evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Propojení výzkumu a vzdělávání v oblasti medicinální chemie
reg. číslo: CZ.1.07/2.3.00/30.0060

Zpráva z mezinárodní konference/ Report from international conference

Účel cesty/Aim of travel:
X. Diagnostic, Predictive and Experimental ONCOLOGY Days

Účastník/Participant: Mgr. Jana Štěpánková, Ph.D.

Doba trvání cesty/Duration of travel: 02. - 03. 12. 2014

Místo/Location: Olomouc, Česká Republika

Zpráva/Report:

Na konferenci jsem prezentovala výsledky dosažené v oblasti studie inhibitorů anhydrázy uhličitanu vápenatého 9, konkrétně karboranů se sulfonamidovým zbytkem. Konference byla zaměřena na problematiku nádorového onemocnění. Účastnili se ji především mladí vědečtí pracovníci.

Prezentované téma: Carborane-Based Carbonic Anhydrase IX Inhibitors

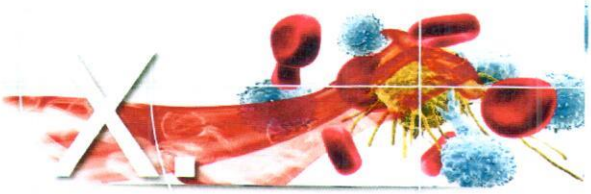
V Praze/Olomouci dne
In Prague/Olomouc, date: 12. 12. 2014

Jméno, podpis/
Name, signature Jana Štěpánková

ve zlínském kraji

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

Fotodokumentace/Photos:



Diagnostic, Predictive and Experimental ONCOLOGY Days

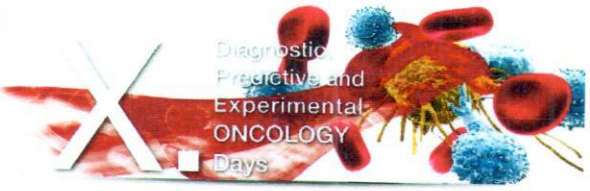
ABSTRACT BOOK

December 02 - 03, 2014

hotel NH Collection Olomouc Congress
Legionarska 21, 779 00 Olomouc,
Czech Republic

INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

MedChemBio



Diagnostic, Predictive and Experimental ONCOLOGY Days

Pořadatel

Ústav molekulární a translační medicíny LF UP a FN Olomouc
Ústav klinické a molekulární patologie LF UP a FN Olomouc
MedChemBio - zájmové sdružení právnických osob

Odborná garance

Sekce diagnostické a prediktivní onkologie České onkologické společnosti ČLS JEP
Komplexní onkologické centrum Olomouc

Prezident akce

doc.MUDr. Marián Hajdúch, Ph.D.

Organizační výbor

doc. Mgr. Jiří Drábek, Ph.D., RNDr. Radek Trojanec Ph.D., MUDr. Kateřina Bouchalová, Ph.D., MUDr. Petr Džubák, Ph.D., MUDr. Josef Srovnal, Ph.D., doc. RNDr. Ondřej Slabý, Ph.D., doc. MUDr. Marek Svoboda, Ph.D.

Organizátor

Ústav molekulární a translační medicíny Lékařské fakulty Univerzity Palackého v Olomouci
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PROGRAM

X. DNY DIAGNOSTICKÉ, PREDIKTÍVNÍ A EXPERIMENTÁLNÍ ONKOLOGIE /
X. DIAGNOSTIC, PREDICTIVE AND EXPERIMENTAL ONCOLOGY DAYS

ÚTERÝ / TUESDAY - 2. prosince 2014 / December 2, 2014

9.45 Zahájení X. Dnů diagnostické, prediktivní a experimentální onkologie / Opening ceremony

10:00 – 12:00 Biomarkery nádorových onemocnění I / Cancer biomarkers I
Předsedající / Chairs: Marek Svoboda, Ondřej Slabý

10.00 – 10.30 Luminální/Her-2 negativní karcinom prsu a možnosti využití komplexních prognostických systémů založených na analýze genetických molekulárních biomarkerů.
Marek Svoboda

10:30 – 11.00 Plasma proteomic biomarkers for early detection and characterization of colorectal cancer
Marián Hajdúch

11:00 – 11:15 Circulating tumor cells in pancreatic cancer
Josef Srovnal

11:15 – 11:30 Circulating tumor cells - a tool to monitor cancer treatment
Anna Jakabová

11:30 – 11:45 MiR-31-3p a miR-31-5p predikují čas do progrese u pacientů s metastatickým kolorektálním karcinomem s nemutovaným onkogenem KRAS léčených cetuximabem
Jitka Mlčochová

11:45 – 12:00 MikroRNA u solidních nádorů: Od biomarkerů po terapeutická cíle
Ondřej Slabý

12:00 – 13:00 OBĚD / LUNCH

13:00 – 14:30 Nové modely a technologie / New models and technologies
Předsedající / Chairs: Viswanath Das, Karel Kobera

13:00 – 13:15 Mouse transgenic models to study tumor initiation and progression in the gut
Michaela Krausová

13:15 – 13:30 Technology of programmable nucleases and reporter mouse lines engineering for cancer research
Dominika Fričová

13:30 – 13:45 Genome editing and gene modification technologies: an overview and update
Sylvia Petreszelyová

13:45 – 14:00 Understanding biological heterogeneity with the CyTOF 2 Mass Cytometer
Mark D. Lynch

14:00 – 14:15 Three-dimensional cultures: physiologically-relevant in vitro tumor models
Viswanath Das

14:15 – 14:30 Advantages of high-throughput qPCR for gene expression profiling. Analysis and applications
Vlasta Korenková

14:30 – 15:00 PŘESTÁVKA / BREAK

15:00 – 17:00 Molekulární cíle a protinádorová léčiva I / Molecular targets and anticancer drugs I
Předsedající / Chairs: Marián Hajdúch, Tomáš Eckschlager

15:00 – 15:30 Lactate transport in hypoxic cancer cells is facilitated by non-catalytic function of carbonic anhydrase IX
Holger Becker

15:30 – 15:45 Carborane - Based Carbonic Anhydrase IX Inhibitors
Jana Štěpánková

15:45 – 16:00 5-Azacytidine Nucleosides and their Derivatives: Molecular hallmarks of Drug Resistance & Alternative Therapeutic Regimen
Kushboo Agrawal

16:00 – 16.15 The activity of disulfiram towards breast cancer
Zdeněk Škrott

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

Molekulární cíle a protinádorová léčiva I
Molecular targets and anticancers drugs I

Předsedající / Chairs: Marián Hajdúch, Tomáš Eckschlager
Úterý / 2. prosince 2014 / Tuesday / December 2, 2014 / 15.00 - 17.00 hod.

Lactate transport in hypoxic cancer cells is facilitated by non-catalytic function of carbonic anhydrase IX

úterý / 2. prosince 2014 / 15.00- 15.30 hod.

Somayeh Jamali[1], Michael Kiler[1,2], L. Felipe Barros[3], Robert McKenna[4], Joachim W. Deitmer[5], Holger M. Becker[1]

[1] *Division of Zoology / Membrane Transport, University of Kaiserslautern, Kaiserslautern, Germany,*
[2] *Division of General Zoology, University of Kaiserslautern, Kaiserslautern, Germany,*
[3] *Centro de Estudios Cientificos (CECS), Valdivia, Chile,*
[4] *Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, USA*

Introduction

The most aggressive and invasive tumor cells, which often reside in hypoxic environments, rely on extensive glycolysis to meet their large demand for energy and biosynthetic precursors. Thereby they release vast amounts of lactate and protons via monocarboxylate transporters (MCTs), which exacerbates extracellular acidification and supports the formation of a hostile environment. In the present study we investigated the mechanisms that regulate lactate transport in cancer cells during the switch from normoxia to hypoxia.

Materials/methods

Changes in intracellular pH and lactate concentration in single MCF-7 breast cancer cells were monitored by pH imaging and with the lactate-sensitive FRET nanosensor Laconic, respectively. Hypoxia-induced changes in the expression levels of various enzymes and transport proteins were analyzed in MCF-7 cells by qRT-PCR and western blot. Functional interactions between MCTs and carbonic anhydrase CAIX were determined in Xenopus oocytes by single-site mutation and subsequent measurements of intracellular pH with ion-sensitive microelectrodes.

Results and conclusions

Under hypoxia, expression of MCT1 and MCT4 in MCF-7 breast cancer cells remained unchanged, while expression of carbonic anhydrase IX (CAIX) was greatly enhanced. Real-time measurements of intracellular pH and lactate concentration show that CAIX augments lactate flux via MCT1 by a non-catalytic interaction. Mutation studies in Xenopus oocytes indicate that CAIX, via its intramolecular H_+-shuttle His200, functions as a „proton-collecting/distributing antenna" to facilitate rapid lactate flux via MCT1. Knockdown of CAIX significantly reduced proliferation of MCF-7 cancer cells, suggesting that rapid efflux of lactate and H_+, as enhanced by CAIX, contributes to cancer cell survival under hypoxic conditions.

Carborane - Based Carbonic Anhydrase IX Inhibitors

úterý / 2. prosince 2014 / 15.30- 15.45 hod.

Jana Stepankova[1], Pavlina Rezacova[2], Brynda Jiri[2], Harvanova Monika[1], Masek Vlastimil[1], Nova Alice[1], Schiller Michal[1], Das Viswanath[1], Dolezal Dalibor[1], Grüner Bohumír[3], Sicha Vaclav[3], Konecny Petr[1], Znojek Pawel[1], Dzubak Petr[1], Hajduch Marian[1]

[1] *Faculty of Medicine and Dentistry Palacky University Olomouc, Institute of Molecular and Translational Medicine, Olomouc, Czech Republic,*
[2] *Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., Structural Biology, Prague, Czech Republic,*
[3] *Institute of Inorganic Chemistry of the AS CR, v.v.i., Department of Syntheses, Husinec Rez, Czech Republic*

Introduction

Carbonic anhydrase IX (CA IX) is highly overexpressed in different solid tumors. It is a transmembrane isoform of carbonic anhydrase with an extracellular-facing catalytic site and therefore is well positioned to act in the control of tumor pH. Function of CA IX can be inhibited by CA IX-selective sulphonamides. Inhibition its function perturbs in vitro survival under hypoxia conditions. The aim of this study is to evaluate the effect of new carboranes with functional sulfonamide residues on CA IX function. Carboranes, icosahedral clusters containing boron, carbon and hydrogen replace various hydrophobic structures in biologically active molecules.

Materials/methods

The most interesting drugs were chosen based on enzymatic assay. To characterize their CA IX inhibition mode in cellular level, several cell biology methods were used. Drug cytotoxicity and consequently extracellular pH of carboranes treated cell lines under hypoxia conditions was evaluated. Data shows reversed hypoxia-induced decline of extracellular pH in treated HT-29 and 4T1 cell lines. Moreover, we set up a new method of Raman spectroscopy locating carboranes distribution in cells. To extend this study, pharmacokinetics and pharmacology profile describe ADME methods and experiments on animal model.

Results and conclusions

The results shows the ability of these compounds inhibit CA IX in enzymatic level and cellular level. Thus, novel carboranes with functional sulfonamide residues indicate new selective CA IX inhibitors as potential anticancer drugs.

Acknowledgement: ProMedChem, reg. n.: CZ.1.07/2.3.00/30.0060, BIOMEDREG, reg. n.: CZ.1.05/2.1.00/01.0030

5-Azacytidine Nucleosides and their Derivatives: Molecular Hallmarks of Drug Resistance & Alternative Therapeutic Regimen

úterý / 2. prosince 2014 / 15.45- 16.00 hod.

Khushboo Agrawal[1], Dušan Holub[1], Petr Vojta[1], Ivo Frydrych[1], Zuzana Maceckova[1], Miroslav Otmar[2], Petr Džubák[1], Marián Hajdúch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic,*
[2] *Institute of Organic Chemistry and Biochemistry, ASCR v.v.i., Prague, Czech Republic*

Introduction

Aberrant DNA methylation turning 'off' the gene expression remains the consistent hallmark due to its frequent involvement in all types of cancer. The prototypal DNA methylation inhibitors, 5-azacytidine and 2'-deoxy-5-azacytidine, are currently one of the most effective epigenetic drugs, for the treatment of blood malignancies. However, the chemotherapeutic resistance to these medicines is the major obstacle, forefending the successful epigenetic therapy.

Materials/methods

We developed several HCT116 p53 wild-type cell clones, resistant towards 2'-deoxy-5-azacytidine. Principal methods used to study the molecular alterations during the development of resistance included, flow cytometry based analyses, high throughput RNA sequencing based transcriptomics, and mass spectrometry based proteomics, utilizing stable isotope labelling of amino acids in cell culture (SILAC). Further, we used MTT cytotoxicity assays to determine the cross-resistance or sensitivity of the resistant clones towards other epigenetic inhibitors.

Results and conclusions

Flow cytometry based studies revealed significant up-regulation of DNA and RNA synthesis. Molecular profiling of resistant clones unveiled 8010 genes and 3352 proteins, which were differentially expressed (ANOVA $p<0.05$) compared to parental cell line. The major affected cellular pathways were (i) Cell cycle: nucleocytoplasmic transport of CDK/cyclins, role of 14-3-3 proteins in cell cycle regulation, G1/S transition and initiation of mitosis (ii) Apoptosis and survival: granzyme A signaling, BAD phosphorylation, p53 dependent apoptosis (iii) Transcription: role of heterochromatin protein I family in transcriptional silencing (iv) DNA damage: role of SUMO in p53 regulation. During MTT cytotoxicity assays, resistant clones exhibited cross-resistance towards all the tested epigenetic inhibitors, however, significant sensitivity was exceptionally observed for bromodomain inhibitors, which was further validated by down-regulation of BET bromodomain, BRD4 gene, in all the resistant cell clones. Validation of relevant genes and/or proteins as biomarkers of drug resistance, and bromodomains as alternative therapeutic target, for re-sensitizing the cancer patients, resistant to DNA methylation inhibitors is currently ongoing.

The present study will aid to the understanding of the molecular basis of acquired tumor resistance to 2'-deoxy-5-azacytidine and help in predicting its clinical response, as well as in designing alternative treatment regimens for overcoming resistance, hence furthering clinical development.

The activity of disulfiram towards breast cancer

úterý / 2. prosince 2014 / 16.00- 16.15 hod.

Zdenek Skrott[1], Martin Mistrik[1], Boris Cvek[2], Pavla Pouckova[3], Jiri Bartek[1,4]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic,*
[2] *Department of Cell biology and Genetics, Faculty of Science, Palacky University, Olomouc, Czech Republic,*
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[4] *Danish Cancer Society Research Center, Copenhagen, Denmark*

Introduction

Drug repurposing is a new viable approach of drug development. Disulfiram (Antabuse), as an old drug used for decades in alcohol aversion therapy, shows promising anticancer activity. The potent disulfiram's antitumor effect is attributed to the complex formed from the reaction between disulfiram and copper in the human body. It is assumed that disulfiram-copper complex kills cancer cells by inhibition of ubiquitin-proteasome system, which is responsible for degradation of cellular proteins.

Materials/methods

The toxicity of the main metabolites of disulfiram was evaluated in vitro with various breast cancer cell lines (MDA MB231, MCF7, HS578T, T47D, Cal51) and in vivo using MDA MB231 xenografts. The cellular effect of disulfiram-copper complex was measured in selected breast cancer cell lines or Ub-(G67V)-GFP expressing Hela cell line by standard methods (Western blot, fluorescent microscopy).

Results and conclusions

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

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