuszkiewicz, A. Szewczyk. *Biochim. Biophys. Acta*, **2016,** 1857(8), 1247-1257.

2. B. Kulawiak, P. Bednarczyk, A. Szewczyk, *Cells*, **2021**, 10(6), 1554.

3. O. Vadzyuk, S. Kosterin. *Saudi J. Biol. Sci.*, **2018**, 25(3), 551-557.

CONFORMATIONAL DIVERGENCE OF EEF1A PARALOGUES AS A KEY TO THEIR DIFFERENTIAL DISEASE ASSOCIATIONS

Boris Negrutskii **Lecture 15**

Oleksandra Novosylna[1], Vyacheslav Shalak[1], Ilias Patmanidis[2], Dmytro Lozhko[1], Michał Dadlez[3], Boris Negrutskii[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, 03143 Ukraine
[2]iNANO, Aarhus University, Aarhus, 8000, Denmark
[3]Institute of Biochemistry and Biophysics, PAN, Warsaw, 02-106 Poland

The paralogous translation elongation factors eEF1A1 and eEF1A2 share 97% sequence identity and carry out comparable roles in protein synthesis; however, they are expressed in mutually exclusive patterns across human tissues. Despite their high sequence similarity, the two paralogs are implicated in distinct pathological processes: eEF1A1 is primarily associated with viral infections, while eEF1A2 is more closely linked to oncogenesis and neurodegenerative disorders. The molecular mechanisms underlying these functional divergences remain largely unclear, likely due to paralog-specific interactions with distinct protein partners, including the formation of actin bundles with differing architectures.

Using a combination of hydrogen–deuterium exchange mass spectrometry and molecular dynamics simulations we show that eEF1A1 and eEF1A2 differ markedly in their structural dynamics. eEF1A2 adopts a compact, stably folded conformation, whereas eEF1A1 displays significant conformational flexibility—including dynamic domain rearrangements between D1 and D3 and substantial internal and external mobility within domain D2. These dynamic features promote dimerization of eEF1A1, in contrast to eEF1A2, which remains monomeric in solution, challenging previous crystallographic findings.

Together, these results provide molecular insight into the distinct structural behaviors of eEF1A1 and eEF1A2, offering a potential explanation for their differential non-translational functions and disease associations.

This work was supported by National Research Foundation of Ukraine grant [2023.03/0072 “Highly homologous paralogs of translation elongation factor 1A. Structural studies to understand functional differences”].

THE LONG-TERM BIOLOGICAL EFFECTS OF NOVEL GRAPHENE OXIDE NANOMATERIALS

Tetiana Dumych **Poster 1**

Tetiana Dumych[1], Solomiya Paryzhak[1], Nadia Skorokhyd[2], Anton Senenko[3], Uwe Ritter[4], Yuriy Prylutskyy[5], Rostyslav Stoika[2], Rostyslav Panchuk[2]
[1]Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[2]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[3]Institute of Physics of the NAS of Ukraine, Kyiv, Ukraine
[4]Technical University Ilmenau, Institute of Chemistry and Biotechnology, Ilmenau, Germany
[5]Taras Shevchenko National University of Kyiv, ESC "Institute of Biology and Medicine", Kyiv, Ukraine
Email address: *tetiana.dumych@gmail.com*

Graphene oxide (GO) possesses unique structural and physicochemical properties, such as a layered structure and a high concentration of oxygen-containing functional groups. These features make it ideal for the functionalization and controlled delivery of therapeutic agents in nanomedical applications. However, using GO requires a comprehensive investigation of its long-term safety and interaction with biological systems. It was shown that high doses of GO can induce lung toxicity, inflammation, and systemic side effects [1, 2]. Therefore, here we evaluated the long-term *in vivo* safety and biocompatibility of GO as a potential nanocarrier for drug delivery applications.

GO was administered at doses of 2 and 5 milligrams per kilogram (mg/kg) every other day to laboratory mice, resulting in cumulative doses of 10 and 25 mg/kg, respectively. To monitor delayed effects, assessments were conducted 15, 30, and 60 days after the last injection. Behavioral observations and body weight changes suggested dose-dependent effects. There was reduced activity and weight loss in the higher-dose group. Hematological analysis revealed increased neutrophil percentages and decreased lymphocytes in the 5 mg/kg group, indicating an immune response. In contrast, the 2 mg/kg dose showed no significant alterations. Serum biochemical markers for hepatic and renal function remained largely unchanged, suggesting that GO is biocompatible at appropriate doses.

In total, a comprehensive assessment of the biocompatibility of the newly developed form of GO and its novel administration method was carried out in mice to ensure its optimal biosafety as a drug delivery system. However, due to observed signs of toxicity at a cumulative dose of 25 mg/kg, the recommended maximum dose should be limited to 10 mg/kg. At this dosage, achieving a high drug loading efficiency is crucial, at least 50%. Further studies on pre-clinical evaluation of GO conjugates with anticancer drugs are in progress.

Acknowledgment:
The research work was supported by the Scholars Without Borders grant from the Davis Center at Harvard University and the Carnegie Corporation of New York.

References:
1. X. Zhang, J. Yin, C. Peng, W. Hu, Z. Zhu, W. Li, C. Fan and Q. Huang, Carbon N. Y., 2011, 49, 986–995.
2. A. Rhazouani, H. Gamrani, S. Ed-Day, K. Lafhal, S. Boulbaroud, L. Gebrati, N. Fdil and F. AZIZ, Food Chem. Toxicol., 2023, 171, 113553.

EFFECT OF *ATG1, ATG6, ATG15* GENE DELETIONS ON FORMALDEHYDE DEHYDROGENASE AND FORMATE DEHYDROGENASE DEGRADATION IN THE METHYLOTROPHIC YEAST *KOMAGATAELLA PHAFFII*

Uliana Pelypyshyn (*young scientist*) **Poster 2**

Dmytruk Kostyantyn[1], Dmytruk Olena[1], Pelypyshyn Uliana[1], Sibirny Andriy[1,2]
[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, 79005 Ukraine;
[2]Department of Biotechnology and Microbiology, University of Rzeszow, Rzeszow, 35-601 Poland.

Understanding the mechanisms behind the degradation of cytosolic proteins is crucial for both fundamental research and practical applications. This study examined how the cytosolic enzymes formaldehyde dehydrogenase (*Fld1*) and formate dehydrogenase (*Fdh1*) are degraded in various strains of the methylotrophic yeast *Komagataella phaffii*. It was demonstrated that *Fld1* and *Fdh1* are degraded via the vacuolar pathway, regardless of the methanol induction duration. This study was confirmed by analyzing changes in specific enzymatic activity, conducting Western blot analysis, and performing fluorescence microscopy studies.

The disruption of the *Atg1*, *Atg6*, and *Atg15* genes — essential for the initiation steps of autophagy, the formation of autophagosomes, and the production of lipases necessary for degrading autophagic body membranes in the vacuole — impacted the degradation of *Fld1* and *Fdh1*. This was indicated by their specific activity levels, similar to those observed in strain *SMD1163*, which has a defective autophagy pathway. These results imply that *Fld1* and *Fdh1* are degraded through the vacuolar microautophagy pathway.

EFFECTS OF PYRAZOLE-CONTAINING BISPHOSPHONATES ON THE MEVALONATE PATHWAY, APOPTOSIS, AND GENE EXPRESSION IN J774A.1 MACROPHAGES *IN VITRO*

Dmytro Labudzynkyi[1], Elvira Pasichna[1], Serhii Ivonin[2], Dmytro Volochnyuk[2], Mykola Veliky[1]
[1]Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine;
[2]Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
Email address: *labudzinskidmytro@gmail.com*

Disruption of the mevalonate pathway is a well-established mechanism of action for bisphosphonates (BPs) in osteoclast precursors. This study investigates the effects of novel pyrazole-containing BPs on isoprenoid and cholesterol biosynthesis, FPPS enzymatic activity, apoptosis, and gene expression in J774A.1 macrophages.

[^{14}C]-acetate incorporation assays were used to quantify isoprenoid and cholesterol synthesis following 6-day and 18-hour treatments of J774A.1 macrophages with BPs at concentrations of 10 and 100 μM, respectively. Farnesyl pyrophosphate synthase (FPPS) activity was assessed using a modified biochemical assay after 10 μM BP treatment. Apoptosis was evaluated via Annexin V-FITC staining, and mRNA expression levels of *NFATc1*, *carbonic anhydrase II*, and *IL-1β* were analyzed by RT-PCR after 24-hour exposure to 10 μM BPs. Compounds 11N3, 11N, and 11N5 significantly reduced isoprenoid biosynthesis by up to 63% after 6 days. In the short-term treatment (18 h), 11N3 and 11N5 decreased isoprenoid synthesis by 58% and 41%, respectively, comparable to zoledronic acid (53%). Cholesterol synthesis was not significantly affected. At 10 μM, all compounds markedly inhibited FPPS activity, with 11N and 11N5 reducing activity by 69.5% and 65.2%, respectively; zoledronic acid showed 92% inhibition. All tested compounds increased the proportion of Annexin V-positive cells, with 11N3 and 11N doubling this population, and zoledronic acid causing a 377% increase. RT-PCR analysis revealed a notable downregulation of *carbonic anhydrase II* and *NFATc1* (up to 48%) by 11N, 11N3, and 11N4, and a strong upregulation of *IL-1β* mRNA (4–6-fold) by all compounds.

Pyrazole-containing bisphosphonates effectively inhibit key enzymes of the mevalonate pathway and modulate apoptotic and inflammatory responses in macrophages. Compounds 11N and 11N5 demonstrate the most promising profiles as potential antiresorptive agents with reduced cytotoxicity compared to classical nitrogen-containing BPs.

Acknowledgment:
The research was funded by the scientific research project of young scientists of the National Academy of Sciences of Ukraine 2021-2022 (project No. 0121U112008).

EFFECTS OF CALIXARENE-METHYLENE-BISPHOSPHONIC ACIDS ON THE PHAGOCYTIC AND METABOLIC ACTIVITIES OF RAW264.7 MACROPHAGES *IN VITRO*

Dmytro Labudzynkyi[1], Andrii Siromolot[1], Elvira Pasichna[1], Vasyl Bilous[1], Anna Selikhova[2], Serhii Cherenok[2], Mykola Veliky[1]

[1]Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine;
[2]Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
Email address: labudzinskidmytro@gmail.com

Osteoporosis results from an imbalance in bone remodeling, with excessive osteoclast activity leading to decreased bone density and increased fracture risk. Bisphosphonates, synthetic analogs of pyrophosphate, are first-line treatments. Given the increasing interest in supramolecular chemistry and the current absence of calixarene-based bisphosphonates, this study investigates the effects of newly synthesized calixarene-methylene-bisphosphonic acids (CMBAs) on metabolic viability and phagocytic activity in RAW264.7 macrophages, which serve as osteoclast precursors.

RAW 264.7 macrophages were cultivated with CMBA in DMEM medium (4.5 g/L glucose). The inhibitory effect of the four synthesized CMBA (C145, C1249, C1336 and C1281) was compared with the action of the zolendronic acid at concentrations of 0.1-1,0 mM during 24-hour incubation. Metabolic activity of cells was studied by resazurin test. The enzymatic activities of malate dehydrogenase 2 (MDH2) and farnesyl pyrophosphate synthase (FPPS) were also assessed using classical biochemical approaches. Phagocytic activity was evaluated with FITC-labeled *E.coli* by flow cytometry. A significant reduction in RAW264.7 cell viability was observed at the highest concentration (1 mM) of all tested compounds compared to zoledronate. At the lowest concentration (10 μM), compound C145 showed the strong cytostatic effect, inhibiting whole cell dehydrogenase activity in RAW264.7 macrophages by 23%. Compound C1249 also demonstrated high efficacy, reducing cell viability similar to zoledronate by 21 and 19%, respectively. Incubation with 10 μM of compounds C145, C1249, and C1281 resulted in a reduction of MDH2 enzymatic activity by 15.0%, 22.2%, and 25.5%, respectively, compared to the control. Compounds C1336 and zoledronic acid caused a smaller decrease of 7.0% and 11.0%, respectively. Additionally, in a cell-free *in vitro* system, the activity of FPPS — a key enzyme of the mevalonate pathway—was markedly reduced at the same 10 μM concentration of CMBAs: by 85.0% (C145), 76.0% (C1281), and 72.0% (C1249), compared to control values. Notably, incubation of LPS-activated RAW264.7 macrophages with the same concentration of each CMBA compound led to an increase in phagocytic activity, showing 2.2-, 1.4-, 2.1-, 1.8-, and 2.0-fold elevations for zoledronic acid, C1336, C1249, C145, and C1281, respectively, relative to control.

The investigation revealed that among the novel calixarene-methylene-bisphosphonic acids, C1249 and C145 exhibited the most effective inhibitory effects on the proliferation and metabolic activity of RAW264.7 macrophage cells.

Acknowledgment:

The research was funded by Grants of the National Academy of Sciences of Ukraine to research laboratories / groups of young scientists of the National Academy of Sciences of Ukraine for conducting research in priority areas of science and technology 2024-2025 (project No. 0124U002507).

IMPACT OF ACTIVE VITAMIN D_3 FORMS ON THE VITAMIN D-ENDOCRINE SYSTEM, OSTEOCLAST FUSION, AND SURVIVAL DURING RANKL-INDUCED RAW 264.7 OSTEOCLASTOGENESIS

Dmytro Labudzynskyi, Olha Lisakovska, Ihor Shymanskyi, Denys Kolibo, Mykola Veliky
Palladin Institute of Biochemistry of NAS of Ukraine, Kyiv, Ukraine
Email address: *labudzinskidmytro@gmail.com*

Osteoclastogenesis is a complex process that plays a critical role in bone remodeling. Vitamin D hormonally active forms of (HAD_3) have a strong regulatory effect on osteoclast maturation and functioning, but this issue is still not well understood. The aim of our study was to determine the effects of different forms of HAD_3 on the RAW 264.7 cells, it`s proliferative activity, viability and level of multinuclear cells formation during RANKL-stimulated osteoclastogenesis.

The murine macrophage cell line RAW 264.7 was cultured with HAD_3 (50 nM 25OHD (25D), 10 and 100 nM $1,25(OH)_2D_3$ (1,25D)) in DMEM (4.5g/L glucose). Cell proliferative activity and apoptosis were assessed and visualized with Incucyte® ZOOM Live-Cell Analysis System. Bovine bone slices were stained with *TRAP* and Hoechst 33258 for TRAP-positive multinuclear cells detection. The levels of VDR, VDBP, CYP27A1, CYP27B1 and GLUT1 mRNA expression were examined by RT-PCR analysis. Exposure of RAW 264.7 cells with 50 nM 25D and 10 and 100 nM 1,25D during 70 h decreased cell proliferation and apoptosis by 4,7,11% and 10, 19, 35% respectively compared with control ($p<0.05$). While MTT test showed an increase in viability level under the action of all tested HAD_3 on RAW 264.7 cells. On the other hand, it was revealed a significant 21% and 31,8 % increase in the number of multinuclear osteoclast-like cells on bone slices under the effect of 50 nM 25D and 100 nM 1,25D respectively compared with control ($p<0.05$). 25D led to upregulation of VDR and GLUT1 and downregulation of VDBP and CYP27B1 mRNA expression; 10 nM 1,25D led to upregulation of CYP27A1 and GLUT1 and downregulation of VDBP and VDR mRNA expression; 100 nM 1,25D led to upregulation of VDR and downregulation of VDBP mRNA expression after 24 hours of RANKL-stimulated osteoclastogenesis compared with control ($p<0.05$).

Our results demonstrate that HAD_3 reduce the proliferation and apoptosis level in population of RAW264.7 macrophages, while increasing the level of they viability. At the same time, HAD_3 significantly affect the expression level of key D-endocrine system genes and on RANKL-induced fusion RAW264.7 macrophages.

INFLUENCE OF TRANSFORMING GROWTH FACTOR BETA 1 ON VASCULAR ENDOTHELIUM ADHESION IN THE TUMOR MICROENVIRONMENT OF HUMAN ACUTE LEUKEMIA

Vira Barilka[1], Volodymyr Matlan[2], Olha Shalay[1], Sofiya Prymak[1], Rostyslav Stoika[3], Vasyl Novak[1]
[1]State Institution "Institute of Blood Pathology and Transfusion Medicine, National Academy of Medical Sciences of Ukraine", Lviv, Ukraine
[2]Lviv National Medical University named Danylo Halytskyi, Lviv, Ukraine
[3]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: *virabarilka@ukr.net*

The tumor microenvironment of acute leukemia (AL), where vascular endothelium (VE) is an important component, undergoes remodeling under the significant influence of secreted cytokines, such as TGFβ1. TGFβ1 is an important cytokine for life. During the early stages of tumorigenesis, TGFβ1 acts as a tumor suppressor of various types of epithelial cells. However, at later stages, TGFβ1 often accumulated in the blood of AL patients (pts) and correlated with the negative course of the AL. TGFβ1 functions as a tumor promoter by inducing the loss of cell-cell contact and enhanced motility/adhesion of leukemic cells to the vascular endothelium (VE). At the same time, the final

role of TGFβ1 in the microenvironment of AL and adhesion of the AL to VE has not been established. The aim of the work was to establish the relationship between the concentration of TGFβ1 in plasma and 24-h supernatants (SP) of leukemia cells of AL pts and the intensity of adhesion of K562 and L1210 cells to the activated monolayer of VE by these samples in vitro. The concentration of TGFβ1 was determined by a biological method using a culture of mink lung cells of the CCL 64 line. VE was obtained from human umbilical vein (E.A.Jaffe, 1973). The study included 10 AL pts. Median of pts age was 68 years. The number of blasts in peripheral blood - 58.62±10.53% (not normally detected). We identified the attachment of labeled by 3H-thymidine cells lines K562, L1210 to the monolayer of VE n vitro after treated of plasma or SP of AL pts. The attachment of K562, L1210 to HUVECs was estimated by the index of adhesion (IA). K562, L1210 were not activated before the investigation. For control, VE cells that were not treated with experimental samples were used. TGFβ1 level in plasma of AL pts was higher than in healthy donors ($p<0.001$). Plasma showed stronger influence on adhesion to VE of K562 cells (IA=2.37±0.24), in comparison to L1210 (IA=1.28±0.8; $p<0.05$). Nevertheless, TGFβ1 levels in SP of blasts AL pts was two-fold higher than in plasma of AL pts and inversely correlated with leukemic cells number ($r= - 0.40$). Blasts of AL pts influenced on adhesive activity of HUVECs to K562 and L1210 (IA=1.99±0.38 and 1.92±0.24, respectively). SP of blasts secreted a higher level of TGFβ1 than it was in plasma pts ($p<0.05$).

Thus, leukemic cells were the powerful source of TGFβ1 secretion which was able to modulate the adhesive properties of VE and may pathogenetically influence on spreading of AL. TGFβ1 may be important in modifying the tumor microenvironment of leukemias and indicate important targets in the treatment of AL.

Acknowledgment:
We sincerely thank the National Academy of Medical Sciences of Ukraine for supporting the research and we sincerely thank the Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine, for the opportunity to present the results of the research work under the state registration number 0124U003739.

References:

1 W. Li, S.-Yu Ma, H.-Yi. Zhao, *World J. Clin Cases*, **2024**;*12* (20), 4121 – 4129.

ASSESSMENT OF FOLLICULOGENESIS IN OVARIAN TISSUE TRANSPLANTS AT DIFFERENT STAGES OF HISTOGENESIS

Yuliia Bozhkova, Viktoria Kiroshka, Evgeniy Legach, Galyna Bozhok,
Tetiana Bondarenko, Viktoria Ustichenko, Natalia Komaromi,
Alexandr Pakhomov, Iryna Rula
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine,
Kharkiv, Ukraine
Email: *bozhkova.jul@gmail.com*

There are numerous studies on the mechanisms of folliculogenesis in the ovaries. However, to solve women's reproductive problems, studying the factors influencing follicle growth and steroidogenesis in ovarian tissue after transplantation remains highly relevant.

The aim of our work was to assess the effect of the initial hormonal status of recipient animals on the follicle profile and sex hormone secretion in ovarian tissue transplants at different stages of histogenesis. To achieve this, allotransplantation of either mature ovarian tissue or neonatal ovaries was performed under the renal capsule, both simultaneously with ovariectomy and two months after it.

In animals with allografts of mature ovarian tissue implanted simultaneously with ovariectomy, normal folliculogenesis and preservation of endocrine function were observed on both the 30th and 60th days. In contrast, transplantation of mature tissue two months after ovariectomy resulted in cyst formation and a decrease in sex hormone levels by day 60.

Transplantation of neonatal ovaries at the time of ovariectomy led to the development of follicular cysts by day 30. When neonatal ovaries were transplanted two months after ovariectomy, follicle development and cyst formation were observed by day 30, followed by pronounced fibrosis of the transplant tissue by day 60.

Our findings demonstrate that the development of ovarian tissue transplants is determined by the age of the donor tissue and the initial hormonal status of the recipient. Mature ovarian tissue better preserves its morphofunctional characteristics when transplanted simultaneously with ovariectomy, whereas neonatal ovaries develop more successfully when transplanted into animals with a previously reduced level of sex hormones and elevated follicle-stimulating hormone levels.

SEARCH FOR MECHANISMS OF CELL DEATH INDUCED BY NOVEL PYRROLIDINEDIONE–THIAZOLIDINONE LES-6287 COMPOUND IN VARIOUS TYPES OF HUMAN BREAST CANCER CELLS

Yuliia Kozak[1], Natalya Finiuk[1], Agnieszka Gornowicz[2], Rostyslav Stoika[1], Roman Lesyk[3], Krzysztof Bielawski[2], Anna Bielawska[2]
[1] Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2] Medical University of Bialystok, Bialystok, Poland
[3] Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Email address: *juliana.kozzak@gmail.com*

Introduction: Mechanisms of cytotoxic effect of novel pyrrolidinedione–thiazolidinone hybrid Les-6287 compound had not been thoroughly studied. Here, we explored if autophagy might be involved in killing human breast cancer cells of different origin. Certain chemotherapeutics are known to activate autophagy as a part of their cytotoxic action. Autophagy plays a dual role in cancer development and therapy, contributing both to cell survival and cell death.

Aim: To evaluate the effect of Les-6287 on autophagy-related markers in hormone receptor-positive MCF-7 and triple-negative MDA-MB-231 human breast cancer cell lines.

Methods: Breast cancer cells were treated with Les-6287 or reference drug doxorubicin (1–2 μM) for 24 h. The expression level of the autophagy-related *BECN1* and *MAP1LC3B* genes was assessed using qPCR. Level of Beclin-1 and LC3B proteins was measured with ELISA.

Results: Les-6287 compound had no significant effect on the expression of *BECN1* and *MAP1LC3B* genes and measurement of the content of their protein products confirmed this trend. Les-6287 slightly reduced Beclin-1 and LC3B level in a dose-dependent manner, however, to a notably lesser extent than doxorubicin which significantly upregulated *BECN1* and *MAP1LC3B* expression in both hormone receptor-positive MCF-7 and triple-negative MDA-MB-231 human breast cancer cell lines. In the MCF-7 cells, Beclin-1 levels dropped from 4.08 ng/mL (control) to 2.78 ng/mL under treatment with 2 μM Les-6287, while the LC3B level was decreased from 574.84 ng/mL to 537.74 ng/mL. A similar slight reduction in the level of these autophagy-related proteins was observed in the MDA-MB-231 cells.

Conclusion: Les-6287 compound does not induce autophagy in breast cancer cells only slightly reducing the level of autophagy-related markers. Thus, we suggest that Les-6287 exerts its cytotoxic effects via autophagy-independent mechanisms.

Keywords: Autophagy, breast cancer, MCF-7, MDA-MB-231, LC3B, Beclin-1, Les-6287, thiazolidinone hybrid.

Acknowledgment: This work was partly supported by the Polish National Agency for Academic Exchange under the Strategic Partnerships program (grant agreement no. BPI/PST/2021/1/00002/U/00001).

Ethical Committee Approval: Not applicable.

ВПЛИВ ВИВІЛЬНЕНОГО НІКОТИНУ ТА ПОБІЧНОГО ПРОДУКТУ ФОТОЛІЗУ RU(BPY)$_2$NIC$_2$ НА СКОРОЧЕННЯ ГЛАДЕНЬКИХ М'ЯЗІВ ТРАХЕЇ ЩУРА

Д.І. Кравчук[1], І.Б. Філіппов[1], Г.В. Соткіс[1], А.П. Кусяк[2], О.Д. Щеглов[2], П.П. Горбик[2], Я.М. Шуба[1]

[1]*Інститут фізіології ім. О.О. Богомольця НАН України, м. Київ, Україна*
[2]*Інститут хімії поверхні ім. О.О. Чуйка НАН України, м. Київ, Україна*
not.now.d@biph.kiev.ua

Нікотин відомий своєю здатністю викликати залежність і підвищувати ризик захворювання на рак. Проте він все більше привертає увагу завдяки своїм численним нейрофармакологічним і фізіологічним ефектам. Як агоніст нікотинових ацетилхолінових рецепторів (нАХР), він демонструє здатність покращувати когнітивні функції, зокрема увагу, пам'ять та навчання. Крім того, зростає кількість досліджень, які вказують на можливі нейропротекторні властивості нікотину, що стимулює його вивчення у контексті таких нейродегенеративних захворювань, як хвороби Паркінсона та Альцгеймера. Окрім впливу на центральну нервову систему, нікотин виявляє аналгетичні властивості в доклінічних і клінічних дослідженнях, ймовірно, через вплив на ноцицептивні шляхи та модуляцію больової чутливості. Водночас, відомо широкий спектр негативних ефектів нікотину на організм. Його системна дія пов'язана з підвищеним ризиком серцево-судинних захворювань, включно з гіпертензією, атеросклерозом та порушеннями серцевого ритму. Крім того, тривалий вплив нікотину під час вагітності або в ранньому віці несе негативні наслідки на розвиток нервової системи, зокрема на когнітивне та поведінковове функціонування. Також значна кількість досліджень свідчить про потенційно канцерогенні властивості нікотину, які реалізуються через вплив на клітинну проліферацію, ангіогенез і зниження ефективності апоптозу. Тому терапевтичне використання нікотину значною мірою обмежене його неспецифічною дією, що охоплює різні тканини та системи організму. У зв'язку з цим, перспективним підходом є локалізоване й контрольоване у часі вивільнення нікотину за допомогою фоточутливих кейдж-сполук. Ці сполуки є біологічно інертними, але у відповідь на світлове опромінення вони вивільнюють активну молекулу, яка діє на клітину-мішень. Однією з таких сполук є Ru(bpy)$_2$Nic$_2$ — комплексна сполука рутенію (біс(2,2'-біпіридину-N,N')біс(S-нікотину-N1) рутенію(2+) дихлорид), яка є фоточутливою формою нікотину, активується світлом у видимому спектрі (473–532 нм) і характеризується квантовим виходом 0.23.

У даній роботі вивчались ефекти Ru(bpy)$_2$Nic$_2$ та продуктів його фотолізу — вільного нікотину і фотолабільного залишку Ru(bpy)$_2$Nic — на гладенькі м'язи дихальних шляхів щура. Вибір ґрунтувався на даних про експресію α7 та α3β4 підтипів нАХР у цих тканинах та збільшення внутрішньоклітинної концентрації кальцію в наслідок впливу нікотину. Експерименти проводилися на кільцях грудної частини трахеї щура з використанням тензометричного методу для реєстрації скорочень у відповідь прикладання звичайного нікотину або Ru(bpy)$_2$Nic$_2$, активованого джерелом видимого світла.Було встановлено, що прикладання 100 мкМ нікотину викликає швидке фазне скорочення м'язів трахеї, тоді як продукти фотолізу 100 мкМ Ru(bpy)$_2$Nic$_2$, після 2-хвилинного опромінення, викликають фазо-тонічне скорочення. Тривалість тонічної компоненти залежала від часу опромінення. За наявності блокатора нАХР гексаметонію (10 мкМ) на тлі дії опроміненого Ru(bpy)$_2$Nic$_2$ гладенькі м'язи відповідали лише тонічним скороченням, ймовірно, за рахунок побічних продуктів фотолізу Ru(bpy)$_2$Nic$_2$. Видалення епітелію трахеї призводило до того, що реакція на опромінений Ru(bpy)$_2$Nic$_2$ зникала. Базуючись на тому, що нікотин має складний механізм дії на гладенькі м'язи трахеї, було зроблено припущення, що побічні продукти фотолізу Ru(bpy)$_2$Nic$_2$ можуть мати вплив як на гладенькі м'язи, так і на епітелій. Так за умов блокування циклооксигенази 1/2 індометацином та пуринорецепторів епітеліальної тканини опромінений Ru(bpy)$_2$Nic$_2$ викликав незначне фазне скорочення. Таким чином, показано, що фотоліз Ru(bpy)$_2$Nic$_2$ супроводжується вивільненням активних компонентів, які здатні викликати пролонговане фазо-тонічне скорочення гладеньких м'язів трахеї, на відміну від звичайного нікотину.

EFFECT OF THE CORNELIAN CHERRY FRUIT EXTRACT FROM THE `UHOLOK´ CULTIVAR ON OXYGEN TRANSPORT AND NITRIC OXIDE METABOLISM IN ERYTHROCYTES OF RATS WITH TYPE 1 DIABETES MELLITUS

Anna Moroz[1], Iryna Brodyak[1], Alicja Z. Kucharska[2], Natalia Sybirna[1]
[1] Ivan Franko National University of Lviv, Lviv, Ukraine
[2] Wrocław University of Environmental and Life Sciences, Wrocław, Poland
Email address: Anna.Moroz@lnu.edu.ua

Prolonged hyperglycaemia and oxidative stress in type 1 diabetes mellitus (DM) lead to erythrocyte dysfunctions, resulting in impaired oxygen (O_2) delivery to tissue. Excessive blood glucose concentrations cause non-enzymatic glycation of haemoglobin (Hb): altering its physicochemical properties and increasing its affinity for oxygen, which leads to insufficient O_2 supply to tissues [1]. Hypoxia evokes compensatory mechanisms to restore physiological tissue oxygenation, one of which is nitric oxide (NO) synthesis by NO synthase enzyme (NOS). Overactivation of the inducible isoform of NOS (iNOS), excessive production of reactive oxygen species, and increased nitrite and nitrate formation lead to Hb oxidation into methaemoglobin (MetHb), which is incapable of transporting O_2 [2].

To normalize erythrocytes function and prevent diabetes-related complications, the search for new antidiabetic medicines is essential. Medicinal plants of the *Cornus* genus are considered promising. The bioactive compounds of cornelian cherry fruit exhibit a wide range of biological properties [3]. Therefore, the aim of our study was to investigate the effect of the fruit extract from *Cornus mas* L. `Uholok´ cultivar on the oxygen-transport functions of erythrocytes and nitric oxide metabolism in these blood cells of rats with diabetes.

Experiments were conducted on male Wistar rats. DM was induced by intraperitoneal administration of streptozotocin (STZ) at a dose of 55 mg/kg b. w. Diabetic rats were *per os* administered the extract of fruit from the `Uholok´ cultivar of *C. mas* L. at a dose of 20 mg/kg b.w. for 14 days. The total Hb content and its ligand forms: oxyhaemoglobin (HbO_2), glycated haemoglobin (HbA1*c*), and MetHb were analyzed. Also, we determined Hb affinity for oxygen and detected the activity of NOS, and the contents of nitrite and nitrate in erythrocytes.

In rats with STZ-induced diabetes, a leftward shift in the HbO_2 dissociation curve and a decrease in the P_{50} value were observed. Elevated HbA1*c* and MetHb levels, iNOS activity, and an increase in the contents of NO_2^- and NO_3^- indicate the development of oxidative-nitrative stress in erythrocytes. Our result showed the right shift of the HbO_2 dissociation curve and a significant raise of the P_{50} value in DM animals that were treated with fruit extract from the `Uholok´ cultivar. Additionally, the extract caused a notable decreased in the percentages of HbA1*c* and MetHb compared to untreated diabetic rats. Also, a positive effect of fruit extract was also revealed in the normalization of the NOS activity and the nitrite and nitrate levels. So, extract exhibits a corrective effect on the oxygen-transport functions of erythrocytes and NO metabolism in these cells. Conducted researches may contribute practical application of fruits of the `Uholok´ cultivar of *C. mas* L. in non-pharmacological approaches to diabetes management.

References:

1. Y. Wang, P. Yang, Z. Yan, Z. Liu, Q. Ma, Z. Zhang, Y. Wang, Y. Su, *J. Diabetes Res*., **2021**, 6656062.
2. R. McNulty, N. Kuchi, E. Xu, N. Gunja, *J. Food Sci*., **2022**, 87 (4), 1423–1448.
3. I. Brodyak, A. Moroz, K. Bernacka, A. Z. Kucharska, N. Sybirna, *Food & Funct*., **2025**, 16, 2136–2155.

ВПЛИВ ГЛЮКОЗИ НА ГІПЕРТОНІЧНИЙ ГЕМОЛІЗ ЕРИТРОЦИТІВ СОБАКИ.

Олена Ніпот[1], Наталя Єршова[1], Сергій Єршов[1], Світлана Федосова[2], Олена Чабаненко[1], Наталія Шпакова[1]

[1]Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

[2]Харківський національний університет імені В. Н. Каразіна, м. Харків, Україна

Email address: *nipotel71@gmail.com*

Глюкоза є унікальним джерелом енергії для еритроцитів, нестача або надлишок цієї речовини веде до порушення метаболізму, зміни морфології еритроцитів, їхньої здатності до деформації, що призводить до скорочення тривалості життя клітин. Захисні середовища, які використовуються для тривалого зберігання еритроцитів як людини так і тварин, зазвичай містять глюкозу у якості компонента, що забезпечує сталість метаболізму клітин та їх стійкість до стресових впливів [1].

У роботі використовували еритроцити собаки (Canis lupus familiaris). Заготівлю крові і всі маніпуляції проводили згідно з вітчизняними та міжнародними біоетичними нормами щодо проведення експериментів на тваринах. Аліквоту еритроцитів поміщали у фізіологічний розчин, що містив чи не містив (контроль) глюкозу в концентрації 5%, та інкубували за 37ºС від 1 до 120 хв. Гіпертонічний шок еритроцитів собаки здійснювали перенесенням клітин у розчин, що містить 4,0 М NaCl, 0,01 М фосфатний буфер, pH 7,4 за температури 37 чи 0°С на 5 хв (гематокрит 0,4%). Статистичну обробку отриманих числових даних проводили за допомогою програми "Statistica" (версія 6.0).

Отримані дані не показали значного впливу інкубації з глюкозою на рівень гіпертонічного пошкодження еритроцитів собаки. Так рівень гемолізу еритроцитів при 37ºС складає: без глюкози 1хв - 75±6%; 30хв - 74±4%; 60 хв -72,5±4%; 90 хв - 73,5±7%; 120 хв - 89±5%; з глюкозою 1хв - 59±5%; 30хв - 64±4%; 60 хв -62±4%; 90 хв - 59±7%; 120 хв - 66±5%;. При 0ºС: без глюкози 1хв - 61,5±6%; 30хв - 66,5±5%; 60 хв - 64,5±4%; 90 хв - 72±7%; 120 хв - 88±5%; з глюкозою 1хв – 54,5±1%; 30хв - 66±2%; 60 хв -61,5±4%; 90 хв - 68,5±7%; 120 хв - 75,5±5%.

Максимальне зниження ушкодження клітин спостерігається при часі інкубації 1 хв: за 37 °С на 27%, за 0 °С на 13%, при більш тривалій інкубації цей ефект поступово нівелюється. Ймовірно це пов’язано з додатковим осмотичним навантаженням у розчинах з глюкозою. При інкубації клітин без додавання глюкози рівень пошкодження зростає тільки при інкубації 120 хвилин, але при додаванні в інкубаційне середовище глюкози цього процесу не відбувається. Можна припустити, що присутність глюкози запобігає метаболічному виснаженню клітин після 120-хвилинної інкубації.

Показово, що спостерігається незначна різниця між пошкодженням клітин при температурі 0 і 37 °С, а при інкубації з глюкози вона не спостерігається зовсім. Це говорить про низьку залученість фосфоліпідних компонентів мембрани до розвитку процесу гіпертонічного пошкодження еритроцитів собаки.

Посилання:

1. L.N.T. Tran, C. González-Fernández, J. Gomez-Pastora. *Biomolecules*. **2024**, 14 (7), 813.

HUMAN VALYL-TRNA SYNTHETASE: CDNA CLONING AND EXPRESSION IN CULTURED HUMAN CELLS

Vyacheslav Shalak, Nazarii Kolodka, Tetyana Bondarchuk, Boris Negrutskii
Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine
Email address: *shalak@imbg.org.ua*

Valyl-tRNA synthetase (VRS) is an essential housekeeping enzyme that catalyzes the attachment of valine to its cognate tRNA. In mammals, VRS is exclusively associated with a heavy form of the translation elongation factor complex eEF-1H that ensures the direct transfer of valyl-tRNA to the elongation factor eEF-1A and facilitates formation of the ternary complex valyl-tRNA∗eEF-1A∗GTP [1]. In humans, VRS is also involved in the development of multiple myeloma cells by affecting the regulation of valine metabolism [2] and promotes therapy resistance in melanoma [3]. Biallelic mutations in VRS gene were found to be responsible for the neurodevelopmental disorder (neurodegeneration with microcephaly, seizures, and cortical atrophy) with reduction of VRS catalytic activity, signifying partial loss of enzymatic function [4]. These data suggest the importance of further functional and structural studies to be done with this enzyme. The aim of our work was to clone cDNA encoding full-length and truncated forms of human valyl-tRNA synthetase, express them in different expression systems in order to obtain isolated recombinant proteins required for functional and structural studies.

The open reading frame of human VRS was amplified using the total RNA preparation from HEK293 cells. The obtained PCR product was successfully cloned into pBluscrip SK(+) vector and sequenced. The sequence of obtained VRS cDNA was found to be identical to *Homo sapiens* valyl-tRNA synthetase 1 mRNA (NCBI Reference Sequence: NM_006295.3) except one point mutation A612G that does not result in amino acid alteration. Expression of human VRS cDNA in bacterial cells using pET28a(+) vector led to non-soluble recombinant enzyme production with no catalytic activity. As an alternative, we created a set of genetic constructs on the base of modified pcDNA3.1(+) vector that included cDNA fragment of full-length VRS, its N-terminally truncated form and catalytic domain of this enzyme. HEK293 cultured cells were transiently transfected by three above-mentioned constructs and cell extracts were prepared 24-, 40-, 48- and 64-hours post-transfection. Western-blot analysis showed the presence of full-length recombinant VRS and its two truncated forms in the cell extracts after 24 hours post-transfection. The level of all VRS forms in cells reached the maximum at 40 hours post-transfection and remained stable until 64 hours. No protein degradation of full-length valyl-tRNA synthetase and its truncated forms was observed along the transfection experiment.

In conclusion, cultured human cells proved to be the best choice for the recombinant VRS production and may be used for expression of its mutant forms in order to understand the functional and structural significance of the different point mutations in this enzyme.

1. Negrutskii BS, Shalak VF, Kerjan P, El'skaya AV, Mirande M. Functional interaction of mammalian valyl-tRNA synthetase with elongation factor EF-1alpha in the complex with EF-1H. J Biol Chem. **1999**;274(8):4545-50.
2. Shi R, et al. High expression of VARS promotes the growth of multiple myeloma cells by causing imbalance in valine metabolism. Zhong Nan Da Xue Xue Bao Yi Xue Ban. **2023**; 48(6):795-808.
3. El-Hachem N, et al. Valine aminoacyl-tRNA synthetase promotes therapy resistance in melanoma. Nat Cell Biol. **2024**;26(7):1154-1164.
4. Friedman J, et al. Biallelic mutations in valyl-tRNA synthetase gene VARS are associated with a progressive neurodevelopmental epileptic encephalopathy. Nat Commun. **2019**;10(1):707.

IN SILICO DESIGN OF A SYNTHETIC CIRCULAR RNA PLATFORM FOR ENHANCED IPSC REPROGRAMMING

Volodymyr V. Zeenko, Oleksandr Yu. Petrenko
Department of Cryobiochemistry, Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv, Ukraine
Email address: v.v.zeenko@nas.gov.ua

The generation of induced pluripotent stem cells (iPSCs) from somatic cells holds great promise for regenerative medicine, disease modeling, and drug discovery. However, current iPSC generation methods suffer from low efficiency and prolonged reprogramming times. This study explores a novel approach utilizing synthetic circular RNA (circRNA) combined with engineered transcription factors to enhance the efficiency of reprogramming human endothelial colony-forming cells (ECFCs) into iPSCs.

We computationally designed a polycistronic synthetic circRNA vector encoding sequence-optimized reprogramming factors (either OCT4, SOX2, KLF4, c-MYC or OCT4, NANOG, SOX2, LIN28) linked by P2A peptides and driven by a coxsackievirus B3 (CVB3) internal ribosome entry site (IRES) for efficient, cap-independent translation. To further amplify reprogramming potential, we incorporated rationally engineered, high-activity variants of key transcription factors (OCT4-VP16, super-Sox2, ePOU) and synergistic co-factors (GLIS1, TEAD2/4, ZIC3) into the design. The synthetic circRNA was engineered to be produced using either the permuted intron-exon (PIE) method or advanced trans-splicing ribozyme-based circularization techniques, which could enable the efficient production of circRNA molecules exceeding 8,000 nucleotides. The circRNA vector sequence underwent rigorous *in silico* optimization for enhanced stability, translational efficiency, and reduced immunogenicity using a suite of bioinformatics tools (e.g., LinearDesign, mRNAid, ViennaRNA (RNAfold), NUPACK, RNAstructure). The proposed workflow involves gene synthesis, modular cloning for flexible vector assembly, and subsequent *in vitro* transcription (IVT) and purification. This computationally designed platform is intended for future testing using lipid nanoparticles (LNPs) for delivery into ECFCs, followed by a thorough evaluation of reprogramming efficiency.

Furthermore, leveraging this modular platform, we will systematically investigate the impact of various circRNA design elements—including vector topology, IRES elements, open reading frames (ORFs), and 3′-untranslated regions (UTRs), as well as the incorporation of RNA modifications—on circRNA translation efficiency, including the potential for rolling circle translation, across different human cell types.

In summary, this work details the *in silico* design and proposed construction methodology for a novel engineered circRNA platform aimed at achieving potent, sustained, and potentially safer expression of reprogramming factors. By combining the inherent advantages of circRNA with optimized sequence design and engineered transcription factors, this approach offers a significant ***potential*** advancement in iPSC generation strategies. This methodology holds substantial promise for developing scalable, safe, and effective strategies in stem cell therapy, regenerative medicine, and as a versatile tool for next-generation RNA therapeutics.

IMPROVEMENT (HARMONIZATION) OF CONDITIONS FOR THE FORMATION OF SPHEROIDS OF MESENCHYMAL STEM CELLS AND NEURAL CELLS OF RATS

Mayorova O. R., Sukach O. M., Ochenashko O. V., Vsevolodska S. O.
Institute of Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv

Neural cells (NCs) in natural conditions function in a specific microenvironment, which consists of glial cells, vascular endothelium and mesenchymal stromal cells (MSCs), in particular pericytes. Reproducing this environment in laboratory conditions using 3D spheroids (SFs) containing both NCs and MSCs makes it possible to study their interaction, differentiation and survival processes in more depth. Culture of NKs in combination with MSCs in spheroids significantly increases their potential for regeneration, which opens new prospects for the treatment of central nervous system (CNS) injuries and the development of novel biotechnological and neuroengineering approaches.

MSCs were isolated from the liver of rat embryos on day 15-16 of embryonic development (ED 15-16). NKs were isolated from the brain of newborn rats. Both cell types were cultured in DMEM/F12 medium supplemented with 10% fetal calf serum (FBS).

SFs were formed from 3–4-passage MSCs and 1-passage NKs by the hanging drop method (drop volume 20 μl, 3000 cells/drop) for 72 hours. To promote 3D self-organization of cells, methylcellulose (MC) was added to the culture medium.

In order to optimize the conditions for the formation of SF, culture media with different contents of FBS (2.5%, 5%, 10%) and MC (0.125%, 0.25%, 0.5%, 0.75%) were tested. It was experimentally established that the optimal combination for the formation of both monoculture (MSCs or NCs) and compatible MSCs and NCs (in a 1:1 ratio) SFs is 0.5% MC and 5% FBS. The use of other concentrations was characterized by unstable formation of SFs, which were characterized by loose cell packing, low mechanical stability and heterogeneity in size and shape. Under optimal conditions (0.5% MCs and 5% FBS), dense spheroids of regular spherical shape and uniform size (100±20 μm) were formed already during the first day of cultivation. The cell viability of such spheroids, assessed by FDA/PI staining, was 80±10%.

Upon further cultivation on the adhesive surface, all obtained SFs attached, after which their cells migrated and spread, forming a monolayer of MSCs (SF MSCs) or neural and glial cells (SF NC). Morphologically, the spread cells of compatible SFs were mainly characterized by a fibroblast-like phenotype with a small proportion of neuron- and neuroblast-like cells.

The obtained results open new prospects for further research in the field of 3D cell modeling and the creation of three-dimensional cellular structures. Optimized conditions for the formation of both mono and compatible SFs of MSCs and NCs create the basis for the development of innovative strategies in neuroregenerative medicine and for the development of advanced biomedical technologies.

International Scientific Conference on Advances in Cell Biology, Microbiology, and Biotechnology (ISCACMB 2025)

Session 2
Molecular Oncology and Medicine

12-13 June 2025
Lviv, Ukraine

CYTOLOGICAL DETERMINANTS IN BIOCRYOIMMUNOLOGICAL MEDICINE

Mykola Korpan **Lecture 1**

Mykola M. Korpan
[1]International Institute of Cryosurgery, Rudolfinerhaus Clinic, Vienna, Austria
[2]Department of General Surgery №1, Bohomolets National Medical University, Kyiv, Ukraine
Email address: *institut@cryosurgery.at*; *kafedra1nmu@gmail.com*

The immune system plays a pivotal role not only in pathogen defense but also in malignant tumor surveillance and, more recently, as a core component of immunotherapy. As the global population ages, immunological strategies—particularly vaccines—are becoming increasingly essential for enhancing quality of life and combating age-related diseases, notably cancer.

Imaging cytometry (IC) has emerged as a valuable and efficient method for studying signaling pathways in immunophenotypically defined subpopulations of cryo-preserved immune cells. In this cytological study, tumor cell groups were characterized by extensively anaplastic, transformed glandular cells with giant nuclei and numerous eccentric, multinucleated elements. IC enables the quantitative image analysis of cryo-particles as markers of immune activity. This webinar will introduce viewers to the process of using IC to assess the activity of transcription factors in clinical samples, including drug-induced stimulation or inhibition.

A human malignant cell model was constructed in vitro. Spherical clusters of breast cancer cells were documented prior to exposure to ultralow temperatures, using Pappenheim cytological staining. Extracorporeal, aseptic, rapid freezing was performed at -180 °C for three minutes, followed by passive, delayed thawing. Complete cryo-cancer cell dissipation (CCCD) was observed after the freeze-thaw cycle, accompanied by the formation of new cryo-cancer cell fragments, identified as bio-cryo-cancer antigens (BCAAg).

The generation of novel bio-cryo-immune substances represents an emerging concept in science. These substances may define a new category in both bio-cryo-immunology and bio-cryo-anticancer immunology, including the development of bio-cryo-immune antigens and antibodies—laying the groundwork for future innovations in bio-cryo-pharmacology and the bio-cryo-pharmaceutical industry.

The production of bio-cryo-vaccines and bio-cryo-anticancer vaccines, derived through targeted and controlled cooling, may represent a quantum leap in the effectiveness of immunologic interventions in both bioscience and medicine. When combined with bio-cryo-immunology, anticancer treatments may become significantly more synergistic and efficient. Notably, no adverse side effects have been observed thus far.

Bio-cryo-immunology is a newly emerging discipline at the intersection of bioscience and clinical medicine.

Reference:
Korpan NN. BioCryoImmunology: Impact of Cytology. Cryobiology, 2020; 92: 276. doi: 10.1016/j.cryobiol.2019.11.021.

ANTICANCER METABOLIC THERAPY: PROBLEMS AND DEVELOPMENTS

Oleh Stasyk **Lecture 2**

Galyna Shuvayeva, Olena Vovk, Nikita Polishchuk, Dmytro Demash, Olena Stasyk, Oleh Stasyk
Institute of Cell Biology, National Academy of Sciences of Ukraine
Email address: olehst11@gmail.com

Anticancer metabolic therapy based on individual amino acid deprivation has been proposed and validated in preclinical and clinical studies. One of its modes involves application of the two recombinant arginine-degrading enzymes, human arginase I (ARGI) or bacterial arginine deiminase (ADI). Arginine (Arg) deprivation therapy (ADT) was demonstrated to be very effective in abrogating proliferation of various cultured tumor cells. We demonstrated that tumor cells cannot convert ornithine to Arg due to deficiency in mitochondrial enzyme ornithine transcarbamylase (OTC), and thus are Arg auxotrophs in culture. However, Arg precursor citrulline, present in human blood stream, and status of argininosuccinate synthase (ASS), determine tumors' sensitivity to ADT *in vivo*. Despite promising results in pre-clinical studies and clinical trials, prolonged ADT monotreatment has limited success *in vivo* and is associated with possible adverse effects.

We proposed several rationale combination therapeutic modalities based on ADT and chemotherapeutic drugs or irradiation aimed to enhance ADT effectiveness. For instance, we were first to propose that application of Arg analogs of plant origin, canavanine or indospicine, may solve the problem of ASS upregulation and acquired ADT resistance. However, we also found out that adaptation to ADT may lead to more aggressive radio-resistant phenotype in certain carcinoma cells. New developments in ADT 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*» and by the bilateral project "I-DRUG: The German Ukrainian Radiosensitizer Development Initiative" (BMBF funding 01DK24023).

SUPPRESSION OF NK/LY LYMPHOMA BY NOVEL PYRROLIDINEDIONE-THIAZOLIDINONE HYBRIDS *VIA* DNA DAMAGE, PARP1 INHIBITION, AND CELL CYCLE ARREST IN G1

Natalya Finiuk **Lecture 3**

Natalya Finiuk[1], Iryna Ivasechko[1], Yuliia Kozak[1], Nazar Manko[1], Olha Klyuchivska[1], Ihor Yushyn[2], Roman Lesyk[2], Rostyslav Stoika[1]
[1] Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2] Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Email address: *nataliyafiniuk@gmail.com*

Lymphomas belong to a group of malignant tumors originating from lymphatic system cells. They are classified into Hodgkin and non-Hodgkin types, and demonstrate such symptoms as enlarged lymph nodes, weight loss, and fatigue. The murine ascitic NK/Ly (Nemeth–Kellner lymphoma) model was employed as the experimental tumor system. Following intraperitoneal inoculation, this tumor exhibits rapid growth and readily adapts, inducing clinical manifestations observed in the mammalian lymphomas (Nemeth and Kellner, 1961). NK/T-cell lymphoma belongs to a category of non-Hodgkin lymphoma, specifically classified as an extranodal lymphoma. It is an aggressive form resistant to traditional chemotherapies and often associated with Epstein-Barr virus infection. Thus, developing new treatment methods is important to improve patient outcomes.

Here, we evaluated therapeutic efficiency and 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) and Les-6294 (1-(4-chlorophenyl)-3-[5-[2-chloro-3-(4-nitrophenyl)prop-2-enylidene]-4-oxo-2-thioxothiazolidine-3-yl]pyrrolidine-2,5-dione).

Les-6287 and Les-6294 compounds were found to be highly toxic for NK/Ly cells adapted for *in vitro* cultivation, with an IC_{50} of 0.65-1.35 μM. They were also effective in inhibiting the development of NK/Ly lymphoma in C57BL/6 male mice. Changes in the morphology of NK/Ly lymphoma cells indicate progressive cell damage and degradation during tumor growth. DNA damage *via* single-strand breaks and fragmentation, and inhibition of the PARP1 enzyme participating in DNA repair, were detected in the ascitic NK/Ly cells. The p21 protein was up-regulated, while cyclin D1 was decreased under treatment with Les-6287 and Les-6294, which could mediate the G1 arrest in these cells.

Administering 20 mg/kg of Les-6287 and Les-6294 to C57BL/6 male mice did not lead to weight loss, changes in the mass index for lung, heart, spleen, kidney, and animal survival. Unlike Dox, Les-6287 and Les-6294 compounds did not show acute toxicity.

Thus, Les-6287 and Les-6294 compounds effectively inhibited the development of NK/Ly lymphoma in mice *via* mitochondria-dependent apoptosis, DNA fragmentation, and modulation of p21/cyclin signals.

The *in vivo* experiments were approved by the BioEthics Committee at the Institute of Cell Biology, NAS of Ukraine (Protocol N 2 dated 27.01.2019).

Acknowledgment: The research was partly funded by a grant from the National Research Foundation of Ukraine (project No. 2023.03/0104).

Reference: Nemeth L, Kellner B. New mouse ascites tumor to be used as a screening tool. Neoplasma 1961;8:337–43.

EXPRESSION PROFILE OF TRANSCRIPTION FACTORS IN B-LYMPHOCYTES AT CHRONIC LYMPHOCYTIC LEUKEMIA

Larysa Kovalevska **Lecture 4**

Larysa Kovalevska[1], Sofia Lytvyn[1], Iryna Titorenko[2], Iryna Kryachok[2]
[1]RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine; [2]The state non-profit enterprise "National Cancer Institute", Kyiv
E-mail: kreyl@yahoo.com

Treatment of oncohematological diseases is one of the most complex problems of modern medicine. Today, the most common form of hemoblastosis in European countries and in Ukraine is chronic lymphocytic leukemia (CLL) comprising about 30% of all cases of oncohematological diseases. CLL involves dysregulation of various signaling cascades in B cells, which leads to their prolonged survival. Despite the almost century-old history of CLL diagnosis, the molecular mechanisms that determine the appearance and properties of such leukemic cells are still not fully known. The question of why B-lymphocytes at CLL do not undergo apoptosis nor are activated, remains open.

The study was conducted on 20 blood samples from CLL patients treated at the National Cancer Institute; each gave informed consent to use the clinical data for scientific purposes. The reverse transcription and quantitative real-time polymerase chain reaction were used to detect the relative expression of PU1, SPiB EBF1 BLIMP1, STAT3, STAT5A, and STAT5B transcription factors at mRNA levels in the peripheral blood. Data were calculated using the ΔΔCt method with TBP as a reference gene.

We found that relative expression was lower in CLL patients compared to conditionally normal B lymphocytes isolated from peripheral blood for PU.1 (7.5-fold), Spi-B (4-fold), EBF1, BLIMP1, and STAT5A (>2-fold). Our studies showed no expression at the RNA level of STAT3 and STAT5b, suggesting an altered transcriptional landscape directed towards a more immature B cell phenotype that may promote survival.

This profile points to damaged B cell differentiation, impaired immune signaling and immune evasion mechanisms that support the progression of the pool of abnormal B cells in CLL. This contributes to the immature phenotype of CLL cells and suggests that dysregulation of key transcription factors may be a driving force in the pathogenesis and persistence of the disease. These cells are likely to be less responsive to BCR signaling and more adaptive, and therefore potentially more aggressive.

These findings also highlight potential targets for therapeutic strategies aimed at restoring immune function or targeting specific signaling pathways in CLL.

The work was supported by the grant No. 0124U003787 of the National Research Foundation of Ukraine “Activation of signaling cascades for the elimination of transformed B cells in chronic lymphocytic leukemia”.

THERAPY OF CHRONIC INFLAMMATION AT THE EARLY STAGE OF ALZHEIMER'S DISEASE USING LIPOSOMAL FORM OF CURCUMIN AND microRNA AEROSOL

Sergiy Shulga **Lecture 5**

Sergiy Shulga[1], Hanna Andriiash[1], Olena Tigunova[1], Viktoriya Sokolik[2], Katerina Kot[3], Yuriy Kot[3], Yaroslav Blume[1]

[1]Institute of Food Biotechnology and Genomics National Academy of Science of Ukraine, Kyiv, Ukraine
[2]State Institution "Institute of Neurology, Psychiatry and Narcology of the National Academy of Medical Sciences of Ukraine", Kharkiv, Ukraine
[3]V. N. Karazin Kharkiv National University of Ministry of Education and Science of Ukraine, Kharkiv, Ukraine
E-mail address: *Shulga5@i.ua*

The main pathological manifestations of Alzheimer's disease (AD) are chronic inflammation, amyloidosis and hyperphosphorylation of tau proteins. The aim of the present work was to investigate the effect of nanoliposomes loaded with miR-101 and curcumin on the proinflammatory, neurodysfunctional and neuroprotective state of the rat brain in the model of Alzheimer's disease induced by Aβ42. A new drug, an aerosol of liposomal form of curcumin and microRNAs, was created for the treatment of neurodegenerative diseases.

The liposomal emulsion was obtained by hydration of lipid films, followed by sonication and extrusion through membranes with pore openings of 200 nm, 100 nm, and 50 nm in steps. It was shown that curcumin is incorporated into the lipid bilayer and miR-101 into the liposome aqueous core. It was found that the spray angle and aerosol deposition profiles in the nasal passages depend on the concentration of active pharmaceutical ingredients and the state of the nasal epithelium.

Intranasal testing of liposome spray with three doses of curcumin and miR-101 C1 (1.1 mM Cur + 2.5 nM miR-101), C2 (0.55 mM Cur + 1.25 nM miR-101) and C3 (0.275 mM Cur + 0, 625 nM miR-101) for the case of 'old' rats with AD model showed that the most effective dose for inhibition of amyloidosis, neuroinflammation and oxidative stress was the dose of C2. The effective duration of the course application of the aerosol of the liposomal form of the complex containing curcumin and miR-101 in animals with AD model was determined to be 15 days. Macromorphological suppression of the inflammatory process in brain tissues, levelling of hippocampal asymmetry due to the restoration of the left hippocampus mass, in contrast to the probable asymmetry of the hippocampi, was shown. The positive detection of specific fluorescence of curcumin and miR-101 in the hippocampus and neocortex of rats indicates the presence of these compounds in the nervous tissue of the brain. Positive detection of curcumin and miR-101 was observed 40 minutes after the last administration of the therapeutic complex. Positive detection of curcumin- and miR-101-specific fluorescence in the hippocampus and neocortex of rats treated with the therapeutic complex for 5, 15 and 25 days (2 hours after the last administration), respectively, indicates the presence of these compounds in the nervous tissue of the brain after the therapy. On day 25, a significant increase in curcumin- and miR-101-specific fluorescence was detected in the hippocampus and neocortex compared to days 5 and 15 of administration. The fluorescence intensity in the hippocampus was higher than in the neocortex. Thus, it was found that in the hippocampus and neocortex of rats treated with the therapeutic complex for 25 days, the content of reactive nitrogen species and the area of amyloid aggregates significantly decreased (2 hours after the last administration), which indicates the therapeutic effect of liposomal complex aerosol.

This work is supported by the National Found of Research of Ukraine founded by project 2021.01/0282.

ANTIDIABETIC POTENTIAL OF BIOLOGICALLY ACTIVE COMPOUNDS ON THE ALKALOID-FREE EXTRACT OF *GALEGA OFFICINALIS*

Halyna Hachkova **Lecture 6**

Halyna Hachkova, Natalia Sybirna
Ivan Franko National University of Lviv, Lviv, Ukraine
Email address: halyna.hachkova@lnu.edu.ua

This study aimed to investigate the hypoglycaemic and antioxidant effects and mechanisms of corrective influence of biologically active substances of the nonalkaloid fraction of *Galega officinalis* L. extract on metabolic and structural disorders of pancreatic and liver cells in experimental diabetes mellitus (EDM).

The pronounced hypoglycemic effect of *G. officinalis* extract, devoid of alkaloids, at a dose of 600 mg/kg in EDM has been proven. The established effect is evidenced by a decrease in the concentration of glucose and glycosylated hemoglobin in the blood, an increase in glucose tolerance of cells, and an increase in insulin content in the plasma of rats' blood. The cytoprotective effect of the studied extract on pancreatic cells at a dose of 1200 mg/kg was experimentally confirmed (an increase was found in the number of Langerhans islets, their average area, diameter, volume, and the number of β-cells relative to these indicators in animals with diabetes). The ability of the biologically active substances present in the studied extract to inhibit α-glucosidase activity *in vitro* was shown. It was found that the severity of the antihyperglycaemic effect of the non-alkaloid extract of *G. officinalis* is not inferior to that of the official phytopreparation Arfazetyn, and the impact of this extract on the content of glycosylated hemoglobin under conditions of EDM significantly exceeds the effectiveness of the comparison drug.

The functional activity of the electron transport chain of hepatocyte mitochondria under EDM conditions was found to be reduced, which was accompanied by a decrease in the activity of its forming enzymes – NADH-CoQ oxidoreductase (complex I), succinate-CoQ oxidoreductase (complex II), and cytochrome c-CoQ oxidoreductase (complex III). The administration of the nonalkaloid fraction of *G. officinalis* extract to animals with EDM led to an increase in the activity of NADPH-CoQ oxidoreductase and cytochrome c oxidoreductase and did not affect the activity of succinate-CoQ oxidoreductase.

The antioxidant potential of the alkaloid-free extract of *G. officinalis* was confirmed under *in vitro* and *in vivo* conditions in liver and pancreatic cells.

The obtained results indicate the advisability of further investigation of this extract to substantiate its potential use as a component of functional foods or dietary supplements for the prevention and complementary therapy of pathologies associated with hyperglycemia.

INHIBITION OF ERN1 AFFECTS THE HYPOXIC REGULATION OF HOMEOBOX TRANSCRIPTION FACTOR GENES EXPRESSION IN ERN1-DEPENDENT MANNER

Daria Krasnytska (*young scientist*) **Lecture 7**

Krasnytska D.A., Viletska Y.M., Minchenko O.H.
Department of Molecular Biology, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Objective. The endoplasmic reticulum stress and hypoxia are essential factors in the tumor growth and chemotherapy resistance. One of the major mechanisms through which tumor cells respond to ER stress involves genome reprogramming via altered expression of transcription factors, including Homeobox family. **The aim** of study was to elucidate how the inhibition of ERN1 endoribonuclease and protein kinase activity affects the hypoxic regulation of some of the genes encoding Homeobox family transcription factors, such as *ZEB2, MEIS1, MEIS3, LHX1, LHX6, SPAG4* and *TGIF1* genes in U87MG glioblastoma cells.
Methods. We used two sublines of this glioma cell line. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dominant-negative constructs (dnIRE1). Second subline was obtained by selection of stable transfected clones with overexpression of dnIRE1 and has suppressed both protein kinase and endoribonuclease activities of this bifunctional sensing and signaling enzyme of endoplasmic reticulum stress.
Results. It was found that hypoxia down-regulated the expression level of *LHX6, MEIS2* and *TGIF1* genes but up-regulated *MEIS1, LHX1, MEIS3,* and *SPAG4* gene expression in control glioblastoma cell. It was also shown that inhibition of ERN1 enzymatic activity alters the expression of studied Homeobox genes. ERN1 knockdown of glioblastoma cells removed the effect of hypoxia on the expression of MEIS1 and *LHX1* genes, but increased the sensitivity of *MEIS2, LHX2, LHX6* and *TGIF1* genes to hypoxia. However, the expression of *MEIS3, ZEB2*, and *SPAG4* genes had decreased sensitivity to hypoxia in ERN1 knockdown glioblastoma cells. The most pronounced changes under the conditions of ERN1 inhibition were detected for the pro-oncogenic gene *SPAG4*. Since hypoxia caused different magnitude and direction of changes in the expression of the studied Homeobox transcription factor genes, we determined the protein level of the alpha subunit of the transcription factor HIF1 in control cells and in cells with suppressed ERN1 under normal conditions and hypoxia. Hypoxia dramatically increases the protein level of the alpha subunit of the HIF transcription factor in both control glioblastoma cells and cells with suppressed IRE1 signaling protein. Moreover, the inhibition of both enzymatic activities of the ERN1 signaling protein decreases the level of HIF1 alpha protein in glioblastoma cells, which is in line with the data on the reduction of the proliferative potential of these cells.
Conclusions. The ERN1-dependent nature of the sensitivity of glioblastoma cells to hypoxia is the basis for revealing the mechanisms of tumor cell resistance to the toxic effects of hypoxia under conditions of endoplasmic reticulum stress.

MECHANISMS OF CELL DEATH INDUCTION BY NOVEL THIOSEMICARBAZONES IN COLON CANCER CELLS

Rostyslav Panchuk **Lecture 8**

Panchuk R.R.[1,3], Skorokhyd N.R.[1], Kowol C.[2], Heffeter P.[3], Berger W.[3], Stoika R.S.[1]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
[3]Center of Cancer Research, Vienna Medical University, Vienna, Austria
E-mail address: *rpanchuk@ukr.net*

α-N-heterocyclic thiosemicarbazone derivatives, first discovered as a byproduct of the synthesis of an antituberculosis drug decades ago, nowadays are considered as promising drug candidates due to their specific ability to chelate metals, which can be used to address one of most prominent tumor-specific vulnerabilities – altered iron metabolism. However, recent studies have revealed that increase of complexity of thiosemicarbazone molecule significantly expands mode of action of these compounds. In particular, 2nd generation thiosemicarbazone – triapine, mostly targets ribonucleotide reductase, while 3rd gen compounds – Dp44mT and COTI-2 – are focused mainly on lysosomal-derived oxidative stress and mild p53 reactivation, respectively.

The main aim of this study was to gain more insight in molecular mechanisms underlying nanomolar activity of novel 4th gen thiosemicarbazone – Coti-NMe2, derived from COTI-2.

Colon cancer cells of SW480 line was selected as main model for the study, and induction of various cell death pathways under the action of COTI-2 and COTI-NMe2 was analyzed in time- and concentration dependent mode.

Annexinv V/PI double staining assay had revealed weak pro-apoptotic activity of studied compounds, while cytomorphological studies had shown massive vacuolization of cytosol under the action of COTI-2, while in case of COTI-NMe2 it increased by an order of magnitude. This may be the hallmark of yet another form of cell death – paraptosis, which is induced by ER stress.

We did not found any changes in expression of Protein disulfide isomerase (PDI), BiP and HSP90 chaperones under the action of COTI-2 and COTI-NMe2, while for ATF4, IRE-1a and Ero-1La the situation was opposite. At high doses of COTI-NMe2 (25 μM), a local peak (2-3-fold increase in expression) of Ero-1La and IRE-1a was observed at 6h timepoint, which was declined in time-dependent mode, reaching its local minimum at 24h. The same effects were observed for COTI-2, but for less extent compared to COTI-NMe2. On contrary, expression of ATF4 was decreasing over time even in untreated cells, but under the action of high doses of both compounds it deteriorated even more rapidly.

The obtained data clearly indicate that both studied compounds induce ER stress in colon cancer cells, and this phenomenon is tightly dependent on drug concentrations – the higher it is, the faster is the process of induction of ER stress and subsequent paraptosis. These data also positively correlate with ability of both thiosemicarbazones to induce immunogenic cell death, where the selection of proper concentration of the drug and timepoint was crucial for the ability of drug to vaccinate animals towards tumor growth.

Acknowledgement: This research was supported by FWF grant P31923-B "*Nanocarrier for Tumor-Specific Anticancer Thiosemicarbazones*" and bilateral Austria-Ukraine grants M/88-2023 and M/30-2024 "*Dual targeting of tumor-specific vulnerabilities by novel thiosemicarbazone derivatives*".

PRODUCTION OF NATIVE SPECIES-SPECIFIC IMMUNE SERA FOR EMPLOYMENT IN FORENSIC MEDICINE

Maxim Lootsik **Poster 1**

Maxim Lootsik[1], Maksym Lutsyk (Jr.)[2], Nazar Manko[1], Marina Barska[1], Rostyslav Stoika[1]

[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]Lviv Private Medical University, Lviv, Ukraine
Email address: lootsikmaxim@gmail.com

Species-specific immune sera are used in forensic medicine practice for determination of origin of the protein (human or animals) in traces on the material evidence. Such sera are produced by OOO "HEMATOLOG" (RF) and were supplied to Ukraine by private companies or individuals. With the outset of russian–ukrainian war in 2022, all relations were stopped which led to acute deficit in species-specific immune sera. According to a proposal of the Chief Forensic Medicine Expert of Ukraine one of the authors (L.M.) was involved in elaboration of technology and production of this type of immune sera (since 1993). In 2022, after several years break, the obtaining of such sera with the specificity to human, cow, swine and fowl proteins was renewed. During 2022-2025, samples of produced sera were tested and approved in 18 bureaus of forensic medicine of Ukraine, including the Main Office. The peak volume of the produced sera (305 ml) was achieved in 2023, however, it was insufficient to satisfy requirements of Ukrainian forensic medicine bureaus who need about 5 times more product.

In our work, we pay special attention to quality of the produced sera which must keep to the installed demands: 1) high sensitivity, denoted as serum titer which must be not less than
1:8 000; 2) solution of serum must be clear, the turbidity or opalescence is unacceptable; 3) high specificity that means that titer of serum with a specific antigen must be 10 times higher comparing with the non-specific antigen. Original methods were developed for improvement quality of unconditioned sera to reach the acceptable levels. One approach concerns the clarifying of serum via elimination of lipoproteins, while another method is connected with purification and concentration of immunoglobulin fraction of the serum in order to achieve the titer of 1:8,000. Briefly, the last method consists of the following stages: 1) isolation of immunoglobulin fraction by ammonium sulfate precipitation in the range of 22–43 % of salt saturation; 2) dissolution of protein sediment in minimal volume of water and elimination of salt by dialysis against 4% sodium chloride solution, followed by a stepwise lowering of salt concentration to 0.5%, and thereafter dialysis against distilled water; 3) dialysis against 50 mM sodium acetate buffer, pH 5.0, sediment discarded, clear solution saved; 4) concentration of solution by elimination of water and low molecular weight substances via immersion into solution the visking tube filled with powder of PEG 20,000. The last takes off water from the external solution and increases concentration of protein to a desired degree. More detailed description of the mentioned method is presented in the poster.

In conclusion, providing of forensic medicine bureaus of Ukraine with native species-specific immune sera stays a complex problem that includes not only the technology of sera production but also a need a proper animal care, legalization and registration of the obtained products, as well as their advertising.

NARRATIVE REVIEW OF CREATINE METABOLISM IN HUMAN HEALTH: VARIATING CONSTANT OF HUMAN BODY

Yuriy Boretsky **Poster 2**

Yuriy Boretsky[1], Vira Hashchyshyn[1], Iryna Hlozhyk[1], Nataliia Paraniak[1], Roksolana Tymochko-Voloshyn[1], Volodymyr Boretsky[2], Vitaliy Ivashchenko[3], Stepan Reshetylo[1], Yaroslav Svyshch[4].
[1]Department of Biochemistry and Hygiene, Ivan Boberskyi Lviv State University of Physical Culture, Lviv, Ukraine.
[2]Medical Laboratory «MeDiS». Lviv, Ukraine.
[3]Department of Traumatology, Orthopedics and Military Field Surgery, Danylo Halytsky Lviv National Medical University; Lviv Regional Hospital of War Veterans and Repressed People Named After Yu.Lypa. Lviv, Ukraine.
[4]Department of Athletics, Ivan Boberskyi Lviv State University of Physical Culture. Lviv, Ukraine.
Email address: *biolog@ldufk.edu.ua*

Creatine is an incredibly widely used supplement. Recently its market market was valued at $1 billion. In many cases (but not in all) creatine supplementations enhance energy production, build lean muscle, and enhance athletic performance (Kreider, Stout, 2021). Despite that the role of creatine in human health should be discussed. Creatine plays a vital role in the normal metabolism of the central nervous system, heart, bones, and muscle tissues. The biosynthesis of creatine requires two potentially deficient substrates, arginine and S-adenosylmethionine, whereas a decrease in their content can influence the regulation of human body metabolism dramatically. Although arginine is formed de novo in humans, these synthetic pathways do not provide sufficient arginine quantities. Thus, humans do have dietary needs of it. It can be speculated that substantial amounts of both arginine and S-adenosylmethionine are stored for cellular needs under creatine supplementation conditions. Creatine acts as a negative regulator of its own synthesis and transport. Mutations in the genes encoding the enzyme of creatine biosynthesis and/or its transporter cause psycho-neurological disorders that compromise speaking ability and motor skills (Ferrada et al., 2024). The «phosphocreatine shuttle» serves as an effective «energy buffer» for different tissue cells. Four genes encoding different forms of creatine kinase were identified in the human genome.

Consuming large amounts of creatine in doses higher than recommended can cause nausea, vomiting, diarrhea, and acne. If the recommended doses are exceeded, most of the creatine consumed is excreted from the body within a day. Creatine and its phosphorylated form are relatively unstable and degrade to creatinine at a rate of about 2% per day. The average amount of creatine and creatinine in the body is relatively constant for each individual. In several studies, these indicators were used to evaluate the content of other marker compounds in the body (Boretsky et al., 2025).

References:

1. Y. Boretsky, I. Hlozhyk, V. Hashchyshyn et al. Creatine beyond muscle metabolism: Changing constant of human body. Journal of Human Sport and Exercise, 2025, 20(2), 715-730.
2. E. Ferrada, T. Wiedmer, W.A. Wang et al. Experimental and Computational Analysis of Newly Identified Pathogenic Mutations in the Creatine Transporter SLC6A8. J Mol Biol., 2024, 436(2), 168383.
3. R.B. Kreider, J.R. Stout Creatine in Health and Disease. Nutrients, 2021, 13(2), 447.

AMINO ACID STARVATION TOLERANCE IN THE ADT-RESISTANT HUMAN ORAL CARCINOMA SUBLINE SAS-R9 IS INDEPENDENT OF HSP AND AUTOPHAGY PATHWAYS

Nikita Polishchuk (*young scientist*) **Poster 3**

Nikita Polishchuk[1], Olena Vovk[1], Oleh Chen[1], Dmytro Demash[1], Galyna Shuvayeva[1], Oleh Stasyk[1]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: *polishchuk@cellbiol.lviv.ua*

Arginine deprivation therapy (ADT) is considered as a promising and relatively non-toxic anticancer strategy, demonstrating efficacy against a wide range of tumour types both in vitro and in vivo. However, its clinical effectiveness is often limited by the development of acquired resistance in cancer cell subpopulations, which poses a major challenge to sustained therapeutic success. This resistance may potentially be overcome by combining ADT with additional agents targeting complementary cellular pathways. In our previous studies, we isolated an ADT-resistant subline of human oral squamous carcinoma cells, SAS-R9. These semi-resistant SAS-R9 cells exhibited enhanced metastatic potential and increased aggressiveness compared to the parental SAS cell line.

For the first time, we applied a combination of asparaginase (ASP) and recombinant human arginase 1 (rhARG) to induce simultaneous depletion of several amino acids as a therapeutic strategy. To induce glutamine starvation, we used the glutaminase (GLS1) inhibitor CB-839. Our data suggest no significant difference in sensitivity to glutamine deprivation between the parental and resistant cell lines. However, the combination of rhARG and ASP demonstrated a certain cumulative effect, suggesting that multi-amino acid deprivation may enhance therapeutic impact. In the present study, we aimed to further investigate the mechanisms underlying cellular adaptation to amino acid deficiency, with a particular focus on the regulation of the endoplasmic reticulum (ER) stress response and other molecular pathways contributing to resistance development.

The analysis RNA sequencing of the original SAS cell line and the ADT-resistant SAS-R9 subline revealed an upregulation of a group of heat shock protein (HSP) genes. Our next step was to examine the expression of ER stress-related genes as well as the key HSP proteins at the protein level to better understand their potential role in resistance mechanisms. We observed reduced expression of IRE1, s/uXBP1, and ATF6 genes, along with lower eIF2α phosphorylation, under ADT conditions in SAS-R9. These results indicate that ER stress activation is markedly attenuated in the resistant SAS-R9 subline, suggesting that these cells are more tolerant to stress induced by arginine deprivation. The expression of key HSPs was slightly elevated in SAS-R9 cells compared to the parental SAS cell line.

Since autophagy plays a protective role under amino acid deprivation, we examined the expression of key autophagy-related markers. Our results revealed an increased expression of p62, LC3 and phosphorylated ULK1 proteins specifically in the resistant SAS-R9 subline, while this effect was either reduced or absent in the parental SAS cell line.

Acknowledgment:
The research was partly funded by grants from the Ministry of Education and Science of Ukraine (bilateral Ukrainian-German project SR № 0124U003964) and supported partially by “Presidential Discretionary-Ukraine Support Grants” from Simons Foundation, Award No 1030281 to the authors from ICB NASU.

ENHANCED ANTITUMOR ACTIVITY OF 9-O-OCTYLBERBERINE VIA POLYMERIC NANOCARRIER-BASED MODIFICATION: *IN VITRO* AND *IN VIVO* EVALUATION

Nadia Skorokhyd **Poster 4**

Nadia Skorokhyd[1], Rostyslav Panchuk[1], Solomiya Paryzhak[2], Tetiana Dumych[2], Nataliya Mitina[3], Alexander Zaichenko[3], Wang Lizhen[4], Liu Kechun[4], Jin Meng[4], Rostyslav Stoika[1]

[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[3]Lviv National Polytechnic University, Lviv, Ukraine
[4]Institute of Biology, Shandong Academy of Sciences, Jinan, China
Email address: n.skorokhyd@gmail.com

Berberine, a natural isoquinoline alkaloid, has limited use in oncology due to low bioavailability and poor cell permeability. However, its cytotoxic activity can be significantly enhanced by introducing lipophilic alkyl substituents through chemical modification at the 9-O-position. After preliminary *in vitro* screening, 9-O-octylberberine was selected as lead compound for further enhancement of its pharmacologic properties by immobilization on polymeric carriers, including PC-PEG, PC-pEtOx, and PC-PEGMA. The antitumor potential of nanoscale carriers with incorporated 9-O-octylberberine was evaluated by examining their effect on the viability of various tumor and pseudonormal cell lines using trypan blue dye and the MTT assay. The PC-PEG-9-O-Berb and PC-pEtOx-9-O-Berb nanocomposites demonstrated comparable cytotoxic activity to 9-O-octylberberine, with a concentration-dependent pattern. In contrast, the PC-PEGMA carrier reduced the cytotoxic activity of the alkylated berberine derivative. No significant differences were observed between the nanocomposites and the free compound in pseudonormal cell lines such as HaCat and MCF-10A, whereas a slight decrease in activity was noted in HEK 293 cells. A time-dependent analysis of the entry of 9-O-octylberberine into HCT-116 colon cancer cells was performed using flow cytometry. All three polymeric carriers partially delayed the cellular uptake of the compound. For PC-PEG and PC-pEtOx, this effect was transient and disappeared after 6 hours. However, PC-PEGMA induced a 2.5-fold reduction in cellular penetration of 9-O-octylberberine. This effect was more pronounced at a concentration of 25 μM and persisted for up to 24 hours. These results are consistent with the MTT assay results, in which the PC-PEGMA-based sample showed the weakest cytotoxic effect. Among the tested carriers, PC-pEtOx demonstrated the highest efficacy, ensuring a prolonged drug effect and enhanced cytotoxicity against HCT-116 tumor cells, while also normalizing reactive oxygen species levels without inducing oxidative stress.

Thus, the polymeric nanocarrier PC-pEtOx containing 9-O-octyl-berberine appears to be the most effective and has the potential for use in antitumor therapy. *In vivo* studies using the B16F10 mouse melanoma model revealed that 9-O-octyl-berberine effectively inhibited tumor growth, though it also resulted in the death of 60% of tumor-bearing animals due to its high systemic toxicity. However, encapsulation in a polymeric carrier reduced these adverse effects while preserving strong antitumor activity. Nanoscale complexes of 9-O-octyl-berberine led to a significant reduction in tumor volume compared to the control group of untreated animals, and tumor remission was observed in 60% of animals. Also these nanocomposites effectively doubled the survival time of the treated animals without inducing significant hematotoxicity. Thus, therapeutic activity of 9-O-octyl-berberine is significantly enhanced when delivered in a polymeric complex. This improvement is attributed to the increased water solubility and bioavailability of these biologically active compounds.

The obtained *in vitro* and *in vivo* results support the potential of polymeric carriers, particularly PC-pEtOx, for the targeted delivery of 9-O-alkyl derivatives of berberine in anticancer therapy.

Compensatory effect of carbon sorbent and nanocerium oxide on the ultrastructure of liver and heart cells in tumor-transplanted rats undergoing doxorubicin chemotherapy

Dmytro Klymchuk **Poster 5**

Dmytro Klymchuk[1], Veronika Sarnatskaya[2], Yuliia Shlapa[3], Liubov Paziuk[2], Bogdan Geraschenko[2]
[1]N.G. Kholodny Institute of Botany of NAS of Ukraine
[2]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine
[3]Vernadsky Institute of General and Inorganic Chemistry of NAS of Ukraine
E mail address: microscopy.botany@gmail.com

Doxorubicin remains one of the most effective drugs in the treatment of various types of tumors, characterized by nonspecific cytotoxicity, which affects various types of normal cells of the body, in particular the heart, liver, kidney.

The aim of this work was to assess the compensatory effect of a medical carbon sorbent and nanocerium oxide on the ultrastructural organization of liver and heart cells and other adverse reactions from the administration of doxorubicin to rats carrying Guerin carcinoma. The study was conducted on three groups (5 animals each) of randomized female animals with a body weight of 200±10 g: the first - control, intact animals; the second - animals that on the 5th day after the transplantation of Guerin carcinoma were administered intraperitoneally twice a week with doxorubicin, a single dose of which was 3.25 mg/kg; the third - animals that, the day after doxorubicin injection, were injected with a suspension containing submicron-sized carbon particles with a concentration of 1.4 mg/ml and cerium oxide nanoparticles with an average size of 4.2 nm and a concentration of 0.02 mg/ml. On the 21st day of the experiment, fragments of liver and heart tissue with a volume of 1-2 mm^3 were fixed in a 3% glutaraldehyde solution in cacodylate buffer (pH 7.4) for 4 h, post-fixed with 1% osmium tetroxide for 2 h, dehydrated and embedded in an Epon-Araldite mixture. Ultrathin sections (0.05±0.01 μm) were made on an LKB-8800 ultramicrotome (Sweden), contrasted with lead citrate according to Reynolds and analyzed on a JEM-1230 electron microscope (Jeol, Tokyo, Japan) at an accelerating voltage of 80 kV.

In the samples of the experimental treatment, in which animals with tumors were administered doxorubicin, the ultrastructural organization of hepatocytes compared to the control ones, is characterized by greater heterogeneity of the mitochondrial population, including variability in size, shape, matrix electron density and disruption of the structural integrity of membranes. In the samples of the experimental treatment, in which animals, in addition to doxorubicin, were administered a carbon sorbent with nanocerium oxide, a lower frequency of signs of hepatocyte mitochondrial dysfunction is noted.

The ultrastructural peculiarities of cardiomyocyte mitochondria of the control and experimental animals reflected trends close to those given above for hepatocytes. These results are consistent with the literature data on the dysfunction of mitochondria in organ cells during doxorubicin-induced intoxication.

Thus, the electron microscopic studies conducted indicate that the use of carbon sorbent in combination with cerium oxide nanoparticles leads to a decrease in the frequency of signs of itochondrial dysfunction that develops in the liver and heart during doxorubicin-induced intoxication.

PRECLINICAL INVESTIGATION OF LES-6485'S ANTINEOPLASTIC ACTIVITY VIA PARP1 INHIBITION

Iryna Ivasechko (*молода вчена*) **Poster 6**

Ivasechko I. I [1], Yushyn I.M. [2] , Kozak Yu.S. [1] , Finiuk N.S. [1] , Klyuchivska O.Yu. [1] , Manko N.O. [1] , Lesyk R.B. [2] , Stoika R.S. [1]

[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]Department of Pharmaceutical, Organic and Bioorganic Chemistry, State Non-Commercial Enterprise "Danylo Halytsky Lviv National Medical University, Lviv" Lviv, Ukraine
E-mail address: *irynagrytsyna@gmail.com*

Cancer is a multifaceted disease driven by disruptions in DNA repair, apoptosis, and immune responses, with chemotherapy often facing limitations in efficacy. Among various cancer treatment strategies, DNA repair inhibitors, particularly PARP1 inhibitors, show significant promise. This study explored the cytotoxic mechanism of Les-6485 (4-(2-{1-(2-fluorophenyl)-3-[4-methyl-2-(pyridin-2-ylamino)-thiazol-5-yl]-3-oxopropylsulfanyl}-acetylamino)-benzoic acid ethyl ester), a potential PARP1 inhibitor, on mammalian tumor and normal cell lines. Additionally, the combined effect of Les-6485 with temozolomide and cisplatin was investigated using the soft agar method.

Les-6485 exhibited IC_{50} values ranging from 2.79 μM to 8.05 μM in tumor cells, while demonstrating no toxicity to pseudo-normal cells. Preincubation with Fluzaparib, a known PARP1 inhibitor, reduced Les-6485's activity threefold, suggesting its role as a potent PARP1 inhibitor. Les-6485 also altered DNA nativity and showed synergistic effects with MGMT inhibitor.

The reduced sensitivity of tumor cells to Les-6485 following PARP1 inhibitor preincubation supports its potential as a PARP1 inhibitor. Its mechanism, combined with DNA repair inhibitors or DNA-damaging agents like temozolomide and cisplatin, warrants further exploration for enhanced therapeutic outcomes.

This research was supported by the National Research Foundation of Ukraine (project No. 2023.03/0104, No 2023.05/0021).

EVALUATION OF BIOCOMPATIBLE CHITOSAN–PVA HYDROGELS WITH HYALURONATE COATING FOR LOCAL DELIVERY OF BIOACTIVE AGENTS

Nazar Manko (*young scientist*) **Poster 7**

Nazar Manko[1], Iryna Ivasechko[1], Olga Klyuchivska[1], Dmytro Demash[1] Rostyslav Stoika[1]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: *mankonazarcb@gmail.com*

The effective wound healing remains an extremely relevant problem in modern medical practice, especially due to an increasing number of chronic, infected and difficult-to-heal skin lesions. Based on the results of previous studies (Manko et al., 2025), we synthesized chitosan-based gel films (up to 1 mm thick) demonstrating high wound healing potential, as well as biocompatability and biodegradability. Here, we focused at overcoming some disadvantages of these films, such as their storage in humid environment and problems of sterilization.

In order to overcome these limitations, a biocompatible scaffold based on complex of chitosan with polyvinyl alcohol (PVA) was created which is amenable to UV sterilization, possess storage stability and sorption properties. This hydrogel was further modified via its coating with a hyaluronate-chitosan gel film. The stability of the resulting constructs was tested in aqueous solution, culture medium with and without blood serum. High stability of all created the constructs was confirmed.

In order to define sorption potential of the created hydrogel and its film modifications, their saturation with antibiotic ampicillin (100 μg/ml, 24 h) and riboflavin (vitamin B2, 1 mg/ml, 24 h) was carried out. Subsequently, a release of riboflavin was visualized in 1% agar layer, and more pronounced sorption properties of the film-coated hydrogel were confirmed using UV detection. Thus, the higher ability to sorption and release of low molecular weight compounds from the modified material was proven. In this experiment, riboflavin was used as a model molecule for further development of local delivery of nutrients (amino acids, vitamins, etc.) to the affected areas with insufficient vascular supply, that is characteristic, in particular, for large-scale burns.

The effectiveness of release of ampicillin was evaluated taking into account its antimicrobial activity towards bacteria *S. aureus* (ATCC 25923). It was found that the inhibition zone of bacterial growth in gel modified with this antibiotic was 25% larger than under the effect of the unmodified gel, under the same sorption conditions. This effect confirmed the effective release of the conjugated substance from the gel, while preserving its biological activity.

Finally, the cytotoxicity of gel constructs was assessed towards pseudonormal murine fibroblasts of the Balb/c 3T3 line. We did not reveal any decrease in cell viability, on the contrary, an increase in cell number was observed in the wells with gels, compared to the control. This results may suggest a favorable surface for cell adhesion and proliferation, which is an important characteristic of materials to be used for regenerative.

Thus, a stable, biocompatible hydrogel of chitosan and PVA was developed, which effectively sorbs and releases biologically active substances, is non-toxic and promotes cell proliferation, which makes it promising for the treatment of complex wounds.

References: Manko, N., Ivasechko, I., Antonyuk, R. et al. Chitosan-Based Hydrogels with N-Stearoylethanolamine for Acceleration of Healing of Acute Cutaneous Wounds: Cytological and Histological Examination. Cytol. Genet. 59, 24–35 (2025). https://doi.org/10.3103/S0095452725010086

Acknowledgment: the authors are grateful to Prof. D. Fedorovych for providing riboflavin.

IN SILICO EVALUATION OF A NOVEL THIOSEMICARBAZONE DERIVATIVE AS A SELECTIVE STABILIZER OF MUTANT P53 R175H VIA L1/S3 POCKET BINDING

Mykola Klishch (*young scientist*) **Poster 8**

Mykola Klishch[1], Rostyslav Panchuk[1], Rostyslav Stoika[1].
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

TP53 gene mutations are among the most frequent genetic alterations in human cancers, often resulting in structural defects in the p53 protein and promoting tumor progression and treatment resistance. Missense mutation R175H represents a structural alteration that compromises the tumor-suppressive function of p53 by destabilizing its DNA-binding domain. Therapeutic strategies aimed at reactivating such mutant forms of p53 remain a high priority in oncology research. One promising approach involves targeting the transiently open L1/S3 pocket of the p53 protein, a site known for its binding of small-molecule stabilizers (Wassman et al., 2013).

In this study, the α-N-heterocyclic thiosemicarbazone derivative COTI-NMe_2 was evaluated for its ability to selectively bind and stabilize the R175H mutant of p53. Molecular docking was performed using AutoDock Vina to estimate binding affinity across the surface of wild-type and mutant p53 DNA-binding domains. Docking poses were classified into L1/S3-binding and off-target groups based on a distance threshold of 5 Å from the sulfur atom of the Cys-124 residue. Subsequently, molecular dynamics (MD) simulations were carried out in GROMACS for 100 ns to assess the conformational stability of proteins and ligand-protein complexes.

The docking analysis revealed that the E-isomer of COTI-NMe_2 demonstrated a strong preference for the L1/S3 pocket in the R175H mutant, with higher predicted binding affinity compared to both wild-type p53 and other mutants. MD simulations predicted the stability of the complex, with RMSD values for the ligand remaining within 2 Å throughout the 100 ns trajectory. Furthermore, the presence of the ligand reduced backbone RMSD fluctuations of R175H, indicating a stabilizing effect on the mutant protein structure. These results suggest a selective interaction mechanism, where the compound preferentially binds to L1/S3 pocket and stabilizes the structure of mutant p53 R175H.

COTI-NMe_2, particularly its E-isomer, demonstrates promising characteristics as a selective reactivator of p53 R175H. Its predicted affinity for the L1/S3 pocket and stabilizing effects observed during molecular dynamics simulations support its potential as a lead compound for further in vitro studies.

Acknowledgement: This study was supported by the Austrian-Ukrainian project “Dual targeting of tumor-specific vulnerabilities by novel thiosemicarbazone derivatives” (Reg. No. 0124U003513, 2022–2024).

References:

1. Wassman, C.D., et al. (2013). Computational identification of a transiently open L1/S3 pocket for reactivation of mutant p53. Nat Commun, 4, 1407. https://doi.org/10.1038/ncomms2361

АКТИВНІСТЬ ФЕРМЕНТІВ РОТОВОЇ РІДИНИ ПРИ РОЗЛАДАХ МІСЦЕВОГО ОКИСНОГО МЕТАБОЛІЗМУ НА ТЛІ ОРТОДОНТИЧНОГО ЛІКУВАННЯ ПАРОДОНТОЛОГІЧНИХ ПАЦІЄНТІВ

Олег Годований[1], Олеся Мартовлос[1], Андрій Мартовлос[2], Ольга Ключівська[3], Ростислав Стойка[3]

[1]ДНП «Львівський національний медичний університет імені Данила Галицького», Львів, Україна,
[2]Центр стоматологічної імплантації та протезування «ММ», Львів, Україна,
[3]Інститут біології клітини НАН України, Львів, Україна
E-mail: ohodovanyi@gmail.com

Запалення тканин пародонта є одним із основних джерел активних форм кисню в ротовій порожнині. Разом з тим, розвиток оксидативного стресу (ОС) є важливою біологічною реакцією на встановлення незнімної ортодонтичної апаратури [1]. У світовій літературі показано, що саме флавоноїди, як поглиначі вільних радикалів, мають виражену антиоксидантну, протизапальну, протиалергічну, противірусну та антибактерійну дію [2]. Мета: визначення активності ферментів антиоксидантного захисту ротової рідини – супероксиддисмутази (СОД), каталазної активності (КА), маркерів місцевої стресорної реакції – амілазної активності (АА) та лактатдегідрогенази (ЛДГ) на тлі застосування лікувально-профілактичного комплексу (ЛПК), що містив флавоноїди та бензидаміну гідрохлориду у поєднанні з електрофорезом в ортодонтичних пацієнтів з патологією пародонта до та після встановлення ортодонтичної апаратури. Матеріали і методи: 118 пацієнтів із зубощелепними аномаліями (ЗЩА) та генералізованим пародонтитом I-II стадії були розділені на основну групу (60 осіб) і групу порівняння (58 осіб). Етапи спостереження в основній групі, де використаний ЛПК та у порівняльній групі (традиційна схема лікування) були до та після пародонтологічного лікування перед встановленням брекет-систем і далі на 3-му та 6-му місяцях активного періоду ортодонтичного лікування. За результатами основної групи встановлено, що ЛПК, дозволив скоротити у 3-3,5 рази підготовку до активного ортодонтичного періоду та досягти стабілізації пародонта у різні терміни після встановлення брекетів. В основній групі показники ферментів покращувалися від 1,5 до 2,4 рази проти отриманих у групі порівняння даних, де після традиційної схеми рівень ОС та місцевих окисних метаболічних порушень залишались високим.

Висновок. Зміни рівня активності СОД та ЛДГ знижувалися відносно КА та АА, які зростали у пацієнтів із ЗЩА на тлі патології пародонта є важливими біомаркерами ефективності проведеного лікування та контролю за станом редукованого пародонта на тлі ОС, спричиненого ортодонтичною апаратурою.

Подяки: Автори висловлюють подяку за підтримку від Simons Foundation (Award № 1030281) та Маньку Назару, PhD (Інститут біології клітини НАН України), за технічну допомогу.

Література:

1. Kovac V, Poljsak B, Perinetti G, et al. Systemic Level of Oxidative Stress during Orthodontic Treatment with Fixed Appliances. Biomed Res Int. 2019;2019:5063565. DOI: 10.1155/2019/5063565.
2. Abou Baker DH. An ethnopharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up to date knowledge. Toxicol Rep. 2022;9:445-469. DOI: 10.1016/j.toxrep.2022.03.011.

TARGETING PROTO-ONCOGENE C-SRC WITH ITS KINASE INHIBITOR PP2 IN HIGHLY AGGRESSIVE MOUSE BREAST ADENOCARCINOMA 4T1 CELLS OVEREXPRESSING THE ADAPTOR PROTEIN RUK/CIN85 EFFECTIVELY SUPPRESSES THEIR MALIGNANT PROPERTIES *IN VITRO* AND METASTASIS *IN VIVO*

[1]Horak I, [1]Skaterna T, [1]Krysiuk I, [1]Hudkova O, [1]Latyshko N, [1]Kishko T, [1]Khudiakova O, [1]Geraschenko D, [2]Lugovskyi S, [1]Drobot L.

[1]Palladin Institute of Biochemistry, NAS of Ukraine

[2] D.F. Chebotarov Institute of Gerontology, NAMS of Ukraine

iryna.horak@gmail.com

The non-receptor tyrosine kinase c-Src is involved in the control of proliferation, adhesion, motility and invasion as well as metastasis of tumor cells, which makes it an attractive target for the development of new therapeutic approaches for the treatment of cancer [1]. Our previous studies demonstrated that overexpression of the adaptor protein Ruk/CIN85 in breast cancer cells is associated with a potent increase in their malignancy, indicating the induction of EMT and the development of cancer stem cells features [2]. In the present study, we sought to detect whether c-Src kinase inhibitor PP2 exerts therapeutic effects in highly aggressive mouse breast adenocarcinoma 4T1 cells stably overexpressing the adaptor protein Ruk/CIN85 *in vitro* and *in vivo*.

Using Western blotting, a high content of constitutively active phosphoform of c-Src was detected in lysates of 4T1 cells overexpressing Ruk/CIN85 (RukUp) compared to control cells (Mock). In wound healing assay, PP2 was shown to inhibit the migration of Mock cells by 2-fold and RukUp cells by more than 4-fold. Furthermore, a significant inhibitory effect of PP2 on metabolic activity and invasiveness was demonstrated only in RukUp cells using the MTT assay and Transwell assay, respectively. We next examined the influence of PP2 on metastatic growth of 4T1 RukUp cells using syngeneic BALB/c mice experimental metastasis model. According to the results of histological studies, tumor development was detected in the lungs, hearts, and livers of animals in the control RukUp group nontreated with PP2 (n=10). At the same time, metastases that developed in the lungs were characterized by pronounced atypia of tumor cells and their high mitotic activity, the formation of foci of necrosis in the central areas of the tumor tissue, the absence of a capsule that limits the tumor. On the contrary, histological studies of secondary organs of the group of mice RukUp treated with PP2 failed to detect the development of metastatic loci. Instead, the lungs showed marked edema and lymphocytic infiltration of the lung interstitium, which developed against the background of dilation and perfusion of the lumens of blood vessels with the development of hemorrhages and erythrocyte diapedesis, as well as the formation of perivascular foci of inflammatory lymphocytic infiltration.

Thus, we have demonstrated that c-Src kinase inhibitor PP2 suppresses the proliferation, migration, and invasion of highly aggressive 4T1 RukUp tumor cells *in vitro*, and also affects their metastasis *in vivo* probably by blocking the extravasation of these cells into target organs. These data also allow us to conclude that the kinase c-Src is a key signaling link involved in the development of tumor cell malignancy, dependent on adaptor protein Ruk/CIN85.

This work was supported by the grant from the National Research Foundation of Ukraine (project #2020.02/0195).

1. Finn RS. Targeting Src in breast cancer. *Ann Oncol.* 2008;19:1379-1386. https://doi.org/10.1093/annonc/mdn291

2. Samoylenko A, et. al. Increased levels of the HER1 adaptor protein Rukl/CIN85 contribute to breast ancer malignancy. Carcinogenesis 2012;33:1976–84 https://doi.org/10.1093/carcin/bgs228

STUDY OF THE MELATONIN PROPERTIES AS A PROMISING RADIOPROTECTIVE AGENTS.

Oleksiy Glavin[1], Victor Mikhailenko[1], Emiliia Domina[1], Olena Safronova[2], Yuriy Dumansky[1].

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2] State institution O.O. Shalimov National Scientific Center of Surgery and Transplantation of National Academy of Medical Sciences of Ukraine
Email address: veterok61@ukr.net

Introduction. The reckless and criminal actions of Russian armed formations in the Chernobyl exclusion zone, as well as at the Chernobyl and Zaporizhzhya nuclear power plants, pose an imminent threat of a radiation disaster -- not only for Ukraine but also for numerous European countries. Furthermore, the aggressor country's potential use of tactical nuclear weapons heightens this mortal danger. Given these risks, Ukraine's strategy for radiation protection must focus on developing and reassessing non-toxic, effective drugs to mitigate the frequency and severity of radiation-induced damage. This includes protection for professionals exposed to radiation at the explosion's epicenter and patients undergoing frequent diagnostic X-ray examinations. One promising candidate in this regard is melatonin (MT), a compound with potent antioxidant properties and minimal toxicity.

Goal of the Study. To assess the potential of MT as a radioprotector in an *ex vivo* study on blood lymphocytes‑cells known for their high radiosensitivity.

Materials and methods. The study was conducted on peripheral blood samples obtained from 11 conditionally healthy female participants aged 21 to 63 years (mean age: 46.6 years), all of whom provided informed consent in accordance with bioethical principles. MT was tested at final concentrations of 0.2 mM and 0.4 mM. One hour after MT administration, blood samples were irradiated using an Xstrahl X-ray therapeutic apparatus at doses of 2.0 Gy and 6.0 Gy. The total incubation period of the samples lasted 3.5 hours in darkness at temperatures ranging from 20°C to 22°C. The level of mitochondrial transmembrane potential (MTP), total production of reactive oxygen species (ROS), and the percentage of apoptotic cells (AP) were determined.

Results. The introduction of MT into blood samples resulted in a 1.3- to 1.5-fold decrease in MTP of lymphocytes - both as an independent agent and in combination with blood irradiation at a dose of 2 Gy. However, at the higher radiation dose of 6 Gy, this MT-induced effect was considerably diminished, showing only a 1.2-fold decrease in TMP at the 0.4 mM concentration. Despite expectations of reduced ROS generation due to decreased MTP, no significant changes were observed in the overall ROS production by lymphocytes, whether MT was administered alone or combined with irradiation. Additionally, the study revealed a substantial increase in the proportion of lymphocytes undergoing early apoptosis - 1.6-fold at 2 Gy and 2.4-fold at 6 Gy, as well as 1.3-fold at 0.2 mM MT and 1.4-fold at 0.4 mM MT concentrations. Nevertheless, 0.2 mM MT exhibited a protective effect in irradiated blood samples, reducing the percentage of lymphocytes in early apoptosis by 1.1-fold at 2 Gy and 2.0-fold at 6 Gy. However, this protective effect was minimal when MT was administered at a higher concentration (0.4 mM).

Conclusions. The obtained results indicate the potential of using MT as a radioprotector to prevent radiation-induced complications. However, further studies are necessary to determine the optimal therapeutic regimen for its use.

INFECTED WOUNDS IN MICE – A COST-EFFECTIVE MODEL FOR SCREENING OF NOVEL ANTIBACTERIALS

Manko N.O.[1], Demash D.V.[2], Ivasechko I.I.[1], Antonyuk R.V.[3]

[1] *Department of Cell Signalling, Institute of Cell Biology, NAS of Ukraine, 14/16 Drahomanov str. Lviv, 79005, Ukraine*
[2] *Department of Cell Signalling, Institute of Cell Biology, NAS of Ukraine, 14/16 Drahomanov str. Lviv, 79005, Ukraine*
[3] *State Specialized Institution "Lviv Regional Bureau Of Forensic Medical Examination", 61 Perarska str., Lviv, 79010, Ukraine*

Spreading of resistance to common antibiotics is one of the primary healthcare concerns worldwide. Thus, many studies are focused on searching for new compounds with antimicrobial activity and novel strategies for treating bacterial and fungal infections, especially those caused by microorganisms with acquired resistance. On the other hand, there is an unmet need for cost-effective models to test such novel compounds and strategies during preclinical trials. Below, we will briefly describe the model of the infected surface wound in mice.

An experimental wound was created in C57BL/6 mice by excising a circular section of skin from the right thigh, previously shaved, under anesthesia. Studied bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were mixed in normal saline with a final concentration of 10^7 CFU/mL. 10 µl of bacterial suspension was applied and evenly distributed along the wound surface. Animals were housed with ad libitum access to food and fresh water, and a 12/12 day/night cycle.

Our study showed that control (non-infected) animals showed the quickest dynamics of wound closure measured by the wound area (complete wound closure and scar formation on days 12-15). Both studied bacterial strains slowed the dynamics of wound healing, with S. aureus causing the most significant inhibition (complete wound closure and scar formation on day 18).

The presence of bacterial infection was confirmed up to day 9 by growing washes from the wound surface on LB growth medium. Gram-Weigert and haematoxylin-eosin staining of wound cross-sections also confirmed bacterial presence on the wound surface without infiltration into the adjacent tissues.

Thus, we suggest that the proposed model is reproducible, cost-effective, and provides a suitable therapeutic window (dynamics of wound closure, detection of bacteria in wound washes and cross-sections) for pre-clinical evaluation of novel molecules with possible antimicrobial effects. If needed, intramuscular dexamethasone injections could prolong the duration of the therapeutic window.

Acknowledgement. This study was supported 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».

СТРУКТУРНО-ФУНКЦІОНАЛЬНИЙ АНАЛІЗ МІССЕНС-МУТАЦІЙ ГЕНА *EBAG9*

Руслан Черепанов

Харківський національний медичний університет, Харків, Україна
Email: *rocherepanov.1m24@knmu.edu.ua*

Ген *EBAG9* (Estrogen receptor-binding fragment associated antigen 9) кодує рецептор-зв'язувальний раковий антиген, експресований на клітинах SiSo (RCAS1), що локалізується в апараті Гольджі [1]. Цей ген відіграє одну з провідних ролей у процесах формування та підтримки кісткової тканини, проте доведена його участь в утворенні найбільш поширених видів раку, а також пригніченні активності Т-лімфоцитів при канцерогенезі та інфікуванні вірусами [1]. При цьому сайленсинг гена *EBAG9* сприяє ефективності лікування раку [2]. Разом з тим, системного *in silico* аналізу впливу несинонімічних замін у гені *EBAG9* на його будову та функції досі не проводилося, що й було метою цієї роботи.

У дослідженні використано 235 однонуклеотидних варіантів (single nucleotide variants, SNVs) гена *EBAG9* з бази UniProt, що спричинюють заміни амінокислот. У якості предикторів впливу замін на функціональність протеїну обрано сервіси fathmm, PhD-SNP, Polyphen-2, Provean, SNPs&GO та SIFT. Для передбачення ефекту SNVs на стабільність білка RCAS1 застосовано інструменти I-Mutant 2.0, INPS-MD, iStable та MUpro. Вивчення внутрішньомолекулярної динаміки протеїну здійснено засобами вебпорталу MEDUSA. Аналіз консервативності амінокислот виконаний за допомогою серверу ConSurf.

Визначення ефектів міссенс-мутацій на функціональні характеристики білка RCAS1 показало, що усі шість використаних відповідних предиктори одночасно позначили негативний вплив на досліджуваний показник лише двох SNVs гена *EBAG9* — R33Q та W63C. Перевірка зв'язку несинонімічних замін із стабільністю протеїну RCAS1 дозволила виявити 64 ушкоджуючі SNVs гена *EBAG9* консенсусом чотирьох застосованих відповідних біоінформатичних інструментів. Співставлення результатів двох проведених видів аналізу дозволило встановити, що обидва виявлених варіанти досліджуваного гена водночас знижують стабільність протеїну.

Оцінка величини нормалізованого B-фактора за допомогою алгоритму, реалізованого на вебпорталі MEDUSA, показала, що амінокислоти, які знаходяться в обох виявлених положеннях, надають протеїну RCAS1 гнучкості, тобто є функціонально важливими. Цей висновок підтверджений результатами, отриманими з використанням вебсервісу ConSurf, які показали, що ці амінокислотні залишки є висококонсервативними, зануреними та функціонально активними.

Таким чином, аналіз наявності в пацієнтів з різними видами раку несинонімічних SNVs гена *EBAG9* може стати підґрунтям для розробки нових видів терапії раку.

Посилання:

1. EBAG9-deficient mice display decreased bone mineral density with suppressed autophagy / K. Azuma et al. iScience. 2024;27(2):108871. https://doi.org/10.1016/j.isci.2024.108871
2. EBAG9-silencing exerts an immune checkpoint function without aggravating adverse effects / A. Wirges et al. Molecular Therapy. 2022;30(11):3358–3378. https://doi.org/10.1016/j.ymthe.2022.07.009

CYTOGENETIC FEATURES AS DIAGNOSTIC AND PROGNOSTIC MARKERS IN ACUTE LEUKEMIAS

S. Shmyhelska, O. Zotova, O. Shalay
SI «Institute of Blood Pathology and Transfusion Medicine, NAMS of Ukraine», Lviv, Ukraine
Email address: imungenneoplazlaboratoria@gmail.com

Background: Acute leukemias (AL) are the result of the accumulation of immature progenitor cells. Genetic changes are the cause of uncontrolled cell proliferation. They can be detected by conventional cytogenetic and molecular methods during diagnosis. In patients with acute myeloid leukemia (AML), cytogenetics is an important prognostic factor in predicting response to treatment. Patients with AML whose leukemic cells harbor t(8;21), t(15;17), t(16;16) or inv(16) translocations have a favorable outcome with induction chemotherapy and intensive post-remission consolidation chemotherapy. Among adults with acute lymphoblastic leukemia (ALL), t(9;22) or t(4;11) carry a very poor prognosis. ALL patients with t(9;22) are not always curable with chemotherapy and usually require a bone marrow transplant.

Aim of this study was to detect diagnostic and prognostic significance of chromosomal rearrangements in AML and ALL patients.

Methods: Cytogenetic investigations of bone marrow and/or peripheral blood cells were performed in 25 adult patients with AL, of which 16 had AML and 9 had ALL. The methods of conventional cytogenetics (GTG) and fluorescence *in situ* hybridization (FISH) were used.

Results: The following recurrent cytogenetic rearrangements were detect in patients with AML: translocation t(8;21)(q22;q22) was found in 4 patients with AMLM2, translocation t(15;17)(q22;q21) in 3 patients with AMLM3 and inversion inv(16)(p13q22) in 1 patient with AMLM4. Except recurring cytogenetic rearrangements also were found other unrecurring abnormalities, namely trisomy 8 and additional marker chromosome in 1 patient with AMLM1, translocation t(8;15)(p12;q14) and additional marker chromosome in 1 patient with AMLM4. The remaining patients (3 patients with AMLM1, 1 patient with AMLM1 and 2 patients with AML of unknown type) had normal karyotype without cytogenetic changes. The presence of the above-described translocations t(8;21), t(15;17) and inversion inv(16) was confirmed using the FISH method and the presence of chimeric genes *RUNX1/RUNX1T1, PML/RARA* and *CRFβ/MYH11*, respectively, was established.

Among patients with ALL, recurring translocation t(9;22)(q34;q11) was detected in 5 patients. Only one of them had additional cytogenetic abnormalities in association with t(9;22)(q34;q11), namely additional material on the long arm of chromosome 1 – add(1)(q36.3) and trisomy 17. The remaining 4 patients had normal karyotype without cytogenetic changes. The presence of translocation t(9;22)(q34;q11) was confirmed using the FISH method, namely, the chimeric *BCR/ABL* gene was detected.

Conclusions: In our cytogenetic study, we found translocations that confirm the presence of chimeric genes corresponding to a certain type of leukemia. According to the results, the diagnosis was confirmed and the course of the disease was predicted.

References:

1. Whiteley AE, Price TT, Cantelli G, Sipkins DA. Leukaemia: a model metastatic disease. Nat Rev Cancer. 2021 Jul;21(7):461-475. doi: 10.1038/s41568-021-00355-z. Epub 2021 May 5. PMID: 33953370; PMCID: PMC8722462.

EXTRACT OF *INDIGOFERA SPICATA* EXERTS PROFOUND CYTOTOXIC EFFECT ON CARCINOMA CELLS

Galyna Shuvayeva[1], Mykola Tupychak[2], Olena Vovk[1], Dmytro Demash[1], Svitlana Chernyshuk[1], Andriy Prokopiv[3,4], Nazariy Pokhodylo[2], Oleh Stasyk[1]

[1] Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

[2] Department of Organic chemistry, Chemical faculty, Ivan Franko National University of Lviv, Kyryla and Mefodiya Str., 6, 79005 Lviv, Ukraine

[3] Department of Botany, Biological Faculty, Ivan Franko National University of Lviv, M. Hrushevskoho Str., 4,79005 Lviv, Ukraine

[4] Botanic Garden, Ivan Franko National University in Lviv, Cheremshyny Str., 4,79014 Lviv, Ukraine

Email address*: shuvayeva77@gmail.com.ua*

Metabolic anticancer therapy based on enzymatic arginine (Arg) deprivation (ADT) is being currently evaluated in clinical trials. We have previously established that ADT combination modalities with low doses of the plant Arg analogs, canavanine (Cav) and indospicine (Isp), exhibit enhanced selective efficacy toward malignant cells resistant to Arg deficiency. It has been shown that the specific anticancer effects of Arg analogs are mainly due to their incorporation into nascent proteins in place of Arg, thereby destabilizing their structure and function. We speculate that Isp completely resistant to hydrolysis by therapeutic enzymes as recombinant human arginase I (rhARG1) can probably be the most effective in combination with ADT. Since pure Isp is difficult to obtain, our data emphasize an interesting and reasonable alternative with cancericidal efficacy in the form of the leguminous plant *Indigofera spicata* extract *contains the highest known amounts of Isp.*

Here we demonstrate for the first time that the Isp-containing ethanolic extract from *I. spicata* is growth-inhibiting and toxic for cultured human colorectal and ovarian carcinoma cells. The extract reduces the viability of tested carcinoma cells under Arg-deficient conditions and profoundly inhibits their residual proliferative potential (growth recovery) after the treatment. Pre-exposure of the extract to rhARGI as a therapeutic Arg-depleting agent did not impact the extract's efficacy.

Thus, our data suggest that the *I. spicata* extract, similarly to pure Isp, may be efficient in enhancing rhARGI-based ADT.

Acknowledgment:

This study was partially supported by "Presidential Discretionary-Ukraine Support Grants" from Simons Foundation, Award No 1030281. The 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*», as well as by the bilateral project "I-DRUG: The German Ukrainian Radiosensitizer Development Initiative" (BMBF funding 01DK24023).

References:

Shuvayeva, G.Y.; Bobak, Y.P.; Vovk, O.I.; Kunz-Schughart, L.A.; Fletcher, M.T.; Stasyk, O.V. Indospicine combined with arginine deprivation triggers cancer cell death via caspase-dependent apoptosis. *Cell Biology International* **2021**, *45*, 518-527, doi:https://doi.org/10.1002/cbin.11321.

ADAPTOR PROTEIN RUK/CIN85 REGULATES NOX GENES EXPRESSION IN HUMAN LUNG ADENOCARCINOMA A549 AND MOUSE MELANOMA B16-F10 CELLS

T. Skaterna[1], O. Hudkova[1], I. Horak1[,2], O. Geraschenko[1], A. Zhyvolozhnyi[1,3], L. Knopfová[2], L. Drobot[1]

[1]Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine [2]Masaryk University, Brno, Czech Republic

[3]University of Oulu, Finland

e-mail address: tskaterna@biochem.kiev.ua

NADPH oxidases are key components of redox-dependent signaling networks involved in the control of cancer cell proliferation, survival and invasion. Our previous studies revealed that ROS production by human colorectal adenocarcinoma HT-29 and breast adenocarcinoma MCF-7 cells is positively correlated with the levels of adaptor protein Ruk/CIN85 expression. Systemic multidirectional changes in mRNA levels for NOX genes were revealed in cells overexpressing Ruk/CIN85. Knockdown of Ruk/CIN85 resulted in the reversion of these changes. The aim of this study was to determine whether Ruk/CIN85 up-regulation correlates with *NOXs* expression in cells of different tissue origin: human lung adenocarcinoma A549 cells and mouse melanoma B16-F10 cells.

The ability of Ruk/CIN85 to modulate the expression level of NOX isoforms in the studied cells was established by qRT-PCR. We observed in A549 Ruk/CIN85-Up cells increasing levels of NOX2 (cytochrome b(558)), NOX3 and NOX5 isoforms. Apocynin, a potent NADPH oxidase inhibitor, reduced the expression levels of all isoforms. It was characteristic to find the highest level of the NOX4 isoform expression in B16/F10 Ruk/CIN85-Up cells, and conversely, Ruk/CIN85-Down cells showed the highest level of NOX4 expression under the influence of apocynin. In addition, in Ruk/CIN85 down-regulated apocynin treated cells increase in *NOX1* mRNA and decrease in *NOX3* mRNAs were observed compared to other isoforms. The obtained data allow us to conclude that signaling pathways dependent on the adaptor protein Ruk/CIN85 may participate in the control of the expression of NOX isoforms in the studied cells. We suppose that protein-protein interaction between SH3 domains of Ruk/CIN85 and Pro-rich motifs of the adaptor protein Tks4, which functions as an organizer subunit of the Nox1-mediated NADPH oxidase complex, may lead to the induction of redox-dependent signaling involved in reprogramming of NOX genes expression and related biological responses. Nevertheless, further studies are necessary to elucidate, by which molecular mechanisms Ruk/CIN85 could affect transcriptional regulation of NOXs genes.

Acknowledgment: This study was supported by FEBS Ukrainian Short term Fellowship

1. Bazalii A.V., Pasichnyk G.V., Horak I.R., Komisarenko S.V., Drobot L.B. Transcriptional regulation of *NOX* genes expression in human breast adenocarcinoma MCF-7 cells is modulated by adaptor protein Ruk/CIN85 // Ukr. Biochem. J. – 2016. – Vol. 88, №1. – P. 119-125.

2. Havrylov S, Rzhepetskyy Y, Malinowska A, Drobot L, Redowicz MJ. Proteins recruited by SH3 domains of Ruk/CIN85 adaptor identified by LC-MS/MS. Proteome Sci. 2009; 7:21.

ADAPTED TO ARGININE DEPRIVATION SUBLINE OF HNSCC SAS-R9: DEVELOPMENT AND CHARACTERISATION

Olena Vovk[1], Nikita Polishchuk[1], Oleh Chen[1], Dmytro Demash[1], Galyna Shuvayeva[1], Oleh Stasyk[1]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: vovkoliv@gmail.com

Arginine deprivation therapy (ADT) has gained attention as a promising metabolic approach for treating highly aggressive head and neck squamous cell carcinoma (HNSCC), which ranks as the seventh most common malignancy globally and is responsible for approximately 4.5% of all cancer-related morbidity and mortality. However, despite continuous efforts to improve the efficacy of ADT, the emergence of acquired resistance remains a critical obstacle.

To explore the ways to overcome this resistance, it is essential to establish a suitable *in vitro* models of drug-resistant cell lines. In our study, we developed a stable cell subline adapted to repetitive arginine (Arg) deprivation from the HNSCC SAS line. This was achieved by mimicking the conditions experienced by cancer patients undergoing chemotherapy. The selection process involved multiple rounds of transferring the SAS cells between Arg-free and Arg-supplemented complete medium (CM), followed by allowing the surviving cells to recover growth. ADT conditions were modelled by adding affinity-purified recombinant human arginase type 1 derived from the yeast *O. polymorpha* into CM. After nine rounds, we established ADT-adapted stable subline SAS-R9, which showed higher survival in response to ADT and radiotherapy than its ADT-sensitive ancestor cell line SAS. SAS-R9 cells also exhibited increased clonogenic proliferation rate and cell-cell aggregation, improved adhesion to the extracellular matrix, and elevated expression of epithelial-mesenchymal transition markers and altered regulation of the signaling pathways compared with the parental line.

These findings suggest that repeated long-term ADT as a monotherapy potentially promote the emergence of more aggressive and radiotherapy-resistant HNSCC phenotypes. Investigation focusing on the acquired resistance to metabolic adaptation using drug-resistant cell lines may provide critical insights for improving ADT efficacy in all types of cancers susceptible to ADT, including HNSCC.

Acknowledgment:
The research was partly funded by grants from the Ministry of Education and Science of Ukraine (bilateral Ukrainian-German project SR № 0124U003964) and also supported partially by “Presidential Discretionary-Ukraine Support Grants” from Simons Foundation, Award No 1030281 to the authors from ICB NASU.

International Scientific Conference "Advances in Cell Biology, Microbiology, and Biotechnology" (ISCACMB 2025)

Session 3
Microorganisms and their Biotechnological Applications

12-13 June 2025
Lviv, Ukraine

ELECTROCHEMICAL ENZYME BIOSENSORS FOR AGRICULTURAL APPLICATION

Sergiy Dzyadevych **Lecture 1**

Sergiy Dzyadevych, Valentyna Arkhypova, Oleksandr Soldatkin

Institute of Molecular Biology and Genetics, NAS of Ukraine
150 Zabolotnoho Str., Kyiv 03143, Ukraine

Biosensors is a new branch of analytical biotechnology, one of the directions of which is the development of enzyme electrochemical biosensors. The most important characteristic features of this type of biosensors are their high sensitivity and selectivity, ease of use and speed of analysis, as well as a wide range of substances that could be detected. This determines the possibility, and rather, the necessity, of their application in almost all areas of human activity, including medicine, pharmaceutical, food, biotechnological and chemical production, agriculture, environmental protection, etc. Compared with existing traditional analytical methods, they can provide fast, reliable, sensitive and cheap analysis of various compounds.

In this presentation, our achievements in the development of electrochemical enzyme biosensors have been reviewed, including their agricultural application.

For development of biosensors, a different detection schemes include potentiometric and conductometric transduction modes were used. As bioselective membranes we used various enzymes such as acetylcholinesterase, butyrylcholinesterase, etc.

The principle of operation of biosensors based on the effect of enzyme inhibition is based on measuring responses associated with enzymatic activity in the absence and presence of an inhibitor with its subsequent quantitative determination. We have developed a number of electrochemical biosensors based on various effects of enzyme inhibition, manufactured and carefully studied their laboratory prototypes. It should be noted that the developed biosensor systems are adapted to large-scale production technologies.

Laboratory prototypes of some of the developed enzyme biosensors have passed the metrology stage and have been tested in the analysis of real samples; they demonstrate a high level of correlation between the results of biosensor and traditional methods.

A number of advantages and disadvantages of the developed enzyme biosensors are discussed. It is important that all the developed biosensors are not a counterweight to traditional analytical methods, but complement them. This is an additional system of rapid and early warning of the presence of toxic substances in the environment. Such systems can save time and money in emergency situations due to the possibility of rapid decision-making on local environmental problems. If necessary, more accurate, but time-consuming and expensive traditional methods can be used for further validation and additional research on samples previously tested by biosensors.

DIAPHORASE NANOMIMETICS AND THEIR APPLICATION IN CONSTRUCTION OF BIOSENSORS, BIOREACTORS, AND BIOFUEL CELLS

Mykhailo Gonchar **Lecture 2**

Gonchar M., Stasyuk N., Demkiv O.
Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine. E-mail: mykhailo1952@gmail.com

Diaphorases (DIs), also known as NADH-dehydrogenases or NAD(P)H-oxidoreductases (EC 1.6.99.1), are flavin-bound enzymes involved in cellular redox reactions. They facilitate electron transfer from NADH or NADPH to various acceptors, playing a crucial role in the electron transport chain. These enzymes help in fighting oxidative stress by reducing harmful oxidants and is key in regulating metabolic pathways by maintaining NAD^+/NADH and $NADP^+$/NADPH ratios. Additionally, diaphorases contribute to cellular signaling processes, influencing cell proliferation, differentiation, and apoptosis. Besides, these enzymes are widely used in clinical diagnostic reagents to couple pyridine nucleotide-dependent analyte oxidation to reduction of chromogenic electron acceptors. These enzymes, which occur naturally in all animal tissues, were discovered also in many microorganisms, being promising for many biotechnological applications.

Recently, DI has also received wide attention as the key enzyme mediating the electron transfer and electric energy generation in enzymatic biofuel cells (EBFCs) [Ma et al. 2020]. EBFC is a device that generates electricity by oxidizing fuels at the anode and reducing oxygen at the cathode *via* enzymatic biocatalysis. Due to its high safety, good biocompatibility, and the use of renewable biocatalysts and high-density fuels, EBFC is believed to hold the promise as a next generation of micro-power sources for wearable and implantable electronic devices in the future.

In EBFCs, the key step for electricity generation is the oxidation catalyzed by various oxidases or dehydrogenases at the anode. Among such enzymes, NAD-dependent dehydrogenases can oxidize many biofuels such as glucose, xylose, ethanol, formate *etc*. In such processes, coupling the respective dehydrogenase with DI-catalyzed reaction give a possibility for recycling NAD^+ or $NAD(P)H^+$.

It is known that lowering anodic pH may be a useful strategy for constructing high-performance in EBFCs, as the proton concentration at the lowered pH can be significantly increased at the anode and it therefore provided a high proton transport driving force (Ma *et al.* 2019). Therefore, to further improve the performance of EBFC, it is desirable to engineer rate- and stability-limiting DIs and increase their acidic tolerance. Also directed evolution is a powerful method for engineering of biological systems including enzymes [Yang et al. 2019], other alternative approach is possible based on using artificial nanozymes which are mimetics of DIs.

Artificial NADH-diaphorases, or nanozyme-based diaphorase mimetics, represent a promising direction in the development of next-generation biofuel cells (BFCs). Compared to natural enzymes, these nanozymes demonstrate enhanced stability under harsh conditions, resistance to denaturation, and tunable catalytic properties, making them highly suitable for long-term use in bioelectrochemical systems. They efficiently catalyze NADH oxidation, enabling recycling NAD^+ in the fuel cells. Their multifunctionality also allows simultaneous catalysis, signal amplification, and molecular delivery, significantly enhancing biosensor sensitivity and performance.

In this study, we employed newly synthesized diaphorase mimetic nMoAuCo, possessing a high diaphorase-like activity toward NADH oxidation, to construct a bioanode for microbial fuel cell, based on formaldehyde dehydrogenase (FdDH)-overexpressed yeast *Komagataella phaffii* cells, additionally enriched by co-immobilizing purified FdDH with nMoAuCo. The developed MFC produced an open-circuit voltage of 600 mV and a maximum power density of 0.3 $\mu W \cdot cm^{-2}$ at an optimal formaldehyde concentration of 0.5 mM. Its effectiveness was proved on real wastewater samples, highlighting the potential of this system for environmental monitoring and bioenergy generation.

Acknowledgement. This research was supported by the National Research Foundation of Ukraine (project No. 2021.01/0010), by the US National Science Foundation (NSF) and ONRG - grant CBET-2235349 including IMPRESS-U supplement (project STCU 7113).

RIBOSOME AS A REGULATORY DEVICE: A CASE OF ANTIBIOTIC-PRODUCING STREPTOMYCETES

Bohdan Ostash **Lecture 3**

Vasylyna-Marta Tseduliak[1], Oksana Koshla[1], Roman Makitrynskyy[2], Yoshikazu Tanaka[3], Andriy Luzhetskyy[4], Victor Fedorenko[1], Bohdan Ostash[1]
[1]Ivan Franko National University of Lviv, Lviv, Ukraine
[2]University of Freiburg, Freiburg, Germany
[3]Tohoku University, Sendai, Japan
[4]Saarland University, Saarbrucken, Germany
E-mail address: bohdan.ostash@lnu.edu.ua

Loss-of-function mutations within genes for protein synthesis machinery (ribosome and associated elements, such as tRNA and amino acyl tRNA synthetase) are either lethal or strongly deleterious, reflecting ribosome essentiality. Nevertheless, a few classes of apparently viable mutations are known to occur within genes for ribosome components and to display intriguing pleiotropic effects on cell physiology. Point substitutions within gene *rpsL* for small subunit ribosomal protein S12 of *Escherichia coli* were historically first "ribosomal" mutations. They were isolated almost 60 years ago thanks to their ability to change cell's response to antibiotic streptomycin. Protein S12 is part of ribosome's decoding pocket, and some S12 mutants render ribosomes more accurate at the expense of protein synthesis rate. The other *rpsL* mutations make ribosome more agile yet error-prone. Available evidence supports the idea that all specific effects of *rpsL* mutations on cellular metabolism are rooted into their ability to either induce or suppress mistranslation. This is clearly "*E. coli*-centric" view, which still lacks a detailed mechanistic picture. Furthermore, *rpsL* mutants were isolated or constructed for the other bacterial species and genera, where the understanding of the chain of cause-to-consequence events remains vestigial.

The isolation of *rpsL* mutations is well-known approach to identify antibiotic-overproducing strains of *Streptomyces* bacteria. This approach relies on isolation of spontaneous streptomycin-resistant mutants, among which *rpsL* mutants dominate. A few studied *S. coelicolor* A3(2) *rpsL* mutants possess increased translation accuracy and extended phase of protein synthesis. Yet the link between initial mutation and the terminal phenotype remains elusive. We have chosen *S. albidoflavus* J1074 as new model to elucidate *rpsL* mutations, due to its excellent growth, genetic amenability and importance for drug discovery. Our main findings are as follows. First, genomes of certain spontaneous *rpsL* mutants harbor the other mutations. Hence, what we perceive as an effect of *rpsL* might in fact be caused by several mutations. We developed a genetic engineering platform to generate any viable *rpsL* mutant of J1074, bypassing the need for streptomycin resistance selection. Second, using this platform, we built clean *rpsL* substitutions Lys88Glu, Lys88Arg and Arg94Gly, and studied their properties. Different mutations led to distinct phenotypes in terms of sporulation and specialized metabolism. The production of second messenger c-di-GMP was strongly decreased in Lys88Arg strain, echoing the effect of the side mutations seen in spontaneous mutants. We also observed deep rewiring of transcription in *rpsL* strains, an indicative of stress-like response to the causative mutation. Third, an initial cryo-EM map of the wild type ribosome has been built, revealing its structural similarity to mycobacterial ribosome. Analysis of mutant ribosome structures is in progress to understand how changes in S12 protein might impact decoding center and the ribosome as a whole.

PRODUCTION OF RIBOFLAVIN AND ITS DERIVATIVES BY METABOLICALLY ENGINEERED YEAST *CANDIDA FAMATA*

Dariya Fedorovych **Lecture 4**

Dariya Fedorovych[a], Kostyantyn Dmytruk[a], Andriy Tsyrulnyk[a], Ljubov Dzanaeva[a], Lubov Fayura[a], Sergiy Romanov[a], Andriy Sibirny[a,b]
[a]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov St., 14/16, Lviv 79005, Ukraine
[b]Institute of Biotechnology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601, Poland
Email address: fedorovych.d@gmail.com

Riboflavin serves as biosynthetic precursor of flavin nucleotides FMN and FAD and is important biotechnological commodity with annual market around 250 million US dollars. It is mostly used as component of feed premixes for animals (80%), in food industry as food colorant and in medicine. Currently riboflavin is produced on a large scale by microbial synthesis. The main producers of riboflavin are engineered strains of *Bacillus subtilis* and of mycelial fungus *Ashbya gossypii.* Flavinogenic yeast *Candida famata* has great biosynthetic potential. However, microbial riboflavin producers require increase productivity and genetic stability. Studying the regulation of riboflavin synthesis and construction of more stable and active riboflavin producers as well as its derivatives are highly desirable. The regulation of riboflavin biosynthesis in yeasts is also an interesting fundamental problem, since a number of regulatory and structural genes are involved in the control of this process. Despite many years of research, the physiological feasibility of riboflavin oversynthesis under iron deficiency is still unclear.

A number of stable highly efficient riboflavin overproducers based on yeast *C. famata* were constructed by classical mutagenesis and through genetic and metabolic engineering. Increased riboflavin production can be achieved by enhancing gene expression of structural genes of riboflavin synthesis (*RIB1*and *RIB7, RIB1* and *RIB6*) and regulatory *SEF1*gene as well as genes involved in synthesis of riboflavin precursor GTP (*RPS3, ADE4*) and ribulose-5-phosphate (*GND1*) and riboflavin excretion (*RFE1*). The overexpression *SEF1* gene under control of promoter of the *LAC4* gene encoding β-galactosidase in *C. famata* overproducers led to increase in riboflavin production on media with lactose and cheese whey. Riboflavin accumulation on lignocellulose hydrolysate was increased in the recombinant strains *C. famata* overexpressing the *XYL1* gene, which codes for xylose reductase. Our data indicate that the waste whey from milk industry and lignocellulose hydrolysate can be promising substrates for riboflavin production by *C. famata.* The possibility of using other industrial waste and semi-products of food industry for production vitamin B_2 is also discussed.

Recombinant strains of *C. famata* able to overproduce FMN and FAD after overexpressing the *FMN1* and *FAD1* genes encoding riboflavin kinase and FAD synthetase were constructed. The strains of the *C. famata* producing flavin antibiotics aminoriboflavin and roseoflavin have been constructed as a result of overexpression of *Streptomyces davaonensis* genes *rosB, rosA* and *rosC.*

Acknowledgment:
This work was supported by National Research Foundation of Ukraine, grant 2023.03/0035. This work was also partially supported by "Presidential Discretionary-Ukraine Support Grants" from Simons Foundation, Award No 1030281 and No 1290613.

METABOLIC ENGINEERING OF *CANDIDA FAMATA* FOR EFFICIENT RIBOFLAVIN PRODUCTION ON XYLOSE-BASED MEDIA

Justyna Ruchala **Lecture 5**

Justyna Ruchala[1], Dominik Wojdyla[1,2], Liubov Dzanaeva[3], Andriy Tsyrulnyk[3], Daria Fedorovych[3], Kostyantyn Dmytruk[3], Andriy Sibirny[1,3]

[1] Faculty of Biotechnology, Medical College, University of Rzeszów, Cwiklinskiej 2D, Rzeszów 35-601 Poland;
[2] The Doctoral School of the University of Rzeszów, University of Rzeszów, 35-959 Rzeszów, Poland,
[3] Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005 Ukraine;
* Correspondence to: J. Ruchala, jruchala@ur.edu.pl

Riboflavin is an essential vitamin with wide-ranging industrial applications. To enable cost-effective and sustainable production, *Candida famata* has been genetically engineered to efficiently convert renewable carbon sources, including lignocellulosic sugars, into riboflavin. This study presents an advanced metabolic engineering strategy targeting genes involved in precursor supply, redox balance, and export mechanisms.

Overexpression of ***XYL1*** and ***XYL2***, encoding xylose reductase and xylitol dehydrogenase respectively, enabled effective utilization of xylose, a major sugar in lignocellulosic hydrolysates. To further enhance riboflavin synthesis, genes ***RIB6*** (involved in riboflavin biosynthesis), ***GND1*** (boosting the oxidative pentose phosphate pathway and increasing ribulose-5-phosphate availability), and ***RFE1*** (encoding a riboflavin efflux transporter) were overexpressed. These modifications synergistically improved both the metabolic flux toward riboflavin and its secretion.

Engineered strains, including BRPI derivatives, demonstrated robust growth and high-level riboflavin production not only on glucose but also on xylose and mixed sugar substrates derived from lignocellulosic biomass. This work confirms the feasibility of using *C. famata* for industrial riboflavin production on non-food substrates, contributing to circular bioeconomy initiatives.

METAL-RESISTANT BACTERIA AND THEIR PLANT GROWTH-PROMOTING POTENTIAL

Svitlana Hnatush **Lecture 6**

Svitlana Hnatush, Olha Maslovska, Solomiia Komplikevych
Ivan Franko National University of Lviv, Lviv, Ukraine
Email address: svitlana.hnatush@lnu.edu.ua

The use of preparations based on plant growth-promoting (PGP) microorganisms is a modern approach to improving soil fertility and protecting plants from phytopathogens and adverse environmental factors. Considering the current challenges, particularly global climate change, contamination of crop areas with various pollutants due to anthropogenic activities, especially hostilities, the need to isolate PGP microorganisms resistant to environmental factors is urgent. Environments with unfavorable habitat conditions are a source for isolating biotechnologically promising strains of microorganisms. The work is aimed at isolating bacteria resistant to heavy metal compounds and determining their PGP potential. The bacteria were isolated from the lake of infiltrates of a Lviv solid waste landfill (Lviv region, Ukraine) and Antarctic substrates collected during the Ukrainian Antarctic expeditions in 2019–2023. The PGP properties of bacterial isolates were determined by their ability to solubilize P and Zn compounds, fix N_2, and synthesize siderophores and auxin-like compounds. Fifteen bacterial strains were isolated and identified by the 16S RNA gene sequence and physiological and biochemical properties as *Pseudomonadota, Actinomycetota, Bacteroidota,* and *Bacillota.* All the strains studied were psychrotolerant, moderate halophiles. The bacteria were resistant to Cd^{2+}, Fe^{2+}, Cu^{2+}, Cr(VI), Mn^{2+}. The most resistant to the influence of heavy metal compounds was the strain *Pseudomonas* sp. 3B-in-57 isolated from the soil of the nesting and feeding site of *Larus dominicanus* (Maritime Antarctic). It grew under the influence of 20.0 mM Mn(II), 20.0 mM Fe(II), 6.0 mM Cu(II), 10.0 mM Cr(VI), and 0.5 μM Cd. Among the tested bacterial strains, six fixed N_2. Five bacterial strains solubilize ZnO. The highest ZnO solubilization index (4.0±0.33) was found in *Pseudomonas yamanorum* 79A-102. Cellulases were produced by *Paenarthrobacter* sp. 2B-in-78, *Paenibacillus tundrae* IMV B-7915, *Ochrobactrum rhizosphaerae* IMV B-7956 and *Pseudarthrobacter* sp. IMV B-7981. The index of enzymatic activity of these strains was in the range of 9.6–11.5. Three strains of bacteria of the genus *Pseudomonas*, isolated from different Antarctic substrates (*Pseudomonas* sp. 3B-in-57, *P. yamanorum* IMV B-7916, *Pseudomonas* sp. EO1), were able to fix N_2, solubilize ZnO, and synthesize cellulases. All the studied bacteria synthesize siderophores and auxin-like compounds. The highest content of siderophores was found in the culture medium of bacteria *O. rhizosphaerae* IMV B-7956, and the highest content of auxin-like compounds was found in the culture medium of bacteria *Pseudarthrobacter* sp. IMV B-7981. Treatment of seeds of *Triticum aestivum* cultivar Tybalt with isolated bacterial strains increased seed germination. The greatest seed germination was found in the case of wheat seed treatment with *Pseudomonas* sp. 3B-in-57, *Lysinibacillus* sp. IMV B-8081 and *P. yamanorum* 79A-102. Bacterization of wheat seeds with the studied bacterial strains had a positive effect on the morphology of seedlings and on the pigment content in leaves.

The work was performed under the State Target Scientific and Technical Program for Research in the Antarctic for 2011–2025 (contracts No H/02-2021, H/03-2023, H/02-2024).

NATURAL LACCASE AND ITS MIMETICS IN AMPEROMETRIC SENSORS FOR DETERMINATION OF SEROTONIN AND CATECHOLAMINE BIOMARKERS OF NEUROENDOCRINE DISORDERS

Olha Demkiv **Lecture 7**

Olha Demkiv, Nataliya Stasyuk, Naum Yulia, Galina Gayda, Mykhailo Gonchar
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: demkiv@yahoo.com

Laccase is a multicomponent oxidase enzyme that catalyzes the four-electron reduction of molecular oxygen to water with simultaneous oxidation of a wide range of phenolic and non-phenolic substrates. Due to its versatility, it finds application in various industries. Of particular interest are laccase mimetics (LacNZs) — nanozymes with laccase-like activity, which are characterized by high stability and ~~the~~ ability to precisely regulate their properties. In this regard, laccase and its mimetics are considered as a promising biocatalyst for the creation of new generation biosensors, in particular, for the determination of biomarkers of neuroendocrine tumors and neurodegenerative diseases.

The use of purified laccase from *Trametes zonata* strain 1525 as a bioselective element of amperometric sensors for the determination of 5-hydroxyindoleacetic acid (5-HIAA) and 4-hydroxyindole (4-HI) — metabolites of serotonin and tryptophan, which are important biomarkers of carcinoid tumors, endometriosis, and neuroendocrine disorders, has been demonstrated for the first time. The developed sensors with immobilized laccase on a modified graphite electrode demonstrated high sensitivity (1900 ± 9 $A \cdot M^{-1} \cdot m^{-2}$ for 5-HIAA and 3556 $A \cdot M^{-1} \cdot m^{-2}$ for 4-HI) and a wide linear range (2–50 μM and 2–200 μM, respectively). The sensor for 5-HIAA was successfully applied to urine analysis, which was confirmed by good recovery and reproducibility indicators.

Along with natural laccase, the use of laccase mimetics (LacNZs) based on PtMn and MnO_2 nanoparticles (NPs) for amperometric determination of 5-HIAA in urine was investigated. The synthesized LacNZs showed high laccase activity comparable to the natural enzyme, as well as increased stability over a wide range of pH (3.0–7.5), temperatures (4–70 °C), and ionic strength (up to 500 mM NaCl). Amperometric sensors based on PtMn NPs/graphite electrode demonstrated high sensitivity (25000 ± 12 $A \cdot M^{-1} \cdot m^{-2}$), low detection limit (0.16 μM), and wide linear range (0.3–15 μM) for the determination of 5-HIAA, as well as better stability (up to 20 days). It was also shown that LacNZs, synthesized by chemical reduction (nAuCePt, nPtFe) and hydrothermal method (nAuCu, nPtCu), exhibit different substrate specificity: the former for adrenaline (AD), the latter for dopamine (DA). These NPs were used to create amperometric sensors. The nAuCePt-based sensor has a higher sensitivity, lower detection limit and wider linear range compared to the natural laccase-based sensor, and has been successfully applied for the analysis of AD in pharmaceuticals. The nAuCu and nPtCu-based sensors for the determination of DA demonstrated better sensitivity, selectivity and stability.

The obtained results indicate the high potential of both natural laccase and its mimetics in creating bioselective elements for highly sensitive, stable amperometric sensors suitable for the determination of biomarkers, such as 5-HIAA, 4-HI, AD and DA. Further studies can be aimed at expanding the list of target analytes and implementing the developed sensors in clinical non-invasive diagnostics.

Acknowledgment: The research was partially funded by the US National Science Foundation grant CBET-2235349, including IMPRESS-U supplement (project STCU 7113) and by "Presidential Discretionary-Ukraine Support Grants" from the Simons Foundation (Award No. 652 1030281).

IDENTIFICATION OF NEW GENES INVOLVED IN THE REGULATION OF XYLOSE ALCOHOLIC FERMENTATION IN THE THERMOTOLERANT YEAST *OGATAEA POLYMORPHA*

Roksolana Vasylyshyn (*young scientist*) **Lecture 8**

Roksolana Vasylyshyn[1,2], Justyna Ruchala[2], Kostyantyn Dmytruk[1], Andriy Sibirny[1,2]
[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov St., 14/16, 79005 Lviv, Ukraine. [2]Faculty of Biotechnology, Medical College, University of Rzeszow, Rzeszów 35-601, Poland, Cwiklinskiej St., 2D, 35-601 Rzeszow, Poland.
Email address: vasylyshyn.r.v@gmail.com

Xylose and L-arabinose are important components of renewable feedstocks (lignocellulose and pectin) for biofuel production, being the second and third most abundant sugars in nature. However, since most known yeast strains do not utilize L-arabinose and xylose as carbon sources, a significant amount of carbon in plant residues cannot be used for bioethanol production. Despite significant efforts made in the alcoholic fermentation of basic sugars from lignocellulose hydrolysates and some achievements in this field (especially in xylose fermentation), yeast strains capable of efficient fermentation of L-arabinose have not yet been identified.

Previously, *O. polymorpha* strains were found to be excellent ethanol producers from xylose (Ruchala et al., 2017; Kurylenko et al., 2018), but these strains grew poorly on L-arabinose. In contrast, mutants of *O. polymorpha* obtained by UV mutagenesis robustly grew on L-arabinose and still accumulated half of the biomass compared to the parental strain growing on xylose. However, after analyzing the level of ethanol production during high-temperature alcohol fermentation in an environment with 10% xylose or 5% L-arabinose, it was found that the resulting mutants produced 30% more ethanol from xylose compared to the parent strain. Moreover, the ability to ferment L-arabinose in mutants was 6 times higher than in the parent strain during high-temperature alcohol fermentation at 45°C. By sequencing the genomes of the respective strains, two genes have been identified that enable the growth and fermentation of L-arabinose as the sole carbon source. It has been established that damage to the *IRA1* gene positively affects xylose and L-arabinose alcoholic fermentation in *O. polymorpha* yeast.

Acknowledgment:
The research was funded by the MSCA4Ukraine consortium under the EU's Marie Skłodowska-Curie Actions (project number 1232295) and was partially supported by the Presidential Discretionary–Ukraine Support Grants from the Simons Foundation (award no. 1030281).

EFFICACY OF A PORTABLE UV-LED WATER DISINFECTION SYSTEM IN COMBAT ZONES

Maya Vergolyas **Lecture 9**

Vergolyas M.R.[1], Leonov Yu.I.[1], Hlyva V.B.[2], Balko O.I.[3], Mavrykin Ye.O.[1], Balko O.B.[3], Nechyporenko S.H.[1], Isypova V.M.[1]
[1] SI "Kundiiev Institute of Occupational Health of the NAMS of Ukraine"
[2] Kyiv National University of Construction and Architecture
[3] Institute of Microbiology and Virusology named after D.K. Zabolotnyi NAS of Ukraine
Email address: vergolyas@gmail.com

Water is an essential condition for life, yet its quality often fails to meet sanitary standards, especially in areas lacking adequate water supply infrastructure or in regions affected by armed conflict. Warfare, as a factor contributing to emergencies and often preventing the implementation of preventive measures, significantly increases the risk of contamination of water sources and drinking water, undermines the reliability of water supply systems, and limits access to safe drinking water for the population [1, 2]. This issue is particularly critical in combat zones and frontline areas in the East and South of Ukraine, where water infrastructure has been damaged or destroyed, making it impossible to meet basic human needs for safe drinking water, which often requires effective disinfection [3]. The primary microbiological indicator of water safety is the absence of coliform bacteria in 100 ml of water. *Escherichia coli* is used as an indicator of fecal contamination for several reasons: it is consistently present in the intestines of humans and animals, easily cultivated in laboratory conditions, sensitive to environmental changes, and quickly dies in clean water; its presence indicates the possible existence of other, more dangerous pathogens [4]. UV irradiation is a modern and safe disinfection method offering several advantages: it does not alter the chemical composition or taste of water; it requires no chemical additives; and it effectively inactivates most bacteria, viruses, cysts, and spores. In natural disasters, military operations, or areas without centralized water treatment, portable filters and UV systems are essential for quick water disinfection; they are easy to transport, battery or solar-powered, and suitable for field use by tourists or military personnel. The aim of this study was to evaluate the bactericidal effectiveness of a portable UV water treatment system prototype developed by the authors, using *E. coli* as a model sanitary-indicator microorganism.

Materials and Methods. We used a prototype portable UV water treatment system developed by the authors, based on LED lamps emitting at a wavelength of 279 nm. The test microorganism was *Escherichia coli* strain UCM B-906 from the collection of the D.K. Zabolotny Institute of Microbiology and Virology.

Results. Our findings confirm that the prototype portable UV water disinfection system demonstrates effective bactericidal activity against *E. coli* after a 10-minute exposure at a wavelength of 279 nm, even at a high bacterial concentration of 10^9 CFU. Moreover, the system is energy efficient and can be powered by a 10,000 mAh power bank for at least 20 consecutive disinfection cycles.

References:

1. Polka N.S., Fedorenko V.I., Plastunov B.A. Problems of environmental conservation and national health in the materials of the XV Congress of Hygienists of Ukraine. Environment and Health. 2013. No. 2(65): 68–80.
2. Smith R., Nieuwenhuijsen M., Wright J., Raynor P., Cocker J., Jones K. Validation of trichloroacetic acid exposure via drinking water during pregnancy using a urinary TCAA biomarker. *Environmental Research*. 2013. Vol. 126. P. 145–151.
3. Ivanko O.M., Zorina O.V., Deputat Yu.M. Analysis of the prospects for implementing AMEDP-4.9 "Requirements for the suitability of drinking water during field operations and emergencies" in Ukraine. *Ukrainian Journal of Military Medicine.* 2022. Vol. 3, Suppl. Materials of the 5th Scientific-Practical Conference with International Participation "Academic Readings Named After Volodymyr Pasko". P. 31–33.
4. Zorina O.V., Surmasheva O.V., Ivanko O.M., Polka O.O., Mavrykin Ye.O. Analysis of approaches to the assessment and use of drinking water disinfectants in Ukraine and EU/NATO countries. *Ukrainian Journal of Military Medicine.* 2025. Vol. 6. P. 48–56.

AMPEROMETRIC BIOSENSORS AND BIOFUEL CELLS FOR DETECTION AND BIOREMEDIATION OF EXPLOSIVES IN EXPLOSIVE-CONTAMINATED ENVIRONMENTS

Nataliya Stasyuk **Lecture 10**

Nataliya Stasyuk, Olha Demkiv, Maryna Barska, Andriy Zakalskiy, Mykhailo Gonchar
Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Str. 14/16, 79005, Lviv, Ukraine
Email address: stasuk_natalia@ukr.net

The growing demand for early disease diagnosis, enhanced environmental protection, and better food quality control highlights the need for more widespread use of highly sensitive, selective, rapid, and cost-effective analytical methods. In this context, analytical biotechnology plays a crucial role, as it leverages biomolecular recognition principles to detect analytes of significant practical importance [1]. The implementation of enzymes, especially reductases, into environmental chemistry is one of the main achievements of analytical biotechnology [2]. Both groundwater and soil pollution with nitroaromatic and nitramine explosive compounds is a wide problem around the world [3]. Explosive materials are energetic substances, that, when released into the environment, pose a serious danger to the environment and living organisms. Among different forms of chemical nitro-explosives, 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-triazinane (hexogen, RDX) are the most common.

Miniaturized devices powered by fuel cells for the detection of explosives would play an important role in enhancing safety and security in military operations, improving public safety measures, facilitating environmental cleanup in contaminated areas, and supporting humanitarian efforts in post-conflict regions. Amperometric biosensors based on artificial enzymes are sophisticated and cost-effective tools for a variety of analytical applications. In the current study, biosensors based on highly selective artificial nitrate reductases or natural nitrate reductase for detecting TNT and RDX in post-explosive soil samples, as well as related biofuel cells (BFCs), have been developed. The fabricated amperometric biosensors based on cadmium-cobalt nanocomposites and copper nanostructures possess high sensitivity (6330 $A \cdot M^{-1} \cdot m^{-2}$ and 6070 $A \cdot M^{-1} \cdot m^{-2}$), low limit of detection and good selectivity towards the target analytes, TNT and RDX, respectively. The best developed BFC generates an open circuit potential of 560 mV with a maximum power density of 0.850 $\mu W \cdot cm^{-2}$ at an optimum of 0.1 mM RDX. The constructed biofuel cells were tested for model bioremediation on the real samples of the post-explosive soils.

References

1. Broomandi P., Guney M., Kim J.R. & Karaca F. Soil Contamination in Areas Impacted by Military Activities: A Critical Review. *Sustainability,* **2020**, *12,* 9002.
2. Santos-Beneit F. *Microbiology*, **2024**, *13*(2), e1406.
3. Robinson P.K. Enzymes: principles and biotechnological applications. *Essays Biochem.* **2015**, 59, 1–41.

CONSRTUCTION OF CANDIDA FAMATA STRAINS WITH INCREASED *RESISTANCE TO LIGNOCELLULOSIC* HYDROLYSATE *INHIBITORS.*

Ljubov Dzanaeva (*young scientist*) **Poster 1**

Dzanaeva Ljubov[1], Dariya Fedorovych[1], Kostyantyn Dmytruk[1], Andriy Sibirny[1,2]
[1] *Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov St., 14/16, Lviv 79005, Ukraine*
[2]*Institute of Biology and Biotechnology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601, Poland*
liubadzanaieva@gmail.com

Riboflavin is essential for growth and reproduction of humans and animals. The price of this biotechnological product is dependent on the price of the carbon substrate used. Lignocellulosic biomass is a promising source of sugars, however, during hydrolysis are released toxic compounds, that can inhibit the producing strain. We recently showed, that C. famata BRPI strain with overexpression of the XYL1 gene increased riboflavin production by 10-15% in a medium with diluted hydrolysate. For today there is no data on the genetic regulation in C. famata of tolerance to inhibitors (furfural, hydroxymethylfurfural (HMF), and acetic acid), nor on whether increased resistance would correlate with increased riboflavin production.

Overexpression of ADH6 (coding for alcohol dehydrogenase), HAA1 (coding for transcription factor), and GRE2 (coding for aldehyde reductase) genes were performed for C. famata BRPI strain. Resulted strains were characterized by increased in riboflavin production in YNB medium supplemented with 3 mM and 10 mM HMF, but not in YNB medium with furfural. The production of riboflavin by the constructed C. famata strains was also checked in YNB medium supplemented by 25 % hydrolysate of bagasse. According to preliminary results, the recombinant strains demonstrated higher levels of riboflavin production compared to the parental strain.

Acknowledgment:
This work was supported by “Presidential Discretionary-Ukraine Support Grants” from Simons Foundation, Award No 1030281 and No 1290613.

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

Lubov Fayura **Poster 2**

Lubov Fayura[1], Dariya Fedorovych[1], Dmytro Chepiha[3], Olena Krasovska[3], Andriy Sibirny[1,2], Kostyantyn Dmytruk[1]

[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine,
[2]University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland
[3] PJSC "Enzyme Company"

Email address: *fayural@gmail.com*

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). Twelve successive transfers of the yeast strain *Saccharomyces cerevisiae* IMB Y-5058 were performed on media with incrementally increased concentrations of roseoflavin, with each step increasing by 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 cultivation conditions for the selected strain F57 in the bioreactor - including temperature, cultivation time, pH and aeration were optimized, resulting in 1.4–1.5 times higher riboflavin accumulation compared to the parental 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.

CONSTRUCTION OF YEAST PRODUCERS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) ACTIVATING WOUND HEALING

Olena Dmytruk **Poster 3**

Olena Dmytruk[1], Nataliya Finiuk[1], Rostyslav Stoika[1], Andriy Sibirny[1,2], Kostyantyn Dmytruk[1]
[1] Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[2]University of Rzeszow, Rzeszow, Poland
Email address: verbaolena@gmail.com

The development of new effective methods promoting the regeneration and healing of wounds of various etiologies is an important problem in modern medicine. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth factor found in wounds after acute injury. It plays an important role in wound healing by promoting wound contraction, attracting immune cells, supporting skin regeneration, and stimulating immune responses. GM-CSF also boosts the production and development of blood-forming stem cells. A lack of GM-CSF leads to slower healing and reduced wound immune cell activity. Applying GM-CSF locally has been shown to improve healing significantly. However, its broader use, especially for treating chronic or combat-related wounds, is limited by the high cost of the medication.

The main goal of this work was to construct GM-CSF producers based on methylotrophic yeast with “humanized” protein glycosylation. Nucleotide sequences encoding GM-CSF were designed, and corresponding expression vectors were constructed for both the secretory form of GM-CSF and variants intended for immobilization on the yeast cell surface in the methylotrophic yeasts *Komagataella phaffii* and *Ogataea polymorpha*, engineered for 'humanized' protein glycosylation. Yeast transformants were selected and analyzed for GM-CSF production and localization. The results showed that the majority of GM-CSF remained within the yeast cells. However, flanking the GM-CSF sequence with flexible glycine-serine linkers promoted its secretion into the culture medium. Engineered strains of *K. phaffii* demonstrated higher levels of secreted GM-CSF compared to *O. polymorpha*. Cultivation conditions were optimized for maximal production, and GM-CSF was purified using metal affinity chromatography. The biological activity of the recombinant proteins, as well as the yeast strains displaying GM-CSF on their cell surface, was confirmed by their ability to stimulate the proliferation of the GM-CSF-dependent erythroblasts of the TF-1 line.

Acknowledgment:
This research was supported by a grant from the National Research Foundation of Ukraine (Project No. 2023.04/0032).

ADAPTIVE LABORATORY EVOLUTION OF LACTIC ACID-PRODUCING *OGATAEA POLYMORPHA* YEAST STRAINS FOR INCREASED LACTIC ACID TOLERANCE

Aksyniia Tsaruk (*young scientist*) **Poster 4**

Aksyniia Tsaruk[1,2], Justyna Ruchała[2], Andriy Sibirny[3]
[1]The Doctoral School of the University of Rzeszów, University of Rzeszów, Rzeszow, Poland
[2]Faculty of Biotechnology, Collegium Medicum, University of Rzeszow, Rzeszow, Poland
[3]Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine
Email address: aksyniiat@dokt.ur.edu.pl

Lactic acid is an organic compound with a large and rapidly growing global market due to its applications in a variety of industries as a solvent, pH regulator, preservative, and a monomer of biodegradable plastic alternative, polylactic acid. One of the main limitations in microbial lactic acid production is the ability of lactate-producing organisms to tolerate low pH values during fermentation caused by lactic acid accumulation in the culture medium.

In our study, *Ogataea polymorpha* yeast strains with heterologous expression of lactate dehydrogenase genes from *Bos taurus* and *Plasmodium falciparum* (*LDH_Bt* and *LDH_Pf*, respectively) were subjected to an adaptive laboratory evolution by cultivating yeast cells in minimal medium with a gradual increase in lactic acid concentration from 10 to 20 g/L during 13 subcultures. From the final subculture, single colonies were isolated from each strain on solid medium. Obtained strains demonstrated improved growth rate on both liquid and solid medium containing 20 g/L of lactic acid compared to unevolved strains. Lactic acid and ethanol production, glucose consumption, and biomass accumulation by the evolved strains were investigated during high-temperature fermentation on minimal medium containing 10% glucose. Biomass accumulation during fermentation was lower compared to parental strains. The highest improvement in lactic acid production was demonstrated by one of the evolved *LDH_Bt* strains. It produced 4 g/L of lactic acid, which is 50% more as opposed to the parental strain. Notably, the evolved *LDH_Pf* strains showed a drastic decrease in lactic acid production along with increased glucose consumption and ethanol production.

Adaptive laboratory evolution in combination with metabolic engineering can be a powerful tool for developing lactic acid-producing yeast strains with increased lactic acid tolerance and lactic acid production, in addition to a deeper understanding of lactic acid metabolism in yeast.

THE POTENTIAL OF *PAENIBACILLUS* BACTERIA AS BIOFERTILISERS AND BIOCONTROL AGENTS

Valentina Kovaleva **Poster 5**

Yurii Yusypovych[1], Yuliia Shalovylo[1,3], Oleh Kit[1], Valentina Kovaleva[1,2]

[1]Ukrainian National Forestry University, Lviv, Ukraine
[2]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[3]Sudovovyshnya Lyceum Named after Tadei Dmytrasevych, Lviv, Ukraine

Email address: kovaleva@nltu.edu.ua

Bacteria from the genus *Paenibacillus*, comprising approximately 200 species, are widely distributed across diverse environments. Among them, many species are capable of directly promoting plant growth through various mechanisms [1]. In addition, bacteria of this genus possess significant biocontrol potential through the production of antibiotic compounds [2]. Moreover, they decompose organic matter and mobilize nutrients, thereby improving soil structure and fertility.

Due to these properties, *Paenibacillus* strains have garnered significant research attention as an important group of microbial inoculants with roles in sustainable agriculture, biofertilization, biocontrol, and biofuel production. The majority of *Paenibacillus* species with characterized plant growth-promoting and biocontrol properties have been isolated from the rhizosphere or plant tissues of various species [3]. *Paenibacillus* strains have also been identified in forest litter—the layer of organic material on the forest floor, including freshly fallen or partially decomposed leaves, bark, twigs, flowers, fruits, and other vegetative matter. However, the plant growth-promoting and biocontrol abilities of forest-litter-derived bacteria remain poorly studied.

In this study, three *Paenibacillus* strains—B21, B22, and B23—closely related to *Paenibacillus terrae* based on 16S rRNA gene sequencing, were isolated from forest litter. Determination of their precise taxonomic position through whole-genome sequencing is currently in progress. In vitro trials showed that all isolates demonstrated plant growth-promoting activities, including the production of indole-3-acetic acid, ammonia, and siderophores, as well as nitrogen fixation and phosphate solubilization. In addition, strains B21, B22, and B23 exhibited antimicrobial activity against a broad spectrum of phytopathogenic fungi and bacteria.

Bioinoculation of Scots pine seeds with *Paenibacillus* strains enhanced seed germination in greenhouse conditions, increased the root length of six-week-old seedlings, and altered root system morphology compared to the control group, including increased branching and enhanced lateral root formation. Furthermore, the dry biomass of inoculated seedlings increased by 17–45%, depending on the strain.

Collectively, these findings suggest that the isolated *Paenibacillus* strains hold significant promise for producing high-quality planting material for forestry.

Acknowledgement:

This research was supported by the National Research Foundation of Ukraine, project No. 2021.01/0184 «Silvicultural and molecular genetic principles of increasing the biotic resilience of Scots pine stands in the context of adaptation to climate change and anthropogenic pressure». This work was partially supported by a grant from the Simons Foundation ([award 1030281], [VAK]).

References:

1. E. N. Grady, MacDonald, L. Liu, A. Richman and Z.-C. Yuan, *Microbiol Cell Factories*, **2016**, 15, 203.
2. J. Dobrzyński, A. Naziębło, *Microbial Ecology*, **2024**, 87, 134.
3. P. Kämpfer, A. Lipski, L. Lamothe, D. Clermont, A. Criscuolo, J. A. McInroy and S. P. Glaeser, *Antonie van Leeuwenhoek,* **2023** 116, 883.

FLAVOCYTOCHROME B_2 AS AN L-LACTATE-SELECTIVE BIOELEMENT FOR ELECTRONIC DEVICES AND A TOOL FOR SYNTHESIZING ORGANIC–INORGANIC NANOMATERIALS

Galina Gayda **Poster 6**

Galina Gayda, Tetyana Prokopiv, Olha Demkiv, Mykhailo Gonchar
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Email address: galina.gayda@gmail.com

L-lactate–cytochrome c oxidoreductase or flavocytochrome b_2 (Fcb_2) is a large FMN- and heme-containing enzyme with a complex structure, and its study has been limited due to protein instability. The enzyme from the thermotolerant methylotrophic yeast *Ogataea polymorpha* exhibits sufficient stability for isolation, purification, characterization, lyophilization, and long-term storage. Fcb_2 displays absolute specificity for L-lactate, making it a highly valuable biorecognition element for amperometric biosensors (ABSs) [1-2] and biofuel cells (BFCs) [3].

Bioelectronic devices ABSs and BFCs are distinct primary functions but share common fundamental principles in their operation. They rely on substrate biorecognition, redox reactions, electron transfer, and advanced electrode materials, including innovative nanomaterials (NMs). ABSs and BFCs, utilizing microbial oxidoreductases in combination with electroactive NMs, are both efficient and cost-effective. Here we report, for the first time, the development of laboratory prototypes of BFCs featuring anodes containing Fcb_2 and cathode based on fungal laccase, which were co-immobilized with redox-active NMs on glassy carbon electrodes (GCEs). The best BFC, incorporating a L-lactate-sensitive bioanode Fcb_2/nanoAuHCF/GCE and a biocathode laccase/nanoAuCePt/GCE, demonstrated a specific power density of 1.8 μW/cm². The constructed BFC were shown to generate energy using lactate-containing foods as fuels [3].

Fcb_2 serves also as a tool for the 'green' synthesis of organic–inorganic NMs, specifically, hexacyanoferrates of transition metals (gHCFs). gCuHCF was shown to have the most promising characteristics among the studied gHCFs. The uniqueness of gCuHCF lies in its multifunctionality, serving as a peroxidase mimic, a chemosensor for ammonium ions, a biosensor for L-lactate, and exhibiting perovskite-like properties [4,5]. We recently reported for the first time the exceptional ability of gCuHCF to enhance fluorescence under blue light irradiation [5]. Thus, gCuHCF, synthesized *via* Fcb_2, presents a promising platform for the development of biosensors, bioreactors, biofuel cells, solar cells, and other advanced devices.

Acknowledgment:
The research was partly funded by the US National Science Foundation grant CBET-2235349 including IMPRESS-U supplement (project STCU 7113) and by "Presidential Discretionary-Ukraine Support Grants" from Simons Foundation (Award No. 652 1030281).

References:

1. Demkiv et al. Flavocytochrome b_2-Mediated Electroactive Nanoparticles for Developing Amperometric L-Lactate Biosensors. ***Biosensors*** *(2023)* doi: 0.3390/bios13060587.

2. Gayda G. et al. Peroxidase-like Nanoparticles of Noble Metals Stimulate Increasing Sensitivity of Flavocytochrome b_2-based L-Lactate Biosensors. **Biosensors** (2024) doi: 10.3390/bios14110562.

3. Demkiv et al. Biofuel Cells Based on Oxidoreductases and Electroactive Nanomaterials: Development and Characterization. **Biosensors** (2025) doi: 10.3390/bios15040249.

4. Gayda G. et al. "Green" Prussian Blue Analogues as Peroxidase Mimetics for Amperometric Sensing and Biosensing. ***Biosensors*** (2021) doi: 10.3390/bios11060193.

5. Gayda et al. Copper Hexacyanoferrates Obtained via Flavocytochrome b_2 Assistance: Characterization and Application. **Biosensors** (2025) doi: 10.3390/bios15030157.

CONSTRUCTION OF RIBOFLAVIN OVERPRODUCER STRAINS BY INTRODUCTIONS OF ADDITIONAL COPIES OF THE GENES *RFE1*, *GND1*, AND *RIB6* INTO THE YEAST *CANDIDA FAMATA*

Andriy Tsyrulnyk **Poster7**

Wen Liu[a, b], Andriy Tsyrulnyk[a], Kostyantyn Dmytruk[a], Dariya Fedorovych[a], Andriy Sibirny[a, c]

[a] Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov St., 14/16, Lviv 79005, Ukraine
[b] Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education of Guizhou & School of Basic Medical Science & Institution of One Health Research, Guizhou Medical University, Guiyang, China.
[c] Institute of Biology and Biotechnology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601, Poland

Riboflavin is an essential vitamin (B2) which plays a central role in cell metabolism through regulation of main oxidative reactions. Animal and human cells are not able to produce it by their self. Thus, riboflavin arrives to the cells only from the food. Usually, no deficit in human nutrition is observed. In contrast, the farm animal industry urgently needs food supplements with riboflavin. The pharmacy is also, one of the beneficiaries of biotechnological production of this vitamin.

At present days, recombinant microorganisms, such as *Bacillus subtilis* and *Ashbya gossypii* are used for biotechnological production of riboflavin in industrial scale. According to the data of *Straits Research* the global riboflavin market reached up to US$ 13.46 billion in 2023.Using in past, yeast *Candida famata* dep8 was terminated through the instability of this strain.

Previously, in our lab were constructed by classical mutagenesis and gene engineering technologies a number of stabile highly efficient riboflavin overproducers based on yeast *C. famata* - strains AF4, BRP and BRPI. However, reached vitamin production on the level of 12-14 mg/L is still not sufficient to use them as industrial producers.

In this work, we introduce additional copies of essential genes of riboflavin synthesis pathway and excretion: gene *RIB6* (involved in conversion of Ru5P to DHBP), gene *GND1* (encoding 6-phosphogluconate dehydrogenase which catalyzes the conversion of 6-phosphogluconate into Ru5P) and gene *RFE1* (encoding riboflavin excretase). Genes where introduced to the wild VKM-Y9 and riboflavin overproducing strains *C. famata* AF4 and BRP. Different combination of two genes as well as simultaneous overexpression of all three genes showed increased riboflavin production from 1,2 up to 3 fold in constructed strains. Proposed technological gene manipulation assay led us to significantly improve yeast riboflavin overproducers.

ROLE OF THE GENES *MET2* AND *SEF2* IN THE REGULATION OF RIBOFLAVIN BIOSYNTHESIS

Serhiy Romanov (*young scientist*) **Poster 9**

Romanov S.[1], Volkova D., Dzanaieva L.[1], Tsyrulnyk A.[1], Fedorovych D.[1], Dmytruk K.[1], Sybirnyy A.[1,2]
[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[3] University of Rzeszow, Rzeszow, Poland
Email address*: sergij8romchyk@gmail.com*

Riboflavin (vitamin B_2) is an essential vitamin that forms part of flavin mononucleotide and flavin adenine dinucleotide, which participate in redox reactions critical for energy metabolism. Due to the high demand for riboflavin in the pharmaceutical, food, and feed industries, enhancing its microbial production has become increasingly relevant. The yeast *Candida famata* is a promising candidate for this purpose because of its natural flavinogenic potential.

In present work we have transformed yeast *Candida famata* VKMY-9 with introduction *MET2* and *SEF2* additional copies of genes and measured riboflavin production. To study the involvement of the *MET2* and *SEF2*, genes in the regulation of RF biosynthesis, their expression under the control of their own or a strong *TEF1* gene promoter was enhanced in the wild-type VKMY-9 strain of *C. famata*. We have found that overexpression of the *MET2* gene did not affect the flavinogenic activity of *C. famata*. Expression of the *SEF2* gene under the control of the *TEF1* promoter resulted in a 5-fold decrease in RF production. The results obtained indicate the involvement of the *SEF2* gene in the negative regulation of RF biosynthesis in *C. famata*.

Acknowledgment:
This work was supported by the National Research Foundation of Ukraine, grant 2023.03/0035.

References:
1 L. Averianova et al., *Front. Bioeng. Biotechnol.,* **2020**, *8*, 570828.
2 C. Abbas and A. Sibirny, *MMBR,* **2011**, *75*, 341.
3 K. Dmytruk and A. Sibirny, *Yeast,* **2012**, *29*, 455.
4 Y. Andreieva et al., *Cytology and Genetics*, **2020**, *54*, 379-385.

ARGININE DEPRIVATION ENHANCES ANTIMICROBIAL EFFECTS OF ARGININE ANALOGUES *IN VITRO*

Demash D.V.[1], Vovk O.I.[1], Polishchuk N.V.[1], Shuvayeva G.Y.[1], Stasyk O.G.[1,2], Stasyk O.V.[1,3]

[1] Department of Cell Signalling, Institute of Cell Biology, NAS of Ukraine, 14/16 Drahomanov str. Lviv, 79005, Ukraine
[2] Department of Biochemistry, Biological Faculty, Ivan Franko National University of Lviv, Hrushevsky Street 4, Lviv 79005, Ukraine
[3] Research Institute of Epidemiology and Hygiene, Danylo Halytsky Lviv National Medical University, 12 Zelena str., Lviv, 79005, Ukraine

Plants are known as a source of unique biologically active substances, which are rarely found in other taxonomic groups. Such substances are often structural analogues of known compounds that mimic their structure but possess minor changes that substantially affect their biological activity, sometimes rendering them toxic.

In the present study, we evaluated the antimicrobial activity of L-arginine structural analogs, canavanine, indospicine, and ethanolic extract from leguminous plant Indigofera spicata green biomass, which contains arginine, canavanine, and indospicine, on model bacterial strains *in vitro*.

We demonstrated that the studied agents had little to no effect on the growth dynamics of *Escherichia coli* DH5α and *Pseudomonas aeruginosa* ATCC 9027 grown on full and M9 media in concentrations up to 200 μM (standardized by canavanine). On the other hand, pre-incubation of an extract with recombinant human arginase I resulted in a significant reduction of growth kinetics for both studied bacterial strains.

We suggest that L-arginine, present in the plant extract, acts as a competitive inhibitor, which prevents the bacterial cells from accumulating its toxic analogs. The fact that the complete removal of arginine from the extract and the growth medium increases the antibacterial activity of both canavanine and *I. spicata* extract suggests that combining amino acid toxic analogues and amino acid deprivation might be a useful novel antimicrobial strategy. Such a strategy may potentially have a low risk of resistance phenotype development due to highly conservative pathways associated with arginine metabolism, which are present across different bacterial genera.

These preliminary results support further studies aimed at testing the proposed strategy against clinical bacterial isolates, especially those with acquired resistance to commonly used antibiotics.

Acknowledgement.
This study was supported 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».

TRANSCRIPTIONAL PATTERNS OF SCOTS PINE DEFENSINS IN SEEDLINGS COLONIZED BY *PSEUDOMONAS PUTIDA*

Valentina Kovaleva[1,2], YuriiYusypovych[1], Yuliia Shalovylo[1,3], Oleh Kit[1]

[1]Ukrainian National Forestry University, Lviv, Ukraine
[2]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
[3]Sudovovyshnya Lyceum Named after Tadei Dmytrasevych, Lviv, Ukraine
Email address: kovaleva@nltu.edu.ua

Biopriming of seeds with various beneficial bacteria, particularly plant growth-promoting bacteria (PGPB), enhances plant resilience and performance under adverse conditions [1]. Recently, we demonstrated that bioinoculation of Scots pine (*Pinus sylvestris*) seeds with *Pseudomonas* strains significantly improved seed germination and increased the dry biomass of aerial parts in two-year-old seedlings under field conditions [2]. PGPB are known to interact with host plants, boosting their immunity.

Defensins, widely distributed in animals and plants, play a central role in innate immune defense. Structurally, defensins exhibit a cysteine-stabilized αβ (CSαβ) motif, which confers exceptional stability under extreme temperature and pH conditions [3]. They serve as the first line of defense against invading pathogens and pests. Certain defensins also participate in plant interactions with symbiotic fungi [4]. However, little is known about the transcriptional profiles of defensin genes in plants colonized by endophytic PGPB.

The aim of this study was to analyze the transcriptional patterns of Scots pine defensins (*PsDef1–4*) in seedlings colonized by *Pseudomonas putida* P57, a strain previously isolated from Scots pine tissues. We quantified defensin gene expression in seedling tissues using qRT-PCR and examined their expression dynamics during early colonization. We found that all four *PsDef* genes showed upregulation 48 hours after inoculation: *PsDef1*, *PsDef3*, and *PsDef4* increased1.5–2-fold; *PsDef2* increased up to 4-fold. Bacterized seedlings (colonized by *P. putida*) exhibited enhanced resistance to fungal pathogens (*Ophiostoma clavatum* and *Fusarium verticillioides*). Colonization modulated defensin gene expression during fungal infection: in bacterized seedlings, expression remained stable and moderately elevated; in non-bacterized seedlings, transcript levels fluctuated erratically, showing both upregulation and downregulation. These results clarify a mechanism through which seed biopriming affects plant responses to pathogens.

Acknowledgement:

This research was supported by the National Research Foundation of Ukraine, project No. 2021.01/0184. This work was partially supported by a grant from the Simons Foundation ([award 1030281], [VAK]).

References:

1. M. Sood, V. Kumar and R. Rawal, *Biocontrol Agents and Secondary Metabolites,* **2021,** 181.
2. Y.Shalovylo, Y. Yusypovych, O. Kit and V. Kovaleva, *J. Microbiol. Biotechnol*., **2025**, 35: e240856.
3. V. Kovaleva, I. Bukhteeva, O. Kit, and I. Nesmelova, *Int. J. Mol. Sci..,* **2020**, 21(15), 5307.
4. M. Uhe, C. Hogekamp, R. M. Hartmann, N. Hohnjec, H. Küster, *PLoS ONE* **2018**, 13, e0191841.

SCREENING OF BLACK LOCUST RHIZOSPHERE BACTERIA FOR NITROGEN FIXATION AND PHOSPHATE SOLUBILIZATION

Nataliya Melnykova[1], Peter Ferus[2], Dominika Bosiakova[2], Sergii Kots[1]

[1]Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Mlynany Arboretum, Institute of Forest Ecology, Slovak Academy of Sciences, Vieska nad Zitavou, Slovak Republic
Email address: mnn_knu@ukr.net

Today, rhizosphere microorganisms, which possess plant growth-promoting properties, are widely used in agriculture as biofertilizers to improve crop growth and productivity. Due to the ability to fix atmospheric nitrogen, solubilize phosphorus and other essential minerals from insoluble compounds, produce antibiotics and phytohormones, regulate plant tolerance to abiotic and biotic stress factors, rhizobacteria enhance plant nutrition, health and development. In addition, the application of microbes is an important element of modern agrotechnologies helping to save the environment. Sustainable agriculture required the use of effective microorganisms with broad-spectrum plant growth-promoting properties. Therefore, the search for beneficial rhizobacteria is of great interest. Most often, beneficial microorganisms are isolated from the rhizosphere of herbaceous plants. Meanwhile, microbes associated with the root zone of trees may also have the potential to improve plant growth and development. The aim of this study was to isolate bacteria from the rhizosphere of black locust (*Robinia pseudoacacia* L.) seedlings and to screen these microorganisms for nitrogen fixation and inorganic phosphate solubilization.

A total of 47 bacterial cultures were isolated from the soil samples taken from the black locust root zones in different studies conducted in the territory of Arboretum Mlynany (Slovak Republic) and their ability to fix atmospheric nitrogen was evaluated using the acetylene reduction method. 32 of the tested isolates exhibited nitrogenase activity ranging from 0,6 nmol to 47,1 nmol per hour per 10^8 bacterial cells. Among these, only five isolates showed nitrogenase activity higher than 39,2 nmol per hour per 10^8 bacterial cells. The tested bacteria were grown on Pikovskaya's agar, and inorganic phosphate-solubilizing strains were identified based on the ability of microbial cells in the colony to solubilize tricalcium phosphate and form a clear zone. A large proportion of the bacterial cultures did not show any phosphate-solubilization activity. Among the tested isolates, 10 bacterial strains solubilized tricalcium phosphate at low rate (the width of clear zones was around 1 mm from the edge of the colony). Only three isolates were found as phosphate solubilizers (the width of clear zones was around 3 mm from the edge of the colony).

The results demonstrate that among the microorganisms inhabiting the black locust rhizosphere, there are bacteria capable of converting atmospheric nitrogen into available nitrogen compounds and solubilizing inorganic insoluble soil phosphate. This indicates the possibilities of using the above-mentioned rhizobacteria to enhance plant productivity as well as to restore black locust forests affected by climate change, wildfires, or destroyed during wartime. However, further studies on the plant growth-promoting properties of selected bacterial cultures are needed.

ENHANCING AGRICULTURAL SUSTAINABILITY THROUGH SOYBEAN-BRADYRHIZOBIUM INTERACTION UNDER CLIMATE CHANGE

Tetiana Nyzhnyk, Sergii Kots, Alla Zhemojda
Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
Email address: *tp_nyzhnyk@ukr.net*

A promising Nature-Based Solution for providing legume crops with ecological nitrogen and increasing drought resistance is based on the formation of rhizobial symbiosis. At the same time, to resist drought in a warming climate, plants must adapt under stress and activate key systems for antioxidant protection. The aim of the present study is to explore the role of protective antioxidant systems in the drought resistance of soybean, depending on the effectiveness of forming a symbiotic interaction with nodula bacteria *(Bradyrhizobium japonicum)*.

Our experiment uses microbiological, biochemical, physiological methods and employs various symbiotic soybean systems based on strains of *Bradyrhizobium japonicum*, differing in activity and virulence.

Results showed that the the formation of effective drought tolerance in symbiotic soybean systems is accompanied by the preservation of the optimal prooxidant-antioxidant balance by activating key antioxidant enzymes, *viz.* superoxide dismutase and catalase, and regulating the development of oxidative processes. This helps maintain redox homeostasis and improve the effectiveness of the soybean-*Bradyrhizobium japonicum* symbiosis in drought conditions. Ineffective and less-effective soybean symbiotic systems are unable to fully implement antioxidant protection systems during water stress due to the excessive production of hydrogen peroxide and intensification of lipoperoxidation processes.

Therefore, in soybean-*Bradyrhizobium* symbioses, a key role in the formation of drought tolerance is played by the coordinated activity of the macro- and microsymbionts, which allows them to realize their adaptive potential and regulate redox homeostasis during water stress by activating key antioxidant enzyme systems. The presented approach in research proves that the use of active virulent *Bradyrhizobium strains* is important for increasing the implementation of protective and nitrogen-fixing properties in soybean under drought conditions.

Our research includes elements of modern agrotechnologies of soybean cultivation in the context of obtaining ecologically and economically safe crop production products.

CREATION OF PLASMIDS FOR INCREASED ACCUMULATION OF HEME-CONTAINING PROTEINS IN YEAST

Oleksandra Pidkurhanna, Hanna Andriiash, Olena Tigunova, Sergiy Shulga
Institute of Food Biotechnology and Genomics of Natl. Acad. Sci. Ukraine, Kyiv, Ukraine
E-mail address: pidkurhanna@gmail.com

The isolation of heme and heme-containing proteins from natural sources is inefficient and complex, so the production of hemoglobins based on microorganisms is a pressing issue. The yeast *Saccharomyces cerevisiae* is a model object of eukaryotic organism and an effective producer of food additives and pharmaceutical ingredients [1]. Therefore, it is a promising object for the microbiological production of leghemoglobin. The CRISPR/Cas9 method is one of the common genome editing technologies. The aim of the work was to create a leghemoglobin-producing strain of the *S. cerevisiae* species using the CRISPR/Cas9 technology.

The genes for enzymes involved in heme synthesis have been identified [2]. Overexpression of some or all eight heme synthesis genes is an effective strategy to increase hemoglobin accumulation in yeast cells. It has been established that *HEM2*, *HEM3*, and *HEM12* genes (encoding aminolevulinic acid dehydratase, porphobilinogen deaminase, and uroporphyrinogen decarboxylase, respectively) are rate-limiting for the yeast *S. cerevisiae* [3].

The coding sequences of the genes – *HEM1, HEM2, HEM3, HEM4, HEM12, HEM13, HEM14* and *HEM15* – were cloned. Two plasmid constructs were created, in which four genes of interest were combined using the ConLX and ConRX connectors. These connectors formed complementary sticky ends after treatment with the BsmBI (Esp3I) restriction enzyme. The first plasmid contained the *HEM1, HEM2, HEM3* and *HEM4* genes, each of which is under the control of the inducible pGAL1 promoter and the tADH1 terminator. The URA3 selectable marker and homologous arms were used for integration into the URA3-site. The second plasmid included the *HEM12, HEM13, HEM14, HEM15* genes, which are also under the control of pGAL1 and tADH1. The *LEU2* gene served as a selectable marker and homologous arms were inserted into the plasmid for integration into the LEU2-site. Both plasmids contained the Amp-ColE element for replication and selection in *E. coli* cells.

As a result, two plasmids with the genes *HEM1, HEM2, HEM3, HEM4* and *HEM12, HEM13, HEM14, HEM15* were assembled. In both constructs, the genes were under the control of the inducible promoter pGAL1. The created constructs were mapped. The resulting plasmids will be used as donor DNA for integration into the yeast genome to increase the accumulation of heme-containing proteins.

References:

1. Блюм ЯБ, Гелетуха ГГ, Григорюк ІП, Дмитрук КВ, Дубровін ВО, Ємець АІ, Забарний ГМ, Калетнік ГМ, Мельничук МД, Мироненко ВГ, Рахметов ДБ, Сибірний АА, Циганков СП. Біологічні ресурси і технології виробництва біопалива: Монографія. 2010. 408
2. Lee HJ, Shin DJ, Nho SB, Lee KW, Kim SK. Metabolic engineering of *Saccharomyces cerevisiae* for fermentative production of heme. Biotech J. 2024; 19(10):e202400351.
3. Liu L, Martinez JL, Liu Z, et al. Balanced globin protein expression and heme biosynthesis improve production of human hemoglobin in *Saccharomyces cerevisiae*. Metabolic Engineering 2014; 21:9–16.

Increased levels of the adaptor protein Ruk/CIN85 and altered expression of S6K1 isoforms induce metabolic reprogramming in human breast adenocarcinoma MCF-7 cells

Hudkova O[1], Latyshko N[1], Kishko T[1], Horak I[1], Skaterna T[1], Filonenko V[2], Drobot L[1]

[1]Palladin Institute of Biochemistry, NAS of Ukraine

[2] Institute of Molecular Biology and Genetics, NAS of Ukraine

ogudkova@biochem.kiev.ua

It has previously been demonstrated the regulatory interdependence between adaptor protein Ruk/CIN85 and S6-kinase 1 (S6K1) isoforms in breast adenocarcinoma cells with high aggressive potential. In addition, it was established that up-regulation of the adaptor protein Ruk/CIN85 and both the content and activity level of the p60 S6K1 isoform in non-invasive human breast adenocarcinoma MCF-7 cells correlate with the development of their malignant phenotype associated with epithelial-mesenchymal transition as well as increased features of cancer stem cells (CSCs) [1, 2]. Altered glucose metabolism is a hallmark of cancer, which is characterized by a unique metabolic phenotype known as Warburg effect. In addition, cancer cell metabolism is closely related to redox-homeostasis. However, the possible relationship between Ruk/CIN85, S6K1 isoforms and the regulation of Warburg effect and metabolism of reactive oxygen species (ROS) during tumor progression still remains unknown, which was the goal of our study.

All biochemical studies were performed on stable MCF-7 cell sublines: with up-regulation of Ruk/CIN85 (G4) and its progeny with down-regulation of adaptor protein using shRNA lentivirus technology (G4vir); expressing different S6K1 isoforms (F1, $p85^-/p70^+/p60^+$, F2, $p85^-/p70^-/p60^+$, and F3, $p85^-/p70^-/p60^-$).

A significant increase in ALDH activity, a stemness marker, was found only in G4 and F2 sublines by approximately 2-fold compared to the control. Further, we assessed the activity levels/content of key enzymes/metabolites of glycolysis and oxidative phosphorylation. It was shown that LDH activity was also significantly increased (by 1.9-fold and 3-fold respectively) as well as the content of lactic acid by about 1.6 fold in the same sublines, G4 and F2, compared to the control. At the same time, the activity of the mitochondrial Krebs cycle enzyme MDH2, NAD-dependent malate dehydrogenase, was decreased 2-fold in G4 and F2 sublines compared to control cells. In these sublines, the content of intracellular H_2O_2 was elevated 4-fold. Accordingly, 1.5-2-fold increase in the activity levels of antioxidant enzymes, catalase and glutathione peroxidase, which utilize H_2O_2, was also observed.

The data obtained confirm the regulatory interdependence between the degree of MCF-7 cells malignancy, associated with changes in the expression level of Ruk/CIN85 and S6K1 isoforms, and the development of Warburg effect.

This work was supported by the grant from the National Research Foundation of Ukraine (project #2020.02/0195).

1. Garifulin OM, et al. Alterations in S6K1 isoforms expression induce Epithelial to Mesenchymal Transition and Estrogen Receptor 1 Silencing in human breast adenocarcinoma MCF-7 cells. *Biopolym Cell*. 2023. Vol. 39. N 3. P 189–200 http://dx.doi.org/10.7124/bc.000A9D

2. Drobot LB. The adaptor protein Ruk/CIN85 is a novel key regulator of epithelium-mesenchymal plasticity in cancer cells and a potential therapeutic target. *Biopolym. Cell*. 2024, 40(3):205. http://dx.doi.org/10.7124/bc.000AE2

УЛЬТРАСТРУКТУРА КЛІТИН МЕЗОФІЛУ РЯСКИ SPIRODELA POLYPHIZA ПРИ ТРИВАЛІЙ ДІЇ ВИСОКОЇ ТЕМПЕРАТУРИ

Юрій Акімов

Інститут ботаніки ім. М.Г. Холодного НАН України, Київ, Україна

Email address: yuri.akimov@gmail.com

Листок, а саме фотосинтезуюча хлоренхіма мезофілу, традиційно слугує модельним об'єктом вивчення впливу гіпертермії на вегетативний ріст. При ґрунтовому вирощуванні гіпертермія викликає розбіжність в положенні різних листків, а також може додатково супроводжуватись посухою. Для проростків арабідопсису, зокрема, відмічалася термальна індукція елонгації гіпокотилів та гіпонастична реакція листових пластинок (Aneja et al., 2025; Lippmann et al., 2019). Тому в якості об'єкта дослідженнь було обрано нейстонну ряску Spirodela polyrhiza, її мезофіл знаходиться в одній площині та в однакових умовах, а водне середовище характеризується кращою буферністю, забезпечуючи більш виражений вплив високої температури.

В проведених нами дослідженнях розміри палісадних клітин мезофілу ряски після тривалої дії високої температури (33ºС, 10 діб) були значно, майже вдвічі, менші, ніж у контролі. На рівні ультраструктури клітин суттєві зміни спостерігалися в хлоропластах та мітохондріях. Зокрема, в хлоропластах відмічені заповнені стромою вирости (стромулі). В стромі пластид в 3-4 рази зростав діаметр пластоглобул, в гіалоплазмі з'являлись великі ліпідні краплі (індикатор змін ліпідного обміну). Ультраструктура мітохондрій характеризувалась слабо вираженими кристами та просвітленим матриксом, що може свідчити про пригнічення процесів дихання. Дослідні мітохондрії часто мали неправильну форму.

Дія високої температури посилювала варіабельність у вмісті крохмалю в дослідних зразках ряски, серед яких спостерігалось як значне зменшення, так і суттєве збільшення розмірів гранул транзиторного крохмалю. Більшість літературних даних свідчить про зменшення вмісту транзиторного крохмалю при тривалій гіпертермії, що вважається ознакою набутої термотолерантності (Акімов, 2021; Seydel et al., 2022). Зазначені вище дані щодо зростання варіабельності у вмісті транзиторного крохмалю в клітинах мезофіли ряски Spirodela polyrhiza потребують подальших дослідженнь.

Література:

1. Акімов Ю.М. УЛЬТРАСТРУКТУРА КЛІТИН МЕЗОФІЛУ АРАБІДОПСИСУ (Arabidopsis thaliana L.) ПРИ ГІПЕРТЕРМІЇ Bulletin of Taras Shevchenko National University of Kyiv, 2021, Vol 85, Issue 2, p15 doi: 10.17721/1728_2748.2021.85.15-22

2. Aneja, P., R. Sanyal, and A. Ranjan. 2025. Leaf Growth In Third Dimension: A Perspective of Leaf Thickness from Genetic Regulation to Ecophysiology. New Phytologist 245, no. 3: 989–999. https://doi.org/10.1111/nph.20246.

3. Lippmann, R. et al. Development of Wild and Cultivated Plants under Global Warming Conditions Current Biology, Volume 29, Issue 24, R1326 - R1338 DOI: 10.1016/j.cub.2019.10.016

4. Seydel, C., Kitashova, A., Furtauer, L. & Nagele, T. (2022) Temperature-induced dynamics of plant carbohydrate metabolism. Physiologia Plantarum, 174(1), e13602. https://doi.org/10.1111/ppl.13602

TOWARD THE ESTABLISHMENT OF A UKRAINIAN IPSC HAPLOBANK

Volodymyr V. Zeenko, Oleksandr Yu. Petrenko
Department of Cryobiochemistry, Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv, Ukraine
Email address: v.v.zeenko@nas.gov.ua

Allogeneic cell therapies derived from induced pluripotent stem cells (iPSCs) hold immense promise for advancing regenerative medicine. However, their clinical success is heavily dependent on overcoming immune rejection, which is primarily driven by mismatches in Human Leukocyte Antigen (HLA) profiles. Developing iPSC haplobanks, comprising cell lines homozygous for common HLA haplotypes, offers an innovative solution to provide HLA-matched therapeutic cells to a substantial portion of a population. For Ukraine, building such a resource—tailored to the unique HLA genetic profile of its residents—is pivotal for enhancing national cell therapy capabilities and ensuring equitable patient access.

This initiative aims to establish the foundation for a comprehensive Ukrainian iPSC haplobank by generating and extensively characterizing iPSC lines derived from donors with prevalent HLA haplotypes in the Ukrainian population. The project will capitalize on existing Ukrainian and international infrastructures, including collaborations with HLA-typed bone marrow donor registries and cord blood (CB) banks. In partnership with immunogenetics specialists and clinicians, suitable biological materials—primarily CB units homozygous for common Ukrainian HLA haplotypes—will be identified and obtained. Advanced methodologies for generating high-quality human iPSCs (hiPSCs) from CB and other sources will be systematically explored and optimized. This will include incorporating state-of-the-art techniques, such as (m)RNA-based reprogramming, to ensure compatibility with therapeutic manufacturing standards. Additionally, protocols for the efficient cryopreservation of iPSCs and their derivative products will be refined to guarantee their long-term viability and functionality for clinical applications.

The expected outcome is the establishment of a foundational collection of HLA-homozygous iPSC lines uniquely suited to the Ukrainian population, forming the basis of a national iPSC haplobank. This critical resource will advance the clinical application of allogeneic iPSC-derived therapies in Ukraine, offering improved HLA matching for patients and fostering potential integration into the global network of iPSC haplobanks. Furthermore, the optimization of iPSC generation and cryopreservation techniques is anticipated to contribute significant advancements to the broader fields of stem cell research and regenerative medicine.

Acknowledgement: This research benefited from the support of COST Action CA21151, "Generation of Human Induced Pluripotent Stem Cells from Haplo-Selected Cord Blood Samples" (HAPLO-iPS).

Index of authors

Acknowledgement


БЛАГОДІЙНИЙ ФОНД КОЗИЦЬКОГО

L'ORÉAL УКРАЇНА

SHIMADZU Excellence in Science

For Women in Science FONDATION L'ORÉAL

FAMIDEZ.UA

BIONIX LAB

LLG Ukraine

ALSI®

РЕАЛАБ

ХЕМА

hlr рішення для ветеринарної медицини

ALT UKRAINE