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CONFERENCE OF YOUNG SCIENTISTS OF INSTITUTE OF CELL BIOLOGY

May 25, 2017

ABSTRACTS

Lviv

CONFERENCE PROGRAM

10:00-10:10	**OPENING OF THE CONFERENCE** **Director of Institute of Cell Biology of NAS of Ukraine, Professor, Member of NAS of Ukraine, Andriy A. Sibirny**	
10:10-10:20	**Lilya Lehka** IN VIVO STUDY OF ANTITUMOR ACTIVITY OF LANDOMYCIN A ON MICE WITH B16F10 MELANOMA AND NK/Ly LYMPHOMA	**8**
10:20-10:30	**Yuliia Andreieva** DEVELOPMENT OF CRISPR-CAS9 SYSTEM FOR YEAST *CANDIDA FAMATA* AND *HANSENULA POLYMORPHA*	**9**
10:30-10:40	**Marta Semkiv** CONVERSION OF CRUDE GLYCEROL FROM BIODIESEL INDUSTRY TO ETHANOL BY YEASTS *OGATAEA POLYMORPHA* AND *PICHIA PASTORIS*	**10**
10:40-10:50	**Mariia Borbuliak** INFLUENCE OF THE *TMI1* GENE DELETION ON ALCOHOLIC FERMENTATION OF GLUCOSE AND XYLOSE BY THE YEAST *SCHEFFERSOMYCES STIPITIS*	**11**
10:50-11:00	**Juliana Kozak** DECREASING THE NEGATIVE SIDE EFFECTS OF DOXORUBICIN BY SELENOMETHIONINE AND D-PANTHETINE IN B16F10 MELANOMA-BEARING MICE	**12**
11:00-11:10	**Olena Kurylenko** STUDYING THE ROLE OF CYTOSOLIC TRANSKETOLASE AND TRANSALDOLASE IN XYLOSE METABOLISM AND FERMENTATION IN THE YEAST *OGATAEA POLYMORPHA*	**13**
11:10-11:20	**Nataliya Stasyuk** METHYLAMINE-SENSITIVE AMPEROMETRIC BIOSENSOR BASED ON $(HIS)_6$-TAGGED *OGATAEA POLYMORPHA* METHYLAMINE OXIDASE IMMOBILIZED ON THE PT/RU NANOPARTICLES	**14**
11:20-11:30	**Yuliya Kurlishchuk** THE IMPACT OF CANAVANINE ON THE ABILITY OF ARGININE-DEPRIVED CANCER CELLS TO UTILIZE CITRULLINE FOR ARGININE SYNTHESIS	**15**
11:30-11:40	**Nataliya Finiuk** 4-THIAZOLIDINONE DERIVATIVE LES-3833 AS NOVEL ANTI-MELANOMA AGENT	**16**
11:40-12:10	**COFFEE BREAK**	

12:10-12:20	**Roksolana Vasylyshyn** ENGINEERING OF THE HEXOSE TRANSPORTER HXT1 FOR IMPROVED UTILIZATION OF XYLOSE DURING HIGH-TEMPERATURE XYLOSE ALCOHOLIC FERMENTATION IN YEAST *OGATAEA (HANSENULA) POLYMORPHA*	**17**
12:20-12:30	**Serhii Chornyi, Yuliya Kurlishchuk** THE IMPACT OF ARGININE DEPRIVATION ON CELL VIABILITY AND SIGNALING PATHWAYS ASSOCIATED WITH HYPOXIA AND ENERGY BALANCE	**18**
12:30-12:40	**Yana Petrovska** IDENTIFICATION OF TARGETS OF Sef1p TRANSCRIPTION FACTOR IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*	**19**
12:40-12:50	**Severyn Myronovsky** APPLICATION OF THE MAGNETIC MICROSPHERES FOR PURIFICATION OF ANTI-46 KDa MIO1C ANTIBODIES FROM BLOOD SERUM OF IMMUNIZED MICE	**20**
12:50-13:00	**Kateryna Levkiv** THE INITIAL STAGES OF THE IDENTIFICATION OF REGIONS OF *PICHIA PASTORIS* GSS1 PROTEIN INVOLVED IN GLUCOSE RECOGNITION	**21**
13:00-13:10	**Julia Senkiv** NATIVE FLUORESCENCE OF NINTEDANIB REVEALS LYSOSOMAL SEQUESTRATION AS DRUG RESISTANCE MECHANISM IN FGFR-DRIVEN LUNG CANCER	**22**
13:10-13:20	**Marianna Yurkiv** ENHANCEMENT OF GLUTATHIONE SYNTHESIS IN METHYLOTROPHIC YEAST *OGATAEA (HANSENULA) POLYMORPHA*	**23**
13:20-13:30	**Nina Bulbotka** CONSTRUCTION OF *PICHIA PASTORIS* STRAINS EXPRESSING β-GALACTOSIDASE UNDER CONTROL OF THE METHANOL REGULATED PROMOTERS TO INVESTIGATE OF THE PROTEOLYTIC DEGRADATION OF CYTOSOLIC PROTEINS	**24**
13:30-13:40	**Liubov Dzanaieva** CONSTRUCTION OF *SACCHAROMYCES CEREVISIAE* RECOMBINANT STRAINS ABLE TO XYLOSE UTILIZATION	**25**
	Oleh Chen CANAVANINE CAUSES PROFOUND ER STRESS-MEDIATED HNSCC CELL DEATH UPON ARGININE DEPRIVATION	**26**
	Discussion and closing remarks **AWARDS**	

ABSTRACTS

IN VIVO STUDY OF ANTITUMOR ACTIVITY OF LANDOMYCIN A ON MICE WITH B16F10 MELANOMA AND NK/Ly LYMPHOMA

Lilya Lehka
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Chemotherapy is one of the most effective way to treat cancer, especially in stage IV (metastasis). But it has two main disadvantages: severe side effects as well as rapid development of multidrug resistance (MDR) of tumor cells to chemotherapy treatment which significantly diminish effectiveness of treatment and worsen prognosis of cancer patients. Thus, searching for new anticancer compounds with low general toxicity and ability to overcome MDR is the main aim of modern pharmacology and medicine.

Landomycin A (LA) - a new angucycline antibiotic effectively inhibits *in vitro* the growth of tumor cells of different origin including cell lines with different mechanisms of MDR with IC_{50} value even lower than for gold chemotherapy standard doxorubicin (Dx).

Intraperitoneal injections of LA (cumulative dose 10 mg/kg, 2 injections) in *C57black/6* mice has shown that studied antibiotic significantly suppressed the growth of murine NK/Ly lymphoma. 30 % of animals were alive on the 60th day of the experiment while untreated mice died on the 21st day after tumor inoculation. We have demonstrated the normalization of hematological parameters of experimental animals under LA treatment. Thus, the level of white blood cells of mice treated with studied compound approached almost the control level. Decrease of the percentage of neutrophils with segmented nuclei (from 64±4.0 to 42. ±3.3) and monocytes (from 5.9±0.7 to 2.1±0.6) demonstrated the therapeutic effect of studied antibiotic.

When we modified the scheme of LA administration by increasing the number of injections (5 injections every 48 hour, cumulative dose remained the same, 10 mg/kg) 70 % of animals were alive on the 60th day of the experiment. Similar effect was found at Dx action in the analogous dose. But regarding the leukogram in a group treated with Dx, we detected myelosuppression and cardiotoxicity, which are negative side effects of this antibiotic.

These results suggest a perspective of landomycin A application in chemotherapy of malignant tumors. Taking into consideration obtained data, investigation of LA action on other experimental tumor models that might be especially sensitive to this drug ere desired, as well as studying the molecular mechanisms of its therapeutic activity.

DEVELOPMENT OF CRISPR-CAS9 SYSTEM FOR YEAST *CANDIDA FAMATA* AND *HANSENULA POLYMORPHA*

Yuliia Andreieva
Department of Molecular Genetics and Biotechnology

Recently developed CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has been successfully applied for modification of genomes from bacteria to human as quick method of rational genome engineering. CRISPR system contains bacterial Cas9 nuclease and a synthetic guide RNA (gRNA) that directs Cas9 to cleave regions in the genome. Homologous genome region (up to 20 bp) hybridizes to guide from the synthetic guide RNA when it is followed by the specific sequence NGG (known as protospacer-associated motif (PAM)). Variations of CRISPR-Cas9 have been developed for numerous groups of organisms; however, the system still was not applied and adapted for biotechnologically important yeast species *Candida famata* and *Hansenula polymorpha*. The aim of this work is development CRISPR/Cas system as a genome editing tool for yeast species *C. famata* and *H. polymorpha.* Gene *ADE2* was used as a target locus to assess the efficiency of the system for these yeasts. Disruption of the gene leads to accumulation of red pigment to confer easily detectible phenotype of the *ade2* mutants.

Materials and methods

Model objects: Yeasts *C. famata, H. polymorpha.*

The *Escherichia coli* strain DH5α was used in experiments that required a bacterial host. Standard cloning techniques were applied.

Results and conclusions

It was found that CRISPR-Cas9 system optimized for *Candida albicans*, revealed low efficiency in flavinogenic yeast *C. famata*. Only 5% of selected transformants showed the mutant red colonies phenotype. Obtained result could be explained by inefficient expression of genes encoding components of the system. In *H. polymorpha*, an endogenous gene promoter *GAP1*, encoding glyceraldehyde-3-phosphate dehydrogenase was cloned instead of *ENO1* gene promoter of *C. albicans* to ensure effective expression Cas9 nuclease. Increased gene expression *CAS9* led to an increase in the frequency of mutations in the gene *ADE2*. Among the selected transformants ≈70% colonies accumulated red pigment.

CONVERSION OF CRUDE GLYCEROL FROM BIODIESEL INDUSTRY TO ETHANOL BY YEASTS *OGATAEA POLYMORPHA* AND *PICHIA PASTORIS*

Marta Semkiv
Department of Molecular Genetics and Biotechnology

In recent years, the increased focus on renewable and sustainable energy has boosted the production of biodiesel from rapeseed oil, cooking oil and animal fats. Biodiesel is a viable fuel with lower sulfur content, therefore more environment-friendly. Approximately 10% of the reaction volume in a given biodiesel production process ends up as raw or crude glycerol. This low-grade glycerol also contains water, salts, and organic materials, including residual methanol and free fatty acids. Purification of the crude glycerol fraction from biodiesel production to obtain food-grade glycerol is quite expensive. More preferable is bioconversion of crude glycerol into liquid biofuels, green chemicals and bioenergy on the basis of fermentation processes, which can provide an efficient solution for sustainable management of glycerol and help improve the economics of biodiesel industries.

For example, low-grade glycerol can be converted to fuel ethanol. Methylotrophic yeast *Ogataea (Hansenula) polymorpha* is considered to be a perspective host for this process, as it can tolerate high concentrations of methanol, which often accompanies crude glycerol. Wild-type strain of *O. polymorpha* produces insignificant amounts of ethanol from glycerol (0.8 g/L). Overexpression of *PDC1* coding for pyruvate decarboxylase enhanced ethanol production till 3.1 g/L whereas simultaneous overexpression of *PDC1* and *ADH1* (coding for alcohol dehydrogenase) led to further increase in ethanol production from glycerol. Overexpression of genes *GPD1, GUT1* and *DAK1*, which encode enzymes involved in glycerol catabolism slightly enhanced glycerol consumption rate and increased ethanol production up to 7.5 g/L. Unfortunately, neither WT nor recombinant *O. polymorpha* strains were able to grow on the medium containing high concentrations of crude glycerol.

We analyzed abilities of different yeast strains to grow on the medium that contained 10 % of crude glycerol as a sole Carbon source, and revealed that only yeasts *Sporopachydermia lactativora* and *Pichia pastoris* were able to grow on this medium, and only *P. pastoris* was able to produce some ethanol (up to 3-4 g/L) in such conditions. We performed adaptation of *P. pastoris* to crude glycerol by continuous cultivation and gradual increase of crude glycerol volume in the medium. Obtained adapted strain revealed better growth on crude glycerol-containing medium and 17 % increase in ethanol production.

INFLUENCE OF THE *TMI1* GENE DELETION ON ALCOHOLIC FERMENTATION OF GLUCOSE AND XYLOSE BY THE YEAST *SCHEFFERSOMYCES STIPITIS*

Mariia Borbuliak
Department of Molecular Genetics and Biotechnology

Fuel ethanol from renewable lignocellulosic biomass has a great economic and ecological significance. However, the feasible technology for the bioethanol production from non-starch lignocellulosic materials has not been developed yet. The main reason is the absence of a robust microorganism capable of efficient alcoholic fermentation of all the main sugars of lignocellulose, most importantly, xylose. One of the microorganisms that is capable of xylose utilization is the yeast *Scheffersomyces (Pichia) stipitis*. Among the xylose-fermenting yeasts, *S. stipitis* seems to be the most promising for industrial application, because it ferments xylose with a high ethanol yield and low xylitol production.

The aim of this work is to investigate the role of the gene *TMI1* (Transport into Mitochondria) in the alcoholic fermentation of glucose and xylose by the yeast *S. stipitis*. The possibility of the gene *TMI1* influence upon the efficiency of alcoholic fermentation was revealed using the method of insertional mutagenesis combined with the positive selection of ethanol overproducers based on the usage of 3-bromopyruvate as selective agent. 3-bromopyruvate specifically inhibits key enzymes of glycolysis: hexokinase, pyruvate kinase and pyruvate decarboxylase, so the yeast cells resistant to 3-bromopyruvate should have intensified glycolysis and this may stimulate ethanol production during the alcoholic fermentation.

Among the selected 3-bromopyruvate resistant insertional mutants strain *#4.6* revealed reproducible increase of ethanol accumulation during glucose or xylose fermentation. In this strain, the insertion was found within the ORF of a gene homologous to *Saccharomyces cerevisiae* gene YDL119C, encoding mitochondrial transporter. Confirmation that the observed increased glucose/xylose fermentation performance of strain *#4.6* is a result of insertion cassette integration, rather than the secondary mutation occurring elsewhere in the genome, is an essential part of the study. Wild-type phenotype was restored via complementation of the insertional mutation by the wild allele of *TMI1* gene, however, deletion of *TMI1* on the background of *Ku80* strain did not improve ethanol production on glucose/xylose containing media.

Obtained results revealed that the gene *TMI1* is involved in the regulation of alcohol fermentation of glucose and xylose. The mechanism of the enhanced ethanol production in the insertional mutant strain will be further studied.

DECREASING THE NEGATIVE SIDE EFFECTS OF DOXORUBICIN BY SELENOMETHIONINE AND D-PANTHETINE IN B16F10 MELANOMA-BEARING MICE

Juliana Kozak
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Cutaneous melanoma is a malignant tumor of pigment-producing cells (melanocytes) located predominantly in skin epidermis. When tumor cells have spread to distant lymph nodes or metastasized (stage IV) they become refractory to common chemotherapies and, therefore, incurable. The prognosis for patients with stage IV metastatic melanoma is very poor, with an expected median survival of only 6 to 9 months.

Doxorubicin (Dx) is the most commonly used anticancer drug in clinics. Dx possesses severe side effects (cardio-, hepato- and nephrotoxicity). That is why the novel approaches should be developed to enhance anticancer action of Dx and decrease its side effects. Selenomethionine (SeMet) and D-panthetine (D-Pt) are promising candidates on the role of such agents.

B16F10 melanoma is characterized by a rapid growth, and mice usually die in 20-22 days after tumor inoculation when tumor volume reached 3000 mm^3. Under the action of Dx was less than 900 mm^3 at the 33nd day after tumor inoculation. Treatment with either SeMet or D-Pt partially inhibited B16F10 melanoma growth. It was shown that joint application of Dx and SeMet coused strong synergistic inhibitory effect on melanoma progression. Tumor growth is characterized by 2-fold decrease in number of small lymphocytes and 3-fold increase in the level of segmented neutrophils and ring-shaped nuclei neutrophils. Dx treatment led to a partial normalization of all above mentioned indices, while a combination of Dx and SeMet or (to lower impact) with D-Pt completely reversed the number of neutrophils and small lymphocytes in blood of mice to the appropriate indices found in control (healthy) group. Dx treatment led to a significant monocytosis in B16F10 melanoma bearing animals. Co-treatment of mice with SeMet or D-Pt reversed this parameter to control level.

Usage of selenomethionine and D-panthetine in combination with anticancer drugs for cancer treatment is a promising area of work in medicine that can be used for different models of tumors.

STUDYING THE ROLE OF CYTOSOLIC TRANSKETOLASE AND TRANSALDOLASE IN XYLOSE METABOLISM AND FERMENTATION IN THE YEAST *OGATAEA POLYMORPHA*

Olena Kurylenko
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The pentose-sugar xylose is the second-most abundant monosaccharide in hydrolysates released from lignocellulosic biomass. *Ogataea (Hansenula) polymorpha* is one of the most thermotolerant xylose-fermenting yeast species, however, with low efficiency of xylose alcoholic fermentation in the wild-type strains. The functional role of two key enzymes involved in the non-oxidative part of pentose phosphate pathway, namely transketolase and transaldolase, in xylose metabolism and alcoholic fermentation in *O. polymorpha* remained unclear.

The methylotrophic yeast *O. polymorpha* contains both cytosolic transaldolase (gene *TAL1*) and transketolase (gene *TKL1*) and their peroxisomal counterparts (genes *DAS1* and *TAL2*, respectively). The deficiency or overexpression of these four genes was examined regarding their roles in xylose utilization and fermentation. The *tal1*Δ, *tal2*Δ and *das1*Δ mutants were constructed by gene disruption technique. The conditionally knockout *tkl1*Δ mutant was constructed by replacing the endogenous promoter of *TKL1* gene by regulated *YNR1* promoter of nitrate reductase, repressed by ammonium sulfate as nitrogen source. A significant decrease in xylose-fermenting ability and totally blocked growth on xylose was observed in *tkl1*Δ and *tal1*Δ mutants. Overexpression of *DAS1* gene in *tkl1*Δ mutant led to restoration of growth on xylose, however, only after prolonged lag phase. The ability of xylose utilization in *tal1*Δ strain was easily restored by overexpression of *TAL2* gene. Moreover, overexpression of each of the mentioned genes resulted in improvement of ethanol production from xylose.

METHYLAMINE-SENSITIVE AMPEROMETRIC BIOSENSOR BASED ON $(HIS)_6$-TAGGED *OGATAEA POLYMORPHA* METHYLAMINE OXIDASE IMMOBILIZED ON THE PT/RU NANOPARTICLES

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Recent advances in nanotechnology have enabled the exploration of metallic nanocomposites for diverse applications, especially, for construction of biosensors. Core-shell metal nanoparticles (NPs) possess unique optical, electronic, magnetic and catalytic properties to be used in different technologies.

The aim of the current research is to construct an effective mono-enzyme bioelectrode based on using methylamine oxidase (MAO) and the best variants of nanocomposites, sensitive to hydrogen peroxide - the final product of enzymatic digestion of methyl amine by MAO.

In this research, a number of metallic NPs were synthesized on the surface of carbon electrode by electrochemical polymerization and characterized by atomic force microscopy. The most electroactive bi-metallic chemosensor on H_2O_2 coupled with recombinant MAO was chosen for construction of amperometric biosensor for analysis of methylamine (MA) in real samples of sea fish extracts.

The $(His)_6$-tagged MAO of the yeast *Ogataea polymorpha* was purified from the cell-free extract of the recombinant strain *Saccharomyces cerevisiae* by metal-affinity chromatography on Ni-NTA agarose. The bioselective layer of biosensor includes MAO immobilized in alginate membrane on the surface of Pt/Ru-modified carbon electrode. The developed biosensor exhibited a high sensitivity (700 ± 12 $A^{-1} \cdot M^{-1} \cdot m^{-2}$), a broad linear range (from 20 μM to 500 μM), a high selectivity toward MA, and reveals satisfactory storage stability (more than 14 days). The constructed amperometric biosensor was used for MA assay in real samples of fish products in comparison with chemical method. The values obtained with both approaches demonstrated a high correlation. The H_2O_2-chemosensing system, based on Pt/Ru NPs, seems to be promising for quantitative analysis of different analytes, substrates of oxidases.

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THE IMPACT OF CANAVANINE ON THE ABILITY OF ARGININE-DEPRIVED CANCER CELLS TO UTILIZE CITRULLINE FOR ARGININE SYNTHESIS

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Department of Cell Signaling

The current challenge of arginine deprivation as an anticancer therapy is the ability of some initially sensitive to arginine restriction cancer cells to become resistant to such a treatment. This gained resistance is linked with a recovered expression of argininosuccinate synthetase (ASS1), which together with argininosuccinate lyase (ASL) is required for arginine synthesis from its precursor citrulline. Thus, an approach to selectively inhibit induction of ASS1 expression in cancer cells can significantly improve the outcome of arginine deprivation-based treatment.

We observed that a non-toxic in complete medium concentration of arginine analog, canavanine (0.1 mM) restrained proliferation of monolayer human colorectal cancer cells (HCT-116 and HT29) in arginine-deprived medium supplemented with an excess of citrulline. It was hypothesized that canavanine impacts cancer cell ability for arginine synthesize from citrulline. To verify this hypothesis, the expression of enzymes involved in arginine synthesis, ASS1 and ASL, was analyzed on the mRNA and protein levels using RT-PCR and Western blotting, respectively. The ASS1 mRNA level was increased after exposure to arginine-deprived conditions in monolayer culture of HCT-116 and HT29 cells. Canavanine reduced the amount of ASS mRNA in the arginine-deprived cancer cells to the level detected before an exposure to arginine restriction. At the same time, canavanine had no significant impact on the ASL mRNA level when compared with arginine deprivation alone. Western blot analysis confirmed that reduction in ASS1 transcription under exposure of HCT-116 and HT29 cells to canavanine in arginine-deprived conditions was followed by the drop in ASS1 protein level. In contrast with monolayer culture, canavanine had a lower potential to inhibit the induction of ASS1 expression in three-dimensional multicellular spheroid culture of these cells. It can be explained by a higher level of ASS1 expression of HCT-116 and HT29 cells when maintained as spheroids. Next, we demonstrated that the ability of canavanine to inhibit the ASS1 induction was not transferred to another arginine analog, homoarginine, and investigated how canavanine affected regulation of translation in the arginine-deprived cancer cells. To conclude, canavanine can selectively inhibit the induction of ASS1 expression in cancer cells and its application together with arginine deprivation may prevent them from becoming resistant to arginine restriction.

4-THIAZOLIDINONE DERIVATIVE LES-3833 AS NOVEL ANTI-MELANOMA AGENT

Nataliya Finiuk

Department of Regulation of Cell Proliferation and Apoptosis

Introduction. Because of ineffectiveness of many cancer treatments (ex. melanomas), numerous negative side effects, difficulties in early cancer detection, new strategies have been developed to improve cancer therapy. Thus, modern approaches are aimed on the creation of novel medicines and drug delivery systems. Novel 4-thiazolidinone derivatives were reported to possess promising chemotherapeutic properties including antitumor activity.

Aim. Evaluation of cytotoxic action of 4-thiazolidinone derivative Les-3833 and study of the mechanisms of its pro-apoptotic action towards human melanoma cells.

Methods. Cytotoxic activity of Les-3833 was measured by MTT and trypan blue exclusion assays, fluorescent microscopy. Western-blot analysis and FACS were used to identify a cell death pathways induced by Les-3833.

Results. Novel 4-thiazolidinone derivative Les-3833 was synthesized at Danylo Halytsky Lviv National Medical University.

The Les-3833 shown to possess poor activity towards human myeloid leukemia K562 cells, human ovarian carcinoma *SKOV3 cells (IC_{50} > 5* μg/mL), and it was some more toxic for human lung adenocarcinoma A549 cells (IC_{50} = 2.5 ± 0.19 μg/mL), human colon carcinoma HCT116 cells (IC_{50} = 3.4 ± 0.32 μg/mL) and human breast adenocarcinoma MCF-7 cells (IC_{50} = 4.5 ± 0.36μg/mL). The Les-3833 was found the most active towards melanoma cells with IC_{50}=0.22 ± 0.03 μg/mL for WM793 cells and IC_{50} = 0.3 ± 0.04 μg/mL for *SK-Mel-28 cells. At the same time,* Les-3833 was much less toxic than the doxorubicin towards non-tumor cells (human embryonic kidney HEK293 cells, human keratinocytes of HaCat line and transformed mouse L929 fibroblasts). Les-3833 induced pro-apoptotic nucleus fragmentation, chromatin condensation and lysosome activation in treated melanoma cells. Apoptosis in the melanoma cells was confirmed by Annexin V-FITC/PI staining, as well as activation of caspase 3, MAPK pathway (p ERK1/2, pJNK) and increasing of cleaved PARP, EndoG proteins. In addition, Les-3833 induced ROS production in melanoma cells and their arrest in G_0/G_1 phase of cell cycle.

Conclusions. 4-thiazolidinone derivative Les-3833 is a potent anticancer drug for melanoma treatment. The mitochondria-depended apoptosis and ROS production are the mechanisms responsible for Les-3833 antineoplastic activity.

Acknowledgement. The study was supported by the West-Ukrainian BioMedical Research Center (WUBMRC) grant.

ENGINEERING OF THE HEXOSE TRANSPORTER HXT1 FOR IMPROVED UTILIZATION OF XYLOSE DURING HIGH-TEMPERATURE XYLOSE ALCOHOLIC FERMENTATION IN YEAST *OGATAEA (HANSENULA) POLYMORPHA*

Roksolana Vasylyshyn
Department of Molecular Genetics and Biotechnology

For the last three decades biofuels produced from renewable feedstocks have received much publicity because of their potential to replace conventional fossil fuels. A major issue in the conversion of saccharified cellulosic biomass into biofuel is the utilization of xylose, since lignocellulosic feedstocks contain a significant amount of this pentose sugar.

The ability of the thermotolerant methylotrophic yeast *Ogataea polymorpha* to ferment xylose has made this yeast species a promising organism for high-temperature alcoholic fermentation. Although *O. polymorpha* recombinant strains metabolize xylose more efficiently, uptake, and therefore consumption of xylose, is strongly inhibited by glucose, due to glucose catabolite repression. The low-affinity transport system is shared between glucose and xylose for sugar transport in *O. polymorpha*.

Recently, the first functional hexose transporter Hxt1 was identified in *H. polymorpha*. To increase the specific xylose uptake rate the modified Hxt1 was engineered by substitution of asparagine to alanine at position 358. Furthermore, N-terminal lysine residues of Hxt1 predicted to be the target of ubiquitination were replaced for arginine residues. The modified versions of Hxt1 were overexpressed in *hxt1Δ* mutant and the efficiency of xylose and glucose co-utilization during high-temperature xylose fermentation was studied.

THE IMPACT OF ARGININE DEPRIVATION ON CELL VIABILITY AND SIGNALING PATHWAYS ASSOCIATED WITH HYPOXIA AND ENERGY BALANCE

Serhii Chornyi, Yuliya Kurlishchuk
Department of Cell Signaling

Deregulation of cell metabolism is a key event in the development and progression of cancer. So, one of the hallmarks of some cancers is their auxotrophic for certain amino acids, for instance, arginine. This inferiority can be used to design selective anticancer enzyme therapy. However, the molecular mechanisms of tumor cell response to arginine deficiency not clarified and there is a lot of subsequent possibilities of improvement. The good approach is to target main signaling pathways that can be associated with sensitivity to the arginine deprivation. Thus, the aim of this study was to investigate the changes in cell signaling pathways caused by artificial hypoxic condition or deregulation of cellular energy homeostasis in human cancer cells.

For this purpose, cancer cell lines HCT-116, HT29 and SKOV-3 were cultured in the arginine-supplied (complete) or in the arginine-deplete medium. Artificial hypoxia was induced by cobalt (II) chloride exposure. Metformin was applied to impact cellular energy homeostasis. MTT assay was used for measuring cell viability rates. Gene expression was determined by RT (reverse transcription)-PCR and Western blotting.

To determine the impact of cobalt chloride as a hypoxia mimic factor on cancer cells in the arginine-deplete medium we monitored activation of main signaling pathways in cells (mTOR, MAPK, apoptosis and UPR). It was shown that cobalt chloride inhibits p38-MAPK and mTOR signaling pathways under arginine starvation but didn't lead to increase in the amount of cPARP in used conditions. Also, we found that cobalt chloride has no significant effect on cell viability under arginine deficiency.

We monitored changes in the cellular energy homeostasis caused by metformin under arginine deprivation. Metformin downregulated the energy status as confirmed with activation of AMPK and subsequent inactivation of mTOR. Any decrease in ERK 1/2 protein level was detected. Activation of p38 MAP kinase is cell line specific. However, treatment of metformin under arginine starvation had no impact on cells' proliferation and viability.

Altogether, additional activation of hypoxia or metformin application didn't lead to increase in cytotoxicity towards studied cells under arginine limitation. Proposal approach requires further investigation.

IDENTIFICATION OF TARGETS OF Sef1p TRANSCRIPTION FACTOR IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*

Yana Petrovska
Department of Molecular Genetics and Biotechnology

Riboflavin (RF) or vitamin B2 - is an important compound for all living organisms. Riboflavin serves as a precursor of flavin coenzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) involved in numerous biochemical processes. Riboflavin biosynthesis pathway is well studied in yeast *C. famata*, however, its regulation is poorly understood. Only one regulatory *SEF1* gene have been identified so far.

The aim of this work was to study the mechanism of interaction of protein Sef1p and structural genes of riboflavin biosynthesis.

The one-hybrid system (Y1H) based on yeast *Saccharomyces cerevisiae* was used through this work. Strain BY4742 was co-transformed with plasmids, one of which contains *SEF1* gene under the control of the promoter of the gene *GAL1*, induced by galactose, and the other contains a gene *LAC4 Kluyveromyces lactis*, encoding β-galactosidase under the control of promoters of genes *RIB1*, *RIB2*, *RIB3*, *RIB5*, *RIB6*, *RIB7* and *SEF1*. In the case of protein Sef1p binding with the corresponding promoter, increase in β-galactosidase activity was expected. To find the binding site of protein Sef1p, nucleotide sequence of *RIB1* promoter has been modified by removing a potential binding site or by its modification.

As a result, it was revealed that protein Sef1p directly interacts with promoters of most *RIB* genes and its own promoter, providing evidence of autoregulation.

APPLICATION OF THE MAGNETIC MICROSPHERES FOR PURIFICATION OF ANTI-46 KDa MIO1C ANTIBODIES FROM BLOOD SERUM OF IMMUNIZED MICE

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Blood serum has been extensively explored as a source of bio-markers, as it may contain not only blood proteins *per se*, but also proteins originating from all tissues of the body. It was estimated that up to 10,000 different proteins (and/or their fragments) might be present in blood serum, and most of them are there in very low concentrations. A selection of protein preparation, and especially enrichment procedures, might aid in a successful search for the bio-markers. We have searched for protein markers present in blood serum of the multiple sclerosis (MS), rheumatoidarthritis (RA), and systemic lupus erythematosus (SLE) patients in comparison with healthy human individuals. To do that, we used precipitation/extraction methods and MALDI TOF/TOF mass-spectrometry, and identified a protein with Mr ~46 kDa as an N-terminal fragment of human unconventional myosin IC isoform b (Myo1C). Western-blot analysis with specific anti-human Myo1C antibodies confirmed the identity of this protein. Screening of blood serum samples from different autoimmune patients for the presence of Myo1c revealed its high level in MS. Statistic analysis confirmed that the level of p46 Myo1C in blood serum is a potential marker for diagnostic of MS patients on an early stage. Well known, that specific antibodies produced against bio-markers are invaluable diagnostic tool. To this aim, mice immunization with using a blood serum p46 Myo1C isoform was performed. To effective isolation of mono specific anti- p46 Myo1C antibodies with blood serum of mice, we used magnetic microspheres, obtained in lab of Dr. Daniel Horac (Institute of Macromolecular Chemistry, Czech Republic). The microspheres were coupled with p46/Myo1C antigen and incubated from blood serum of immunized mice. It was found, that this approach allows to effectively, in one step, purified mono specific anti-p46/Myo1C immunoglobulin G (IgG) antibodies from crude antibody preparations of mouse blood serum. High efficiency of this approach was confirmed by SDS/PAGE, Western blot, and dot-blot analyses.

Conclusion: the newly develop of magnetic microspheres, conjugated with a potential disease biomarker p46/Myo1C protein, are promising tool for affinity purification of antibodies, which can improve diagnosis and treatment of MS patients.

THE INITIAL STAGES OF THE IDENTIFICATION OF REGIONS OF *PICHIA PASTORIS* GSS1 PROTEIN INVOLVED IN GLUCOSE RECOGNITION

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Investigation of proteins involved in glucose sensing and/or signalling in the methylotrophic yeast *P. pastoris* is necessary for further understanding of the molecular mechanisms of glucose-dependent pexophagy induction. Nowadays these processes are not fully investigated despite the identification of more than 40 *ATG* genes.

Previously was shown that *P. pastoris Gss1* (homolog of both *Sc*Snf3 and *Sc*Rgt2) involved in normal growth on high and low glucose concentrations, micropexophagy (occurred in the case of shift of methanol grown cells into glucose-containing medium) and glucose catabolite repression. We also studied the role of the of cytoplasmic C-tail of this protein and it was shown that complete removal of its cytoplasmic tail leads to the severe growth defect in medium with mannose, fructose and glucose (both high and low glucose concentrations) as sole carbon source, cased. significant retardation of alcohol oxidase inactivation during adaptation for substrates more favorable than methanol (glucose, fructose or mannose) and has damaged catabolite repression, in contrast to the partial C-tail removal (ΔC_{50}, ΔC_{100}). We also suppose that cytoplasmic tail of the Gss1 protein is necessary not only for glucose signaling but also for hexose uptake in *P. pastoris*.

To reveal which regions of *PpGss1* involved in glucose recognition we decided to hold error prone mutagenesis of the ORF *PpGSS1.* For this purpose we used PCR-mixture with additional Mn^{2+}, increased concentrations of Mg^{2+}, dCTP, dTTP and with decreased dGTP, dATP. Vectors with mutant forms of *PpGSS*1 were constructed, linearized and used for electrotransformation of either ***Δgss1*** or GS200. Then we checked phenotype of the transfomants which were able to growth in mineral media without histidine. We search for 2-DOG-positive strains with damaged micropexophagy and normal growth on glucose. Unfortunately, we didn't reveal mutants with desirable characteristics among approximately 400 His^+-mutants, despite the majority of them possessed some *PpGSS1*-mutant form (verified by PCR). Our attempts to obtain transformants directly on mineral media with methanol and 2-DOG also were unsuccessful, so we decided to change selective marker of the vector. For this purpose, we used zeocin containing plasmid. We are going to use obtained vectors for electrotransformation of GS200 strain and further identification of regions involved in glucose recognition.

NATIVE FLUORESCENCE OF NINTEDANIB REVEALS LYSOSOMAL SEQUESTRATION AS DRUG RESISTANCE MECHANISM IN FGFR-DRIVEN LUNG CANCER

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Studying the intracellular distribution of pharmacological agents, including anticancer compounds, is of central importance in biomedical research. It constitutes a prerequisite for a better understanding of the molecular mechanisms underlying drug action and resistance development. The molecular pharmacology and tissue distribution of many anticancer agents is well established. However, dynamics of subcellular pharmacokinetics often remain obscure even concerning approved anticancer therapeutics. Hyperactivated fibroblast growth factor receptors (FGFRs) constitute a promising therapy target in several types of malignancies including lung cancer. The clinically approved small-molecule FGFR inhibitor nintedanib exerts strong cytotoxicity in FGFR-driven lung cancer cells.

We report for the first time drug-intrinsic fluorescence properties of nintedanib in living and fixed lung cancer cells as well as in cryosections derived from allograft tumors of orally treated mice. Using this feature in conjunction with flow cytometry and confocal microscopy allowed to determine cellular accumulation levels, impact of drug efflux pumps and to uncover nintedanib trapping into lysosomes. Lysosomal sequestration - resulting in an organelle-specific and pH-dependent nintedanib fluorescence- was identified as an intrinsic resistance mechanism in FGFR-driven lung cancer cells. Accordingly, combination of this multi-kinase inhibitor with agents compromising lysosomal acidification (bafilomycin A1, chloroquine) exerted distinctly synergistic effects. In summary we provide a powerful tool to dissect molecular factor impacting on organismal and intracellular pharmacokinetics of nintedanib.

Regarding the clinics, prevention of lysosomal trapping via lysosome-alkalinization might represent a promising strategy to increase drug-target interactions and, thus, to circumvent cancer cell-intrinsic nintedanib resistance.

ENHANCEMENT OF GLUTATHIONE SYNTHESIS IN METHYLOTROPHIC YEAST *OGATAEA (HANSENULA) POLYMORPHA*

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Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is abundant conserved tripeptide present in most living organisms, predominantly, in eukaryotic cells. GSH is an important redox buffer, detoxifier, co-factor of several enzymes and a key source of nitrogen and sulfur under conditions of their depletion in the environment. Due to the antioxidative properties of GSH there is an increasing interest for application of this tripeptide in several industrial areas, including cosmetics, pharmaceutical products, and foods. As an active ingredient of food, drugs and cosmetic products, GSH could alleviate harmful oxidative processes, scavenge toxic compounds at different kinds of human intoxications and strengthen whitening, skin repair antiaging effect. Although GSH can be produced by chemical or enzymatic synthesis, microbiological production using natural or engineered microorganisms (yeasts *Saccharomyces cerevisiae* and *Candida utilis*, bacteria *Escherichia coli* and *Lactococcus lactis*, etc.) is currently the most common method for the commercial production of GSH. At present, GSH is produced mainly by fermentation using yeast. Thermotolerant methylotrophic yeast *O. polymorpha* with naturally high content of GSH is considered as a promising organism for design of competitive GSH producer.

The plasmid for overexpression of *GSH2* gene under control of strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase simultaneously with *MET4* gene was constructed. This plasmid was introduced into genome of *O. polymorpha* wild-type strain by electroporation method. Selection of transformants was performed on solid YPD medium supplemented with nouseothricin. The presence of *GSH2* and *MET4* genes in obtained transformants was confirmed by PCR. Selected transformants were analyzed for their GSH accumulation. Recombinant strains overexpressing both *GSH2* and *MET4* genes under control of strong constitutive promoter of GAP were characterized by significant increase in intracellular GSH concentration compared with the wild-type strain.

CONSTRUCTION OF *PICHIA PASTORIS* STRAINS EXPRESSING β-GALACTOSIDASE UNDER CONTROL OF THE METHANOL REGULATED PROMOTERS TO INVESTIGATE OF THE PROTEOLYTIC DEGRADATION OF CYTOSOLIC PROTEINS

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The methylotrophic yeast has become an important host organism for recombinant protein production. The success of methylotrophic yeasts in the production of recombinant proteins is highly linked to the presence of very strong and tightly regulated promoters of some genes of the methanol utilization pathway such as the alcohol oxidase, formaldehyde dehydrogenase etc. Proteolytic degradation has been a perpetual problem when yeasts are employed to express recombinant proteins. Since the β-galactosidase assay is simple and well visualized method, we decided to use it for investigation the mechanisms of inactivation and proteolytic degradation of cytosolic proteins. For this purpose, we constructed the vectors containing β-galactosidase under control of regulated promotors of the formaldehyde dehydrogenase (P_{FlDH}), formate dehydrogenase (P_{FDH}) and fructose-1,6-bisphosphatase (P_{FBP}) genes. We also obtained strain of *P. pastoris* which synthesizes β-galactosidase under control of strong regulated promotor of the formaldehyde dehydrogenase gene. β-galactosidase (β-D-galactohydrolase, EC 3.2.1.23) is an enzyme that hydrolyzes D-galactosyl residues from polymers, oligosaccharides or secondary metabolites.

The β-galactosidase gene *LAC4* from *Kluyveromyces lactis* strain CBS 2359 was amplified using the High-Fidelity DNA polymerase and the primers N3 and N4. This fragment was cloned into the multiple cloning site of the plasmid pIB1. The promotors of *FlDH, FDH* and *FBP* were obtained by the PCR-amplification of following sequences from the *P. pastoris* genomic DNA. Promotor sequences were cloned into the plasmid pIB1-*LAC4* upstream to the *LAC4* ORF resulting in the constructs pIB1-P_{FlDH}-*LAC4*, pIB1- P_{FlDH} -*LAC4* and pIB1- P_{FBP}-*LAC4* respectively. The wild type strain of *P. pastoris* GS200 *his4 arg4* was transformed with plasmid DNA pIB1-P_{FlDH}-*LAC4*. Colonies of the *P. pastoris* transformants were selected on the minimal medium without histidine and analysed by PCR. β-galactosidase activity assay was held in verified mutants upon short- or long-term duration of glucose starvation. The cells pregrown on the medium with methanol were shifted to the glucose containing medium either with the nitrogen source or without it. The changes of the β-galactosidase activity were compared in the obtained *P. pastoris* pIB1-P_{FlDH}-*LAC4* transformant and in the *Saccharomyces cerevisiae* strain expressing the β-galactosidase under control of the constitutive promotor of the alcohol dehydrogenase (*ADH*) gene.

CONSTRUCTION OF *SACCHAROMYCES CEREVISIAE* RECOMBINANT STRAINS ABLE TO XYLOSE UTILIZATION

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Plant biomass is a renewable energy source and therefore has great potential as a feedstock to produce fuel ethanol. Yeast *Saccharomyces cerevisiae* is unable to catabolize and ferment xylose, the second main sugar of lignocellulosic hydrolysates, due to lack of enzymes catalyzing initial stages of the pentose catabolism.

Therefore, for efficient lignocellulose conversion to ethanol the development of *S. cerevisiae* strains capable of both glucose and xylose fermentation is of great interest. *Spathaspora passalidarum* belongs to the natural xylose-fermenting yeast species with higher ethanol production rate on xylose that on glucose under low-oxygen conditions. *S. passalidarum XYL1, XYL2* and *XYL3* genes, encoding xylose reductase, xylitol dehydrogenase and xylulokinase, respectively, were cloned under control of *S. cerevisiae ADH1* gene promoter and introduced into the genome of *S. cerevisiae* industrial strains, namely AS400; Y563; PE2. Selected transformants were unable to grow on xylose, despite the increased specific activity of xylose reductase. Moreover, the increased specific activity of xylitol dehydrogenase was confirmed for transformants derived from Y563 and PE2 strains. The adaptation of constructed recombinant strains to xylose as carbon source is under the progress.

CANAVANINE CAUSES PROFOUND ER STRESS-MEDIATED HNSCC CELL DEATH UPON ARGININE DEPRIVATION

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Head and neck squamous cell carcinoma (HNSCC) is one of the most aggressive malignant tumors, which is often detected in advanced stage of disease and for which therapy outcome remains poor. Systemic depletion of free circulating arginine with arginine-degrading enzymes has been proposed as metabolic therapy for a number of malignant tumors, recently also for HNSCC. It has also been shown that the natural arginine analogue canavanine (Cav) strongly enhances the cytotoxic effect of arginine starvation in a broad spectrum of cancer cells.

In this study, we examined the molecular mechanisms of Cav-induced HNSCC response upon arginine deprivation by focusing on endoplasmic reticulum (ER) stress pathways. We showed, for the first time, that Cav triggers acute ER stress response in HNSCC upon arginine deprivation, which is associated with HNSCC cell apoptosis. Gene expression analysis revealed that the levels of ER stress marker genes were drammatically up-regulated under arginine deprivation and Cav co-treatment. We provide a clear evidence that ER stress is involved in up-regulation of *PPP1R15A, ATF3* and *CHOP* genes, which are critical for ER stress-induced apoptosis in human HNSCC cells. Knockdowns of *PPP1R15A, ATF3* and *CHOP* by siRNA in human HNSCC cells blocked Cav-induced apoptosis upon arginine deprivation. Moreover, the inhibitor of ER stress-induced apoptosis salubrinal strongly protected HNSCC cells against Cav-mediated cell death upon arginine withdrawal. In addition, pre-treatment with salubrinal increased the viability of HNSCC upon arginine deprivation and Cav co-treatment. However, activated IRE1α pathway did not cause Cav-mediated apoptosis induction upon arginine deprivation. Apparently, triggered IRE1α pathway rather mediates transcription of chaperone-encoding genes, whose products have a role in ER protein folding machinery to eliminate abnormal proteins from the ER lumen. Taken together, our findings suggest that Cav causes massive ER stress-mediated HNSCC cell death upon arginine deprivation and ER stress response pathways could be a novel target to develop more efficient metabolic anti-cancer approaches.

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