SCIENTIFIC PROGRAM & ABSTRACTS

8:30 – 9:10 Registration

9:10 – 9:15 Opening | *Jan Trka, Júlia Starková*

9:15 – 12:15 SESSION I

Chairman: Tomáš Mráček

9:15 – 10:00 abstract

Transcriptional deregulation of metabolism as a hacker of metastasis: decoding the communication networks between tumours and microenvironment

Verónica Torrano, CIC bioGUNE, Derio, Spain

10:00 – 10:30 abstract

Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells

Jakub Rohlena, Institute of Biotechnology, Czech Academy of Sciences, Prague

Short talk:

10:30 – 10:45 abstract

Functional electron transport chain is necessary for stress resistance in quiescent cells

Silvia M. Novais, Institute of Biotechnology, Czech Academy of Sciences, Prague

10:45 – 11:15 Coffee break

11:15 – 11:45 abstract

Lipids in colon cancer development

Jiřina Hofmanová, Institute of Biophysics, Czech Academy of Sciences, Brno

11:45 – 12:15 abstract

Mitochondrial glycerophosphate dehydrogenase as a target for inhibition of cell proliferation in tumors

Alena Pecinová, Institute of Physiology, Czech Academy of Sciences, Prague

12:15 – 13:30 Lunch

13:30 – 14:45 SESSION II

Chairman: Jan Trnka

13:30 – 14:00 abstract

Altered cellular metabolism in malignant transformation and therapy response in acute leukemias

Júlia Starková, Second Faculty of Medicine, Charles University, Prague

Short talks:

14:00 – 14:15 abstract
The metabolic sensor AMPK is a new regulator of oncogene-driven ERK signaling in cancer cells
Stjepan Uldrijan, Faculty of Medicine, Masaryk University, Brno

14:15 – 14:30 abstract
Cancer-associated fibroblasts in malignant melanoma
Karel Smetana, First Faculty of Medicine, Charles University, Prague

14:30 – 14:45 abstract
Mitochondrial 2HG production as a function of IDH2 and HOT in breast cancer cells
Katarína Smolková, Institute of Physiology, CAS, Prague

15:00 – 16:00 MODERATED POSTER SESSION

Chairman: Verónica Torrano

P1. Lactate as a signaling molecule in the tumor microenvironment abstract
Martina Koncošová, University of Chemistry and Technology, Prague

P2. Glutamine as a sensitizing factor for the therapy of solid tumors abstract
Nikola Vrzáčková, University of Chemistry and Technology, Prague

P3. Cancer senescent cells differ from their proliferating counterparts in their response to cell death-inducing agents abstract
Peter Holíček, Institute of Biotechnology, Czech Academy of Sciences, Prague

P4. Role of metabolism in chemoresistance of neuroblastoma cells abstract
Tomáš Eckschlager, Second Faculty of Medicine, Charles University, Prague

P5. Common themes in cancer and virus-infected cells abstract
Zora Mělková, First Faculty of Medicine, Charles University, Prague

P6. Pushing the ERK pathway activity out of the fitness zone with metabolic stressors: new targeted therapy for melanoma? abstract
Barbora Valčíková, Faculty of Medicine, Masaryk University, Brno

P7. Identification the key players of metabolic reprogramming of leukemic cells upon l-asparaginase treatment abstract
Natividad Alquezar Artieda, Second Faculty of Medicine, Charles University, Prague

P8. Exploring metformin action on the regulation of cancer cell proliferation abstract
Andrea Brázdová, Institute of Physiology, Czech Academy of Sciences, Prague

18:00 – 23:00 Social event

ABSTRACTS

TRANSCRIPTIONAL DEREGULATION OF METABOLISM AS A HACKER OF METASTASIS: DECODING THE COMMUNICATION NETWORKS BETWEEN TUMOURS AND MICROENVIRONMENT

Verónica Torrano

CIC bioGUNE, Derio, Spain

The advances towards curative treatments for cancer are nowadays based on three pillars of research: (i) early detection, (ii) stratification of high-risk of recurrence patients, based on the molecular characterization of each individual cancer and ii) the selection of the most appropriate therapeutic strategy based on these molecular characteristics. The advances in the molecular understanding of cancer has led to a paradigmatic change in the way we combat the disease, introducing the concept of **precision medicine: patient's stratification and personalized therapy.** We are interested in the cross-interaction between transcriptional regulation of metabolism and prostate cancer, which has led us to identify the tumour and metastasis suppressive potential of the transcriptional co-activator PGC1α (Torrano et al., Nature Cell Bio 2016; Valcarcel et al., Cell Cycle 2016; Valcarcel et al TEM 2017). The study of this transcriptional co-activator and its biological activity can potentially lead to the development of a therapeutic strategy based on precision medicine. But, three critical questions need to be address in order to develop a precision medicine approach: **WHAT** are the key regulators of the metastatic process induced by the reduction of mitochondrial metabolism? **HOW** can we effectively treat prostate cancers with high risk of recurrence? **WHO** among PCa patients is better fitted to benefit from this therapeutic approach?
To answer these questions we aim first to define the mechanisms underlying the anti-metastatic activity of mitochondrial metabolism in PCa. Based on a multidisciplinary approach, from bioinformatics to cell biology, we are studying the contribution of mitochondrial metabolism to the acquisition of invasive properties and the preparation of the metastatic niche. I propose that the anti-metastatic activity of PGC1α-mediated mitochondrial metabolism goes beyond the activation of catabolic processes presenting the transcriptional co-regulator as a key metabolic player that modulates cell signalling pathways and transcriptional programs involved in cell motility and invasion.

REACTIVATION OF DIHYDROOROTATE DEHYDROGENASE BY RESPIRATION RESTORES TUMOR GROWTH OF MITOCHONDRIAL DNA-DEPLETED CANCER CELLS

Jakub Rohlena,[1] Stepana Boukalova,[1] Jaromira Kovarova,[1] Martina Bajzikova,[1,2] Ana Coelho,[1,3] Sean Oh,[5] Katerina Rohlenova,[1] Sona Hubackova,[1] Gus Maghzal,[6] Kristyna Judasova,[1] Paulo J. Oliveira,[3] Lanfeng Dong,[4] Roland Stocker,[6] Sunghyouk Park,[5] Jiri Neuzil[1,4]

[1]Institute of Biotechnology, Czech Academy of Sciences, 252 50 Vestec, Prague-West, Czech Republic
[2]Faculty of Science, Charles University, 128 44 Prague, Czech Republic;
[3]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech, 3060-197 Cantanhede, Portugal
[4]School of Medical Science, Griffith University, Southport, 4222 Qld, Australia
[5]Natural Product Research Institute, Seoul National University, Korea
[6]Victor Chang Cardiac Research Institute, Darlinghurst, Australia

Cancer cells without mitochondrial DNA (mtDNA) need to reconstitute oxidative phosphorylation (OXPHOS) by acquisition of host mitochondria to form tumors (1,2), but the reasons why functional respiration is crucial for tumorigenesis remain unclear. Using time-resolved analysis of the initial stages of tumor formation by mtDNA-devoid cells and genetic manipulations of OXPHOS components, we now show that pyrimidine biosynthesis, supported by the respiration-linked dihydroorotate dehydrogenase (DHODH), is strictly required to overcome cell cycle arrest, while mitochondrial ATP generation is dispensable for tumorigenesis. Primed DHODH is present in mtDNA-devoid cells and becomes fully active by complex III/IV respiration after mitochondrial transfer, or by the introduction of an alternative oxidase. Conversely, DHODH deletion interferes with tumor formation even in cells with functional OXPHOS, whereas disruption of mitochondrial ATP synthase has little or no effect. Collectively, our results show that pyrimidine biosynthesis via DHODH is an essential pathway that links respiration to tumorigenesis.

1. Tan, A. S., Baty, J. W., Dong, L. F., Bezawork-Geleta, A., Endaya, B., Goodwin, J., Bajzikova, M., Kovarova, J., Peterka, M., Yan, B.*, et al.* (2015). Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab *21*, 81-94.

2. Dong, L. F., Kovarova, J., Bajzikova, M., Bezawork-Geleta, A., Svec, D., Endaya, B., Sachaphibulkij, K., Coelho, A. R., Sebkova, N., Ruzickova, A.*, et al.* (2017). Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. Elife *6*.

FUNCTIONAL ELECTRON TRANSPORT CHAIN IS NECESSARY FOR STRESS RESISTANCE IN QUIESCENT CELLS

Silvia Magalhaes Novais[1,2], Jan Blecha[1,2], Katerina Rohlenova[1], Jiri Neuzil[1,3], Jakub Rohlena[1]

[1]Institute of Biotechnology, Czech Academy of Science, BIOCEV, Vestec, Prague-West, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]School of Medical Science, Griffith University, Southport, Qld, Australia

Mitochondria are organelles central to energy metabolism and cell death. In the present work we investigated the role of functional electron transfer chain (ETC) in cell's adaptation to the quiescent state, using ETC-deficient (ρ^0 cells) endothelial cell line EA.hy926 as a model. Preliminary results showed an increase in glucose consumption and lactate production in ETC-deficient quiescent cells compared to their proliferative counterparts. Unlike control cells, quiescent cells lacking the ETC were highly susceptible to reactive oxygen species (ROS) inducers such as phenethyl isothicyanate (PEITC). This was surprising, as the ETC-deficient quiescent cells, similar to ETC-functional counterparts, showed elevated activity of mitochondrial antioxidant defense. Interestingly, we observed a reduced autophagic flux in quiescent ρ^0 cells. Moreover, pharmacological interference with autophagy or the knock down of ATG5, a protein essential for autophagy, not only reduced autophagic flux but also increased sensitivity to ROS and the ROS-induced cell death in quiescent cells with functional ETC, recapitulating the ETC-deficient phenotype. This suggest that quiescent ETC-deficient cells are metabolically stressed, leading to compromised autophagic flux and limited protection from ROS.

LIPIDS IN COLON CANCER DEVELOPMENT

Hofmanová J.

Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic

Development of colon cancer (CC) is accompanied by typical genetic alterations, which in interaction with environmental factors (life style, diet) deregulate cell proliferation, differentiation and apoptosis and support disease progression. In addition, cancer cell survival and growth are supported by modulation of cellular metabolism. Recently, together with technological progress, the interest in the role of lipids and their metabolism has been increased. Lipids are either obtained from dietary sources or they are synthesized endogenously in the organism. CC development is often characterized by abnormalities in lipid synthesis and metabolism, which may influence energetic balance, structure and function of biological membranes, or production of specific mediators and cell signalling. The changes in lipid profile and metabolism (lipidome) may significantly affect cell behaviour and response to therapy. CC patients often show abnormalities in plasma and red blood cell fatty acid (FA) profiles, and differences in phospholipid, sphingolipid, and FA composition and metabolism between CC tumour and non-tumour tissues were demonstrated and further investigated. CC is also one of the cancer types that could most benefit from the prevention. Some dietary lipids, especially essential polyunsaturated fatty acids (PUFAs) or short-chain FA such as butyrate produced from fibre, can play important supportive or inhibitory role in CC development. During last decade, we defined the effects of dietary PUFAs (such as n-3 docosahexaenoic acid) especially in interaction with butyrate, on a wide range of human colon epithelial cells representing various stages of CC development. Our results showed that PUFAs together with butyrate may specifically alter colon cell FA composition, cell membrane properties, metabolism and intracellular signalling, thus significantly affecting cell behaviour and its response to various exogenous or endogenous stimuli. Recently, our research is focused to the lipid perturbation in CC patients in order to find plasma and tumour biomarkers which may be useful for novel approaches in CC diagnosis and prognosis.

This work was supported by Czech Health Research Council, grant No. AZV 15-30585A.

MITOCHONDRIAL GLYCEROPHOSPHATE DEHYDROGENASE AS A TARGET FOR INHIBITION OF CELL PROLIFERATION IN TUMORS

Pecinová A., Brázdová A., Pecina P., Kovalčíková J., Drahota Z., Alán L., Zima M.,
Houštěk J., Mráček T.

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic.

Rapidly proliferating cells, including many tumors, face the need to produce both ATP by catabolic processes as well as many compounds serving as building blocks for anabolic reactions. These opposing requirements lead to specific adaptations of cellular metabolism, including high rates of glucose utilization. In order to sustain high glycolytic rate, many cancer types depend on functional mitochondrial respiration (aerobic glycolysis) for reoxidation of cytosolic NAD(P)H pool. An important component of this pathway is glycerophosphate (GP)-shuttle, which connects mitochondrial and cytosolic processes (glycolysis, lipogenesis) and plays an important role in cell bioenergetics, both under physiological and pathological conditions. One component of GP-shuttle, namely mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH), has highly variable expression across mammalian tissues and effectively acts as rate limiting component of the whole shuttle. In our research, we focus on the utility of mGPDH as potential druggable target in cancer cells lines.
Recently, several compounds were identified as mGPDH inhibitors: α-tocopheryl succinate (α-TOS), biguanides (metformin and phenformin) and a novel specific compound i-GP1. At first, we focused on direct inhibitory mechanisms of the inhibitors on brown adipose tissue (BAT) mitochondria, an established model of high mGPDH content. We found that physiologically relevant inhibitors are α-TOS and i-GP1 (IC50~10 uM). Unlike biguanides, these inhibitors did not increase oxidative stress.
In parallel, we decided to test whether the proliferation of selected cancer cells is affected by mGPDH inhibitors in order to verify mGPDH as a druggable target. Given that metastasis is one of the cancer hallmarks, we identified prostate cancer cell lines of metastatic origin with high mGPDH content and activity. Regardless of the profound inhibitory effect of α-TOS and i-GP1 on the enzyme activity, cell proliferation was modulated solely by suprapharmacological levels of metformin. However, epidemiological studies have clearly indicated that the type II diabetes patients treated with metformin have lower risk of cancer. Therefore we propose an indirect modulatory mechanism of pharmacological concentrations of metformin as a cause of antitumorigenic activity.

Supported by the Grant Agency of the Czech Republic (16-12726S).

ALTERED CELLULAR METABOLISM IN MALIGNANT TRANSFORMATION AND THERAPY RESPONSE IN ACUTE LEUKEMIAS

Júlia Starková

CLIP – Childhood Leukemia Investigation Prague, Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University, Prague

The cancer metabolic program alters bioenergetic processes to meet the higher demands of tumour cells for biomass production, nucleotide synthesis, and NADPH-balancing redox homeostasis. It is widely accepted that cancer cells mostly utilize glycolysis, as opposed to normal cells, in which oxidative phosphorylation is the most employed bioenergetic process. Still, studies examining cancer metabolism had been overlooked for many decades, and it was only recently discovered that metabolic alterations affect both the oncogenic potential and therapeutic response. Since most of the published works concern solid tumours we aim to study the metabolism of leukemia cells. Leukemia is a malignant disease that ranks 1st and 5th in cancer-related deaths in children and adults, respectively. Current treatment has reached its limits due to toxicity, and there has been a need for new therapeutic approaches. One of the possible scenarios is improved use of established drugs, and another is to introduce new druggable targets. We aim to describe the role of metabolic alterations in leukemogenesis and progression of the disease and highlight cellular processes that could be targeted therapeutically and enhance the effectiveness of current treatments.

THE METABOLIC SENSOR AMPK IS A NEW REGULATOR OF ONCOGENE-DRIVEN ERK SIGNALING IN CANCER CELLS

Uldrijan S.[1,2], Verlande A.[1,2]

[1]*Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2]*International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

Malignant melanoma is an aggressive malignant tumor that rarely responds to standard chemotherapy or radiotherapy. Targeted therapy focuses in particular on the ERK kinase pathway, which is activated in most melanomas by mutations of BRAF or NRAS oncogenes. New BRAF inhibitors manage to slow down the progression of the disease, but the majority of patients develops resistance to the therapy, and alternative treatment options are needed.

Genetic and epigenetic changes that occur during malignant transformation contribute to changes in cell metabolism. Deregulation of metabolic pathways is one of the characteristics of tumor cells, and their metabolism, therefore, appears to be a promising therapeutic target. Several small molecule drugs targeting the energy metabolism of tumor cells have already entered clinical trials.

Our results suggest the existence of a previously unrecognized regulatory mechanism, mediated by proteins from the KSR family and the AMPK kinase, which actively modulates the oncogene-activated ERK pathway under stress induced by energy metabolism inhibitors. In melanoma cells carrying the NRAS mutation, metabolic stress promotes interaction between KSR scaffold proteins and CRAF kinase, resulting in an excessive increase in ERK signaling independently of NRAS and inhibition of cancer cell growth. In BRAF-mutated melanoma cells, metabolic stress also stimulates ERK activity by promoting interactions between the BRAF V600E mutant and KSR proteins. Higher levels of metabolic stress, however, promote the localization of the AMPK energy sensor into the BRAF-KSR complex, followed by the disruption of this complex and inhibition of the ERK pathway. These results indicate that while ERK activity in the melanomas with NRAS and BRAF mutations is affected by metabolic stress differently, the disruption of energy metabolism leads to deregulation of the ERK pathway and inhibition of tumor cell proliferation in both molecular subtypes of melanoma.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic: The Specific University Research project Cellular and Molecular Biology (MUNI/A/0754/2017) and the National Program for Sustainability II project Translational
Medicine (LQ1605).

CANCER-ASSOCIATED FIBROBLASTS IN MALIGNANT MELANOMA

Smetana K. Jr.[1,2], Dvořánková B.[1,2], Lacina L.[1,2,3], Kodet J.[1,2,3], Strnadová K.[1,2], Szabo P.[1,2]

[1] *Charles University, 1st Faculty of Medicine, Institute of Anatomy, Prague, Czech Republic*
[2]*Charles University, 1st Faculty of Medicine, Institute of Anatomy, Vestec, Czech Republic*
[3]*Charles University, 1st Faculty of Medicine, Department of Dermatology and Venereology, Prague, Czech Republic*

Despite the progress in therapy, the possibility to cure the malignant melanoma (MM) is limited. Similarly, to other solid tumors, MM represents not only the cancer cells but also other cell types that form a complicated ecosystem significantly influencing the biological properties of tumor. Cancer-associated fibroblasts (CAFs) are able significantly influence properties of all cells in ecosystem. They produce not only the extracellular matrix as expected but also numerous bioactive substances that influence not only the cancer cells but also the metabolism of patients and can participate in the wasting of cancer patients. From this point of view, CAFs represent a potential therapeutic target of anticancer therapy.

Lacina L, Kodet O, Dvořánková B, Szabo P, Smetana K Jr.: Ecology of melanoma cell. Histol Histopathol 33: 247-254 (2018)

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MITOCHONDRIAL 2HG PRODUCTION AS A FUNCTION OF IDH2 AND HOT IN BREAST CANCER CELLS

Smolková K.[1], Špačková J.[1], Dvořák A.[2], Vítek L.[2], Ježek P.[1]

[1]*Institute of Physiology, Department of Mitochondrial Physiology, Czech Academy of Sciences, Czech Republic*
[2]*Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University in Prague, Czech Republic*

Cancer metabolic alterations result from complex genetic and epigenetic adjustments, and include also mitochondrial pathways glutaminolysis, reductive carboxylation (RC), oncometabolite 2-hydroxyglutarate (2HG) production, and NADPH synthesis. We studied complex mechanisms that promote mitochondrial enzymes isocitrate dehydrogenase 2 (IDH2) and hydroxyacid-oxoacid transhydrogenase (HOT) towards 2HG production in breast cancer cell lines. We demonstrate that IDH2 enzyme produces 2HG *in vitro*, as assumed from functional analysis of isolated recombinant wild-type IDH2. Our analysis of metabolic flux shows that mitochondrial production of 2HG by wild-type IDH2 is largely dependent on mitochondrial NADPH balance, because induction of mitochondrial NADPH by dm-L-malate or overexpression of NADPH-producing enzymes induce IDH2-dependent 2HG synthesis. In addition, we demonstrate that active interplay and competition between IDH2 and HOT for substrate (2OG) exists; overexpression of superactive mutant of glutaminese 1, which induces 2OG production, favors HOT when NADPH levels are low. Moreover, we demonstrate that IDH2 is a direct substrate of mitochondrial deacetylase SIRT3, and that distinct regulation by SIRT3 towards oxidative vs. reductive IDH2 activity exists. Quantity and frequency of acetylated lysines on IDH2 declines when treated with SIRT3. The metabolic flux analysis shows that mitochondrial production of 2HG by wild-type IDH2 depends on SIRT3 presence and activity in mitochondria, as supposed by overexpression of wild-type SIRT3 and SIRT3-inactive mutant in cancer cells. Moreover, an acetylation surrogate IDH2 mutant K413Q tends to decrease levels of 2HG *in vitro*. Taken together, our findings impact the understanding of breast cancer metabolism, since breast cancer express a broad range of IDH2, HOT and SIRT3 levels, and exhibit distinct metabolic phenotypes, including 2HG levels. *Supported by The Czech Science Foundation grant 16-04788S to P.J.*

POSTERS

LACTATE AS A SIGNALING MOLECULE IN THE TUMOR MICROENVIRONMENT

Koncošová M., Zelenka J., Ruml T.

University of Chemistry and Technology, Prague, Czech Republic

Tumor microenvironment is different from the normal one and influences the behavior of the cancer. Main characteristics of the tumor environment are hypoxia, hyperlactemia, hypercapnia and acidosis. Our work is focused on hyperlactemia and acidosis, which are caused by increased glycolysis followed by lactate fermentation even under conditions when oxygen is available. This phenomenon is called Warburg effect. Several reports showed that higher levels of tumor lactate correlate with a higher incidence of metastases, higher resistance to treatment, poor survival of patients and they increase the likelihood of disease recurrence. The aim of our work was to determine the effect of lactic acidosis on tumor cells sensitivity to oxidative stress. This feature was studied in the previously unexplored context of the lactate's ability to activate a master antioxidant and chemoprotective transcription factor Nrf2. We were investigating the viability of cancer cell which were cultivated in medium with different concentration of lactate anion and pH and treated with hydrogen peroxide. In addition, the nuclear translocation of Nrf2 protein was characterized with western blot and the expression of its target genes with real time PCR. It was found that the presence of lactate anion in acidic microenvironment increases the resistance of tumor cells to oxidative stress. Our preliminary data also show that the lactate treatment stimulates Nrf2 signaling pathway. This is, to our knowledge, the first demonstration of Nrf2 induction with lactate and of disjunction between the effects of lactate and acidosis on tumor cell resistance to stress.

GLUTAMINE AS A SENSITIZING FACTOR FOR THE THERAPY OF SOLID TUMORS

Vrzáčková N., Zelenka J., Ruml T.

University of Chemistry and Technology, Prague, Czech Republic

Metabolic transformation of cancer cells includes an increase in the consumption of glutamine, which can even lead to a „glutamine addiction". This study focuses on depletion of glutamine via treatment with asparaginase, which can create an option for sensitization of cancer cells to chemo/radiotherapy. Glutamine is the most abundant amino acid in the circulation and its role in the cell metabolism involves the synthesis and import of other amino acids, proteosynthesis, and synthesis of purine and pyrimidine bases. After deamination and subsequent conversion to 2-oxoglutarate, its carbon skeleton serves as an anaplerotic substrate for the Krebs cycle. In addition to its role in energetic metabolism, 2-oxoglutarate can serve as a resource for the antioxidant system through its role in the regeneration of NADPH.
In this study, *in vitro* effect of asparaginase, a drug commonly employed for the long-term therapy of the acute lymphoblastic leukemia, which decreases the blood levels of asparagine and glutamine, was simulated. The effect of acute and chronic depletion of glutamine and asparagine on the cell resistance was studied using human cell lines, namely pancreatic adenocarcinoma (PaTu), cervix carcinoma (HeLa), and non-cancer fibroblasts MRC-5. The effect was tested in relation with oxidative stress induced by hydrogen peroxide, chemotherapeutic effect of doxorubicin and oxaliplatinum, and radiotherapy.
Our results show, that the simulation of asparaginase significantly increases the sensitivity of cancer cells to oxidative stress and oxaliplatinum. For this effect, a simultaneous depletion of glutamine and asparagine is required. Acute depletion of both amino acids is accompanied by decrease of an antioxidant capacity, lower activity of mTORc1 and changes in levels of intracellular amino acids and intermediate metabolites. On the other hand, all the described phenomena were less pronounced in the non-cancer fibroblasts.
In conclusion, while asparaginase is clinically used for the long-term chemotherapy of leukemia, this study suggests a potential role of its acute exposure as a chemosensitizing factor in the therapy of solid tumors.

CANCER SENESCENT CELLS DIFFER FROM THEIR PROLIFERATING COUNTERPARTS IN THEIR RESPONSE TO CELL DEATH-INDUCING AGENTS

Holíček P. [1,2], Hodný Z.[3], Neužil J. [1], Ladislav Anděra[1]

[1]*Institute of Biotechnology, Prague-Vestec, Czech Republic*
[2]*Faculty of Science, Charles University, Prague, Czech Republic*
[3]*Institute of Molecular Genetics AS CR, Prague, Czech Republic*

Increasing and persisting incidence of senescence cells has been shown to have detrimental effect on an organism contributing to pro-aging features. Senescence normal as well as cancer cells seems to have modified apoptotic signaling at the level of mitochondria and Bcl-2 family proteins. Within tumour the presence of senescent cells positively support its growth and can even stimulate transformation of surrounding cells. Senescence state of tumour cells can be triggered by chemotherapy or spontaneously induced. Thus, for the effective cancer therapy it would be beneficial to eliminate senescent cancer cells along with the ordinary cancer cells. Current strategies for elimination of senescent (cancer) cells target mitochondria exploiting their intrinsic apoptotic signaling as well as mitochondrial bioenergetics.
Two main senescence induction signalings downstream of initial stimuli employ either p16/pRb or p53/p21 pathways. In our model of senescent cancer cells we used mild and controllable approach employing inducible expression of p16 or p21 in mesotheliomal cancer H28 cells and clonal selection. In addition to the analysis of their growth and bioenergetics profile we aimed to screen and analyze their response to various cell death-inducing agents/drugs including those directly targeting mitochondria.
Though p16- and p21-induced pro-senescence phenotype (growth, cell shape and mitochondrial profiles) differed between p16 and p21 H28 clones they responded similarly to various cell death stimuli. In both p16 and p21 clones we observed an increase of mitochondrial mass and ROS production though to a significant lesser extent in p16-expressing cells. We show that senescent cancer cells are more resistant to ROS- mediated cell death induced by mitochondrially-targeted vitamin E succinate (mitoVES) and interestingly also to TRAIL-induced apoptosis enhanced by homoharringtonine. However and interestigly, they became more sensitive to FasL- and Bcl-2/BcL-XL-targeting drug ABT-737-triggered apoptotic signaling. These data together with their bioenergetics profiling might be instrumental in better understanding (cancer) senescence phenotype and pave a way for more effective cancer therapy.

ROLE OF METABOLISM IN CHEMORESISTANCE OF NEUROBLASTOMA CELLS.

J. Plch[1], M. Belhajová[1], J. Hraběta[1], T. Eckschlager[1]

[1]Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague

Introduction :
Metabolic profile of cancer cells reflects their altered bioenergetic requirments. Chemoresistance has been explained by changes in the genome of tumor cells that carry a particular mutation that is selected during the treatment. However, the rapidly acquired resistance to cytostatics is more likely to be attributed to the dynamics of non-genetic heterogeneity of the tumor population, which produces different phenotypic variants. The flexibility in using different energy pathways may indicate a survival adaptation to achieve a higher cellular fitness that might be also associated with chemoresistance.

Aims:
The aim of this study is to understand the connection between metabolic character and chemoresistance.

Materials and Methods:
Metabolism characterization was obtained from UKF-NB-4, UKF-NB-4^{CDDP}, survivors ^{CDDP}, UKF-NB-4^{VCR}, survivors ^{VCR}. The survivor cells have been created by a single high dose of cytostatics (vincristine -VCR/cisplatin -CDDP) and further cultivation in drug-free media for 3 weeks. We used Seahorse XF Glycolysis Stress Test for measuring glycolytic function and Mito Stress Test for measuring mitochondrial function in cells. The rate of O_2 consumption (OCR) can be assigned to OXPHOS and the rate of extracellular acidification (ECAR) to glycolysis. AlamarBlue viability test was performed to determine the differencies in sensitivity of used cells to VCR/CDDP.
Results:
Our data suggests that metabolic changes occur as a result of chemotherapy drugs. We observed significantly lower glycolysis in UKF-NB-4 compared to respective resistant counterpart UKF-NB-4^{CDDP}. This was also reflected in lower basal ECAR values that defined the glycolysis measure at resting state. Along with increased glycolysis, the chemoresistant cell also performed higher OXPHOS. To get an overall sight of the bioenergetics organization of the cells, a ratio of basal glycolysis vs. basal OXPHOS was generated. The results indicate that UKF-NB-4^{CDDP} favor the OXPHOS pathway. VCR treatment did not result in any increase in metabolism, indicating the metabolic changes might be unique to platinum exposure.

Conclusions:
To understand the origin of acquired chemoresistance, we decided to analyze changes in metabolism of UKF-NB-4 and its derived resistant cell lines. The resistant cells seemed to switch to a high metabolically active phenotype, which enables them to survive a chemotherapy insult better than the sensitive cell line. We observed that metabolic state influence chemoresistance. Understanding the cancer cells metabolism can lead to more effective treatment of tumors.
The study was supported by the Charles University, project GA UK No. 812217 and GACR project No.17-12816S

COMMON THEMES IN CANCER AND VIRUS-INFECTED CELLS

Mělková Z.[1], Madleňáková M.[1], Humlová Z.[1], Hubálek Kalbáčová M.[1], Vrbacký M.[2]

[1]First Faculty of Medicine, Charles University, Prague, Czech Republic
[2]Czech Academy of Science, Prague, Czech Republic

Cellular homeostasis and metabolism are key determinants of different aspects of the life of the cell, its survival, death or division. These processes are regulated at various levels, depending on both extracellular and intracellular stimuli, with energy supplies strongly affecting or limiting the course and outcome of individual processes. The energy supplies in form of ATP are generated very efficiently by mitochondrial oxidative phosphorylation or less efficiently by glycolysis. A major by-product of oxidative phosphorylation is generation of reactive oxygen species while glycolysis can promote cellular antioxidant defenses. Different cells and tissues are more or less resistant to the oxidative damage and/or lack of energy supplies. However, usually only high levels of free radicals are harmful, while their low levels stimulate redox-sensitive transcription factors, promoting cellular metabolism and growth as well as angiogenesis. Blood supply then determines delivery of oxygen, glucose and other nutrients, and/or removal of toxic products of metabolism. Cellular metabolism also affects inactivation or exclusion of xenobiotics as well as growth of intracellular parasites.
We have focused on the interactions of viruses with the host, especially on the role of energy metabolism, redox stress, and the type of virus-induced cell death with implications for the cellular and immune responses. Namely, we have

found that the type of energy metabolism determines the typical anti-apoptotic and a paradoxical pro-apoptotic effect of Bcl-2 protooncogene expressed by a recombinant vaccinia virus. Further, we have described inhibitory effects of high levels of nitric oxide on the growth of vaccinia virus and HIV-1, as well as stimulatory effects of low levels of nitric oxide on the growth of these two viruses. Finally, we have employed heme- and iron-induced redox stress to reactivate latent HIV-1 with the aim to eliminate this deadly virus from the organism.
There are many themes and aspects common to both cancer and virus-infected cells. Moreover, many viruses are suspected or proved to induce tumorigenesis while they can be useful for the oncolytic therapy or anti-tumor vaccination. Thus, the investigation of both cancer and virus-infected cells with respect to the underlying cellular metabolism can be fruitful.

PUSHING THE ERK PATHWAY ACTIVITY OUT OF THE FITNESS ZONE WITH METABOLIC STRESSORS: NEW TARGETED THERAPY FOR MELANOMA?

Valčíková B. [1,2], Zacpalová M. [1], Štětková M. [1], Palušová V. [1,2], Verlande A. [1,2], Krejčí P. [1,2], Uldrijan S. [1,2]

[1]*Dept. of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2]*International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

The majority of malignant melanomas are driven by mutations in the MAPK/ERK signaling pathway which dysregulate a number processes, leading to aberrant cell growth and proliferation. The development of mutant BRAF kinase-targeted therapy has prolonged patient survival. However, in most patients, tumor cells acquire resistance after several months of treatment. A new ERK pathway-targeted approach for melanoma therapy has been recently suggested - to push the cancer cells out of their fitness zone by over-activating the ERK signaling to levels that the cancer cell cannot sustain.
We recently demonstrated that melanoma cells respond to metabolic stress induced by inhibitors of cell energy metabolism by enhancing ERK pathway activity. While studying further the mechanisms responsible for this hyperactivation, we identified AMPK as an essential player in this process. We also found a small molecule drug (DRG) that potently enhanced ERK signaling at very low concentrations and inhibited melanoma cell growth and proliferation. The effect of DRG was observed only at the level of ERK, not its upstream signaling, and we propose that the candidate target for DRG is the negative feedback regulator of ERK – dual-specificity phosphatases (DUSPs). Interestingly, when metabolic stressor or AMPK activators were combined with DRG, we observed a robust synergizing effect on the ERK signaling on transcriptional levels. Our results show surprising plasticity of oncogene-driven ERK signaling in cancer cells and suggest new drug combinations that might be suitable for targeting the ERK pathway in melanoma.
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IDENTIFICATION THE KEY PLAYERS OF METABOLIC REPROGRAMMING OF LEUKEMIC CELLS UPON L-ASPARAGINASE TREATMENT.

Alquézar-Artieda N.[1], Vališ K.[2], Pecinová A.[3], Hernychová L.[2], Kavan D.[2], Hložková K.[1], Šperková K.[1], Žaliová M.[1], Mráček T.[3], Novák P.[2], Trka J.[1] and Starková J.[1]

[1]*CLIP - Childhood Leukaemia Investigation Prague, Department of Pediatric Hematology/Oncology, 2nd Faculty of Medicine, Charles University Prague, Prague, Czech Republic*
[2]*Laboratory of Structural Biology and Cell Signaling, Institute of Microbiology, Academy of Science of the Czech Republic, Prague, Czech Republic.*
[3]*Department of Bioenergetics, Institute of Physiology of the Czech Academy of Science, Prague, Czech Republic.*

L-asparaginase (ASNase) is the key component in the treatment of acute lymphoblastic leukemia (ALL) in children. ASNase depletes extracellular asparagine (Asn) and glutamine (Gln). In our previous findings, we had demonstrated that ASNase treatment leads to metabolic reprogramming of leukemic cells in order to compensate the lack of mentioned amino acids and can turn on pro-survival processes which could interfere with the effectiveness of the treatment. We aim to describe the mechanism of action of ASNase in more detail and interactions between bioenergetics processes. In order to complete our aims, we used pre-B ALL leukemia cells lines (REH and NALM6) treated with ASNase in two different time points. The project was contemplated in two different ways. First, we measured the intracellular metabolites of the central carbon metabolism and amino acids (AAs) by employing Liquid Chromatography - Mass Spectrometry (LC/MS). Then extracellular amino acids were measured from cultured media with LC/MS and High-performance LC. Next, using Label-Free Proteome MS Quantification we detected changes in proteins involved in

metabolic processes. Results were confirmed on mRNA and protein level. Treatment with ASNase led to significant decrease of extracellular levels of Asn and Gln followed by increase of aspartate and glutamate. Intracellular Asn and Gln were decreased as well whereas aspartate and glutamate were not affected. Interestingly, levels of non-essential AAs (alanine, serine, and glycine) and also essential AAs (histidine, isoleucine/leucine, methionine, phenylalanine, valine, tryptophan and threonine) were increased. Proteome analysis identified 16 metabolic-related proteins. After validation, we selected arginosuccinate synthase (ASS1), aspartate aminotransferase (GOT1) and phosphoserine aminotransferase (PSAT1) as possible key effectors of the metabolic reprograming. Our results show that ASNase disturbs AAs metabolism. We hypothesize that activation of AAs biosynthetic pathways could allow leukemia cells maintain normal levels of aspartate and glutamate necessary for energy and biomass maintenance and also compensate for depletion of Asn and Gln by synthesis of other AAs. Supported by the Charles University Grant agency (794218) and AZV grant 15-28848A.

EXPLORING METFORMIN ACTION ON THE REGULATION OF CANCER CELL PROLIFERATION

Brazdova A.[1], Pecina P., Kovalcikova J., Houstek J., Mracek T., Pecinova A.

[1]*Institute of Physiology of the Czech Academy of Sciences, Department of Bioenergetics, Prague, Czech Republic, andrea.brazdova@fgu.cas.cz*

The increasing incidence of various cancer types leads to the development of specific therapies focusing on altered metabolism of cancer cells. Rapid growth and fast proliferation of some tumors require specific adaptations of cellular metabolism comprising high rates of glucose utilization and subsequent NADH re-oxidation. To sustain high glycolytic rate, many cancer cells depend on functional mitochondrial respiration, in which glycerophosphate-(GP)-shuttle connects mitochondrial and cytosolic transduction pathways. Mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase (mGPDH) is a rate-limiting component of GP shuttle; hence, its activity may be crucial for efficient tumor cell proliferation.
In this work, we focused on the action of metformin (MF) on tumor cell proliferation as MF has recently been demonstrated to act as mGPDH inhibitor. For this purpose, we identified several cancer cell lines of metastatic origin with high mGPDH content and activity and treated them with MF. We observed decrease in cell proliferation, yet solely associated with suprapharmacological levels of MF and therefore this could be explained by apoptosis/necrosis induction than by any specific MF action. However, as epidemiological evidence from type II diabetes patients on MF treatment clearly points to reduced cancer risk in this cohort, we propose an indirect modulatory mechanism to be causative for the antitumorigenic potential of MF rather than direct enzyme inhibition and subsequent reduced cell proliferation. We examined two different MF actions: (1) how mitochondrial substrate utilization underlies cancer cell sensitivity to MF, (2) its effect on the components of immune system. We showed that MF affected cell proliferation in time dependent manner in nutrient restricted environment. Concerning MF action on immune system, we identified its immunomodulatory properties as it altered immunophenotype together with respiratory and metabolic profiles of various immune populations. Most prominently, MF interfered with the generation of monocyte-derived dendritic cells. Altogether, our results might be an asset for anticancer therapies.

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