REACTOR

6.–8.10. 2021

SKALSKÝ DVŮR HOTEL

IMTM Reactor Conference

INSTITUTE OF MOLECULAR AND
TRANSLATIONAL MEDICINE

PROGRAM

WEDNESDAY 6TH

08:00		DEPARTURE FROM OLOMOUC	
10:00	11:30	SPORTING ACTIVITIES	
08:00	12:00	CHECK-IN	

Chair: Petr Džubák

12:00	12:10	Tomáš Oždian	Welcome Speech
12:10	12:30	Lenka Hrubá	Development and characterization of resistant cell lines to nucleosides based cytostatics
12:30	12:50	Kateřina Ječmeňová	PNH173, mechanism of action
12:50	13:40	LUNCH	

Chair: Martin Mistrík

13:40	14:00	Jiří Drábek	Tsar Nicolas II, DNA evidence, and Czechoslovak legionaires
14:00	14:20	Dušana Majera	Applications of molecular combing of single DNA molecules
14:20	14:40	Tereza Buchtová	CBD attenuates the efficacy of platinum-based chemotherapy
14:40	15:00	Dávid Lukáč	Emetine's anti-DNA replication activity reflects proteosynthesis inhibition not targeting Okazaki fragment formation
15:20	15:40	Matthew Lacey	Mechanism of MCOPPB senolytic activity
15:40	17:00	COFFEE DEGUSTATION	

Chair: Milan Urban

17:00	17:20	Jiří Řehulka	Discovery of novel biologically active compounds and their molecular target
17:20	17:40	Soňa Gurská	Cytotoxicity profiling of new chemical compounds in HTS facility
17:40	18:00	Barbora Lišková	Synthesis of betulinic acid derivatives with aryl moiety via Suzuki-Myiaura cross-coupling reaction and their biological
18:00	18:20	Zuzana Macečková	Bystin regulates AURORA A during mitosis
18:20	18:40	Olena Mokshyna	Exploring protein-protein interactions of bystin using HADDOCK
19:00		DINNER	
20:00		MASOŽRAVÁ BERTA	

THURSDAY 7TH

Chair: Lukáš Najdekr

09:00	09:20	Jana Václavková	Lung cancer and COPD protein biomarkers found in exhaled breath condensate
09:20	09:40	Jiří Dostál	Pro BiomER – CM in ClinData
09:40	10:00	Tomáš Oždian	The proteomic characterization of cervical mucus
10:00	10:20	Jarmila Stanková	Human plasma analysis by HR-MS: An approach for plasma profiling of large population-based cohort
10:20	10:40	Karel Koberna	Anchored linear oligonucleotides: The effective tool for the real-time measurement of uracil DNA glycosylase activity

PROGRAM

10:40	11:00	COFFEE BREAK	

Chair: Miloš Petřík

11:00	10:20	Kateřina Bendová	Radiolabelled siderophores for the diagnosis of Burkholderia cepacia complex infectionscondensate
11:20	10:40	Zbyněk Nový	Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer
11:40	12:00	Narendran Annadurai and Vishwanath Das	Tau aggregation and seeding as a therapeutic target in tauopathies
12:20	12:40	Martin Ondra	Diamonds Are Forever: Development and application of ultra-bright 200-nm fluorescent nanodiamonds for the sentinel lymph nodes visualisation
12:40	13:40	LUNCH	

Chair: Miroslav Popper

13:40	14:00	Dominik Vítek	High-throughput screening of chemical compound libraries in Nematodes
14:00	14:20	Juan Bautista de Sanctis	Adaptative memory cell responses against SARS CoV2 virus
14:20	14:40	Ermin Schadich	Anti-SARS-CoV-2 properties of one anti-inflammatory drug
14:40	19:00	EXPLORATION OF VYSOČINA	
19:00		DINNER	
20:00		WINE DEGUSTATION	

FRIDAY 8TH

Chair: Jana Kotulová

09:00	09:20	Pavel Polishchuk	In silico design of synthetically feasible compounds
09:20	09:40	Mariia Matveieva	Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer
09:40	10:00	Alina Kutlushina	High-throughput screening of chemical compound libraries in Nematodes
10:00	10:20	Aleksandra Nikonenko	Lead optimization of the tubulin inhibitor
10:20	10:40	COFFEE BREAK	

Chair: Alžběta Srovnalová

10:40	11:00	Zdeněk Škrott	Microthermal-induced subcellular protein damage reveals a two-phase HSP70-p97 cellular response
11:00	11:20	Pavel Stejskal	The Circulating Tumor Cells detection in Solid Tumors using CytoTrack TM11
11:20	11:40	Rastislav Slavkovský	Deep amplicon sequencing of predictive markers for fast and effective tumor diagnostics
11:40	12:00	Agáta Kubíčková	CRISPR/Cas9 technology - creating cellular models for human genetic disorders
12:00	12:20	Barbora Koblihová	Clonal somatic variants in hematopoietic cells in relation to age and stroke
12:20	12:30	Tomáš Oždian	Concluding remarks
12:30	13:30	LUNCH	
13:30		DEPARTURE FROM SKALSKÝ DVŮR	

Development and characterization of resistant cell lines to nucleosides based cytostatics

Lenka Hrubá[1], Kateřina Ječmeňová[1] and Petr Džubák[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

Drug resistance is one of the key problems in the cancer therapy. There were more than 19 million new cases of cancer around the world in 2020 and more than 10 million patients died because of this diagnosis. There can be a lot of causes of therapy failure, such as late diagnosis of the disease, limited surgical treatment options or drug resistance, which limits number of suitable drugs. In vitro models of resistant cancer cells seem to be useful model for basic understanding of the drug resistance mechanism and they can be used in the screening of new potential drugs. We are focused on resistance to nucleoside-based drugs in our project. We have already developed resistant cell lines of acute lymphoblastic leukemia (CCRF-CEM) and chronic myeloid leukemia (K562) and basic characterization has been done (expression of MDR proteins, nucleoside transporters etc.). These cells were also used in the screening of new biologically active compounds, which can potentially be able to overcome this drug resistance.

Acknowledgment

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Citation

Ferlay, J. et al. Cancer statistics for the year 2020: An overview. International Journal of Cancer (2021) doi:10.1002/ijc.33588.

PNH173, mechanism of action

Kateřina Ječmeňová[1], Jana Kotulova[1], Marián Hajdúch[1] and Petr Džubák[1]

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Abstract

PNH173 is a nucleoside-based compound and highly active anticancer drug. The aim of this study is the analysis of signaling pathways responding to the treatment by PNH173. Particularly, we have focused on PI3K/Akt signaling pathway, especially on ribosomal protein S6 (rpS6). rpS6 is an indispensable component of the mammalian 40S small ribosomal subunit. rpS6 is subject to phosphorylation in response to multiple physiological, pathological and pharmacological stimuli. Phosphorylation of rpS6 is involved in cell size regulation, cell proliferation or glucose homeostasis.

Acknowledgment

This study was supported by the European Regional Development Fund (Project ENOCH No. CZ.02.1.01/0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN, LM2018130), the Czech Science Foundation (GACR 19-08124S), internal grant of UP (IGA_LF_2021_038) and Technology Agency of the Czech Republic (Project TN01000013)

Tsar Nicolas II, DNA evidence, and Czechoslovak legionaires

Jiří Drábek[1,2]

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[1]*University Hospital Olomouc CSSFG, r.c., Olomouc, Czech Republic*

Abstract

In 1918, Tsar Nicolas II, his family and loyal members of his staff were brutally murdered by communists, following the order of Vladimir Iljič Lenin who feared Czechoslovak legionaires. The dead bodies were destroyed and disposed in two mass graves. Only Pěrestrojka and Glasnosť enabled investigation of the case. Even after 73 years, it was possible to identify the remains using DNA profiling and dismiss alternative tsar family fate scenarios and attempts to verify false princesses.

Acknowledgment

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Applications of molecular combing of single DNA molecules

Dušana Majera[1] *and Martin Mistrík*[1]

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Abstract

Molecular combing is a very powerful method used to visualize the DNA at the single molecule level. It simultaneously stretches and assembles millions of DNA molecules. It is one of the most frequent tool to study DNA replication. In this assay consecutive pulse labelling with two thymidine analogues, iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU), permits measurement of the speed of individual forks and determination of their frequency of stalling, as well as identification of initiation and termination events. DNA fiber analysis is therefore the method of choice to accurately monitor replication and has been successfully used in a variety of organisms. It can also be used to characterize the methylation pattern on single DNA fibers at high resolution. This can be achieved by employing combing of genomic DNA with immunodetection of DNA methylation. This approach is termed epi-combing. The method is also compatible with fluorescence in situ hybridization (FISH), and it is known as fiber-FISH method. It allows the visualization of individual genes or other small DNA elements on chromosomes. We are currently optimising fiber-FISH method to detect ribosomal DNA (rDNA) genes. DNA fibers will be hybridized with human rDNA fragments labelled with biotin and images will be acquired using fluorescent microscope.

Acknowledgment

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Pavla Vašicová, Ph.D.

CBD attenuates the efficacy of platinum-based chemotherapy

Tereza Buchtová[1], Katarína Chromá[1], Martin Mistrík[1], Jiří Bártek[1,2,3]

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Abstract

Cannabidiol (CBD) is a non-psychoactive member of the cannabinoid family presented in the Cannabis sp. plant. Its female buds or buds-derived products are often used by cancer patients to decrease harmful or unpleasant adverse effects of antineoplastic treatment and generally increase the quality of their life. Here we screened against anticancer drugs including cisplatin, carboplatin, and oxaliplatin, to look for potential co-treatment interactions on the cellular level. Importantly, platinum-based drugs are used to treat a wide variety of cancers. Here we show that concomitant treatment of CBD with cisplatin and carboplatin results in attenuated cell death and other treatment effects via an unknown mechanism. Interestingly, this phenomenon was not present in oxaliplatin-treated cells suggesting not only an additional mechanism of action of this particular platinum-based drug but also a drug of choice in the case of concomitant CBD usage.

Acknowledgment

IGA_LF_2021_030

Emetine's anti-DNA replication activity reflects proteosynthesis inhibition not targeting Okazaki fragment formation

Dávid Lukáč[1] *and Pavel Moudrý*[1]

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Abstract

Emetine is a natural product alkaloid, many decades known as a specific chemical inhibitor of Okazaki fragments synthesis. Inhibition of lagging strand can cause replication stress, which is considered as a source of genome instability. One of the markers of the replication stress is formation of single-stranded DNA (ssDNA). Accumulation of ssDNA can occur on either leading or lagging strand by uncoupling the polymerases or by unregulated unwinding by helicase exposing the strands. In this work we propose the idea that DNA replication inhibition by emetine is not caused by strand uncoupling but rather with protein synthesis inhibition. Our in vitro studies focused on strand uncoupling markers after emetine exposure in comparison to adarotene, inhibitor of PolA. Our results revealed that emetine does not cause ssDNA accumulation followed by chromatin bound RPA32 loading and activating DNA damage response pathway. In line with this, emetine did not activate replication checkpoint. Moreover, inhibition of protein synthesis precedes inhibition of DNA replication after treatment with emetine. Collectively, we showed that emetine completely blocks DNA replication and should not be used as lagging strand inhibitor.

Acknowledgment

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Mechanism of MCOPPB senolytic activity

Matthew Lacey[1], Marin Mistrík[1] and Lucie Béresová[1]

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Abstract

Cellular senescence, a stable form of cell cycle arrest, has a complex role within an organism. It is both a natural process, with an increasing number of senescent cells accumulating within an individual as they age, and a driving factor in a number of age-related degenerative diseases. Due to their connection with the deleterious aspects of aging, there is significant interest in senescent cells as treatment targets.

A possible therapeutic avenue exists in the form of compounds which are preferentially lethal to senescent cells, known as senolytics. MCOPPB is an agonist of opioid receptors, primarily the nociception receptor OPRL1, which was recently identified to have senolytic activity. The mechanism of action for MCOPPB senolytic activity, suspected to be via the OPRL1 receptor, is the subject of this research.

Acknowledgment

This work was financially supported by the grant IGA_LF_2021_030.

Discovery of novel biologically active compounds and their molecular target

Jiří Řehulka[1]

[2] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic*

Abstract

In the talk will be presented current state of projects focused on new analogues of estradiol, pyrroloquinolin and betulinic acid. In addition to the published results, there will be discussed the importance of a multidisciplinary collaboration that enables rapid mode of action elucidation and drug design based on structure activity-relationship.

Acknowledgment

This study was supported by the European Regional Development Fund (Project ENOCH No. CZ.02.1.01 /0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, and CZ-OPENSCREEN, LM2018130), and IGA_LF_2021_038 (Palacky University in Olomouc).

Cytotoxicity profiling of new chemical compounds in HTS facility

Soňa Gurská[1], Lenka Lachnitová[1], Petr Džubák[1] and Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

High-throughput screening (HTS) is widely used in the field of pharmaceuticals and academic institutes as a primary tool for early-stage drug discovery. This technique was developed to evaluate the biological activity of thousands of individual small molecules and to identify potential drug candidates in a short time. One of the methods routinely used in our HTS laboratory is in vitro cytotoxicity screening as in vitro cytotoxicity testing has become an essential aspect of drug discovery. It is a convenient, phenotypic and predictive mean of characterizing the toxic potential of new chemical entities. The MTS assay as a cytotoxicity test was validated on 10 cell lines (8 cancer cell lines and 2 non-cancer cell lines) in 384 and 1536 well plate format.

In the primary screen, all compounds were tested at one concentration (50 μM) and the PI (percentage of inhibition) value was calculated. To calculate IC50 values for selected active compounds (PI ≥ 50%), a secondary (dose-response) screen was performed. Data were analyzed by Dotmatics software. To quantify the suitability of cytotoxic assay in HTS, the Z-factor was determined for each plate and cell line. Some results obtained in the cytotoxicity testing will be presented and discussed.

Acknowledgment

Study was supported by grants: This study was supported by the the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, and CZ-OPENSCREEN, LM2018130), and the IGA_LF_2021_038 (Palacky University in Olomouc).

Synthesis of betulinic acid derivatives with aryl moiety via Suzuki-Myiaura cross-coupling reaction and their biological evaluation

Barbora Lišková[1], Lucie Borková[1,2], Barbora Vránová[2], Soňa Gurská[1], Ivo Frydrych[1], Marián Hajdúch[1] and Milan Urban[1]

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[2] *Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, Czech Republic*

Abstract

Betulinic acid is one of the most investigated pentacyclic triterpenes because some of its derivatives are selectively cytotoxic against cancer cells, another serve as anti-HIV or antiprotozoal agents. Conjugates of cytotoxic derivatives of betulinic acid have been used to reach all three goals – to obtain compounds of high activity, high bioavailability, and to study their mechanism of action. All compounds prepared in this study were tested in vitro for their cytotoxic activity by standard MTS assay. Compounds with m-aminophenyl, m-hydroxyphenyl or p-hydroxyphenyl substituent in the position 3 were the most active compounds with cytotoxicity in the range 0.69 – 3.9 μM on CCRF-CEM cell line. Also, these compounds had a cytotoxicity of less than 10 M against the CEM-DNR and K562-TAX resistant cell line.

Knowledge of the ADME properties of semisynthetic triterpenes can be decisive for the selection of leading candidate(s) in our drug discovery program. Potential candidates showed sufficient stability in hepatic microsomes with low category of intrinsic clearance and low ability to diffuse through an artificial cellular membrane in PAMPA. The compounds with m-hydroxyphenyl substituent demonstrated good stability in human plasma and showed a small improvement in cellular permeability in our PAMPA model and cell permeability assay: Caco-2 and MDCK-MDR1 cell lines.
We have more answers for finding which type of the substituent in which position (o, m, or p) gives better result.

Acknowledgment

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Bystin regulates AURORA A during mitosis

Zuzana Macečková[1], Ivo Frydrych[1], Tomáš Oždian[1] and Marián Hajdúch[1]

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Abstract

Nucleolus is multi componential structure in nucleus where assembly of ribosomes takes place. Furthermore, because whole proteosynthesis process is energetically challenging, nucleolus also executing stress sensor function. For that purpose, many structural proteins of nucleolus have advanced objective beyond building block of nucleolus. From oncogene and oncosupersor regulation to control of metabolism. Furthermore, some structural proteins have been reported to regulate mitosis in different steps of division.
Here we present that Bystin is another structural protein of nucleolus regulating mitosis. More specifically, we have noticed that BYSL trans locates to centrioles during mitosis which indicate its role in cell division. Furthermore, to explore its role we performed MS after taxol treatment to elucidate BYSL binding partners during mitosis. This experiment show that BYSL binds AURORA A during mitosis. Further study revealed, that BYSL is necessary for proper mitosis transit and that knock down od BYSL results in slight block in mitosis leading to accumulation of polyploid cells, all without increased apoptosis or changes in cell cycle. This observation indicated that BYSL is necessary for proper AURORA A p288 phosphorylation, which was confirmed by westernblot.

Acknowledgment

The project was financially supported by grants IGA LF UP 2021_019, IGA_LF_2019_003, AZV 15-29021A and ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868.

Exploring protein-protein interactions of bystin using HADDOCK

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Abstract

Bystin is a compact protein that was shown to play an important role in pathogenesis of various diseases. In our previous studies we explored the bystin interaction with c-MYC protein. However, experimental studies suggested an alternative pathways, in which bystin gets included.
In this study we employed the state-of-art software for protein-protein docking software HADDOCK[1]. We explored bystin interactions with two potential protein partners – Aurora A and mTOR proteins. Analysis of the resulting docking scores and poses allowed us to better understand the details of molecular mechanisms. Best obtained poses were analyzed for interactions between proteins. This study will further help to explore and understand mechanism of actions of potential bystin-binding ligands able to influence the explored protein-protein interactions.

Citation

[1.] Cyril Dominguez, Rolf Boelens and Alexandre M.J.J. Bonvin. HADDOCK: a protein-protein docking approach based on biochemical and/or biophysical information. J. Am. Chem. Soc. 125, 1731-1737 (2003).

Lung cancer and COPD protein biomarkers found in exhaled breath condensate

Jana Václavková[1], Petr Džubák[1], Jana Vrbková[1], Pavla Kouřilová[1], Dušan Holub[1], Juraj Kultan[2], Petr Jakubec[2], Ondřej Fisher[2], Vítězslav Kolek[2], František Kopřiva[3], Tatiana Gvozdiaková[3], Vendula Látalová[3] and Marián Hajdúch[1]

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Abstract

The respiratory tract that serves to ensure gas exchange can be a rich source of biomarkers for diagnosis and monitoring of respiratory disease progression. The airway epithelium is an incredibly active layer of cells involved in body defense. The mucous membranes of the respiratory tract are naturally moistened with fluids containing proteins, metabolites cytokines, chemoattractants, and growth factors which are released by the highly active epithelium. Collection of the fluids from the respiratory tract can be used to diagnose the respiratory tract malignant and non-malignant diseases. Direct tissue analysis, which is used nowadays, is frequently coupled with invasive examination methods like bronchoscopy which are not pleasant for the patients. Therefore, intensive research work is conducted to develop non-invasive methods for biomarker detection. We selected an exhaled breath condensate to be further studied as a promising matrix that is collected non-invasively and is suitable for detecting biomarkers of various respiratory and systemic diseases. We have developed a gel-free mass spectrometry based approach for in-depth characterization of the EBC proteome with high reproducibility. Our powerful method led to identification of low thousands of proteins across whole patient cohort consisting of 296 individuals. Using multivariate statistical analysis, the biomarkers of lung cancer, on the background of COPD, were suggested.

Acknowledgment

This work was supported by European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2021_038).

Citation

Mutlu GM, Garey KW, Robbins RA, Danziger LH, Rubinstein I. Collection and analysis of exhaled breath condensate in humans. Am J Respir Crit Care Med. 2001; 164(5):731-7.

López-Sánchez LM, Jurado-Gámez B, Feu-Collado N, Valverde A, Cañas A, Fernández-Rueda JL, Aranda E, Rodríguez-Ariza A. Exhaled breath condensate biomarkers for the early diagnosis of lung cancer using proteomics. Am J Physiol Lung Cell Mol Physiol. 2017 Oct 1;313(4):L664-L676. doi: 10.1152/ajplung.00119.2017. Epub 2017 Jun 15.

Proteomic biomarkers of endometrial receptivity in ClinData

Radovan Pilka[2], Jiří Dostál[2], Jan Vodička[2], Kateřina Smékalová[2], Branislav Šiška[1], Tomáš Oždian[1], Petr Džubák[1], Jana Václavková[1], Petr Pavliš[1], Marian Hajdúch[1], Igor Crha[3], Pavel Ventruba[3], Jana Žáková[3], Michal Ješeta[3] and Barbora Kočvarová[4]

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[3] *Department of Obstetrics and Gynecology, University Hospital Brno, Czech Republic*
[3] *Palacky University Press, Olomouc, Czech Republic*

Abstract

Endometrial receptivity (ER) is the sensitivity of the uterine cavity mucosa for implantation of the embryo. ER is currently investigated by ivasive methods directly in the endometrium morphologically, by genomics and proteomics and in the secretions obtained from the uterine cavity by genomics, proteomics and lipidomics.

The uterine cervix is filled with cervical mucus, which reflects hormonal changes during the menstrual cycle. For this reason, a correlation between the development of the endometrium and the molecular-biological profile of the cervical mucus can be expected.
The goal of our proposed project is to identify biomarkers of ER by proteomic examination of cervical mucus during the treatment of infertility by in vitro fertilization and embryo transfer, with or without making intracytoplasmic sperm injection.

This study will require stewardship of large number of clinical and analytical data. For a purpose of progressive data collection we decided to use ClinData. This software is suitable for our multicenter study by running on a server, a user is connected through a web browser, it is flexible and secure.

Acknowledgment

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The proteomic characterization of cervical mucus

*Tomáš Ožd*ian1*, Dostál J.*[2]*, Slavkovský, R.*[1]*, Holub, D.*[1]*, Hamerníková, B.*[1]*, Vodička, J.*[2]*, Ješeta, M.3, Pilka, R.*[2]*, Hajdúch, M.*[1] *and Džubák, P.*[1]

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[3] *Center of Assisted Reproduction, Brno, Czech Republic*

Abstract

Cervical mucus is a viscous fluid produced by cervical glands located in the myometrium of the uterine cervix. During ovulation, cervical mucus starts to be less viscous, which is a good window for non-invasive sampling. This study focuses on the proteomic characterization of cervical mucus. This mucus may thus act as a non-invasive source of biomarkers of the female genital tract. Proteomic characterization of cervical mucus was therefore focused on two main goals – to optimize a protocol leading to the best identification score possible and to characterize and localize the source of the identified proteins. The optimization of proteomic workflow resulted in using stimulated IVF cycles for monitoring the window of sample aspiration, multi-enzyme proteomic digestion and protein analysis at Orbitrap mass spectrometer. The bioinformatics analysis revealed the composition of cervical mucus. The most intense proteins were extracellular mucins and other extracellular proteins; however, most identified proteins were of intracellular origin. The second and more challenging analysis was to search tissues of the source of proteins present in the cervical mucus. This was done using Human Protein Atlas tissue expression data. In tissues of interest, cervix uterine, endometrium, fallopian tubes, ovary and vagina, we have found approximately 2% of proteins specific to particular tissues, 73% of proteins with middle expression and 25% of proteins not expressed at all. Proteins, which were not expressed in tissues of interest were expressed mainly in the liver. This study characterizes the proteome of cervical mucus and confirms its suitability for biomarker study. This characterization is the most extensive in current literature according to our best knowledge.

Acknowledgment

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Human plasma analysis by HR-MS: An approach for plasma profiling of large population-based cohort

Jarmila Stanková[1], *Jana Václavková*[1], *Jana Vrbková*[1], *Dušan Holub*[1], *Josef Srovnal*[1], *Petr Džubák*[1] *and Marián Hajdúch*[1]

[1]*Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic*

Abstract

Human blood is a rich and easy to come by source of information about individual's health state. Protein profiles obtained from blood components such as plasma or serum can be further used for various purposes. Mass spectrometry-based proteomics has become a powerful tool in a chase for specific and quantitative readout of the plasma proteome. Current workflows allow characterization of large cohorts with preserved proteome depth. A challenging point of studies especially aimed to sample large cohorts is quality assurance. We present a complete methodology for human plasma analysis by HR-MS with emphasis on the quality of obtained data. Czech multi-omics cohort including 127 plasma samples was proceeding with Filter-Aided Sample Preparation protocol, peptides were purified on home-made C18 StageTips or OMIX tips (Agilent Technologies) and measured by LC-MS with HCD fragmentation and precursor, fragment mass detection by Orbitrap. During LC-MS analysis, spiked-in standards were used to follow a quality across the multiple runs. Data were searched by ProteomeDiscoverer 2.5 (Thermo) including the INFERYS node. We provide a standard operation protocol which could be used for prospective studies.

Acknowledgment

Supported by Ministry of Health of the Czech Republic, grant nr. NV18-08-00291A.

Citation

Philipp E Geyer et al Mol Syst Biol. (2017) 13: 942
Philipp E Geyer et al EMBO Mol Med (2019) 11:e10427

Anchored linear oligonucleotides: The effective tool for the real-time measurement of uracil DNA glycosylase activity

Karel Koberna[1], Anna Ligasová[1], Ivan Rosenberg[2], Markéta Bocková[3], Jiří Homola[3] and Karel Koberna[1]

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Abstract

Base excision repair is one of the important DNA repair mechanisms in cells. The fundamental role in this complex process is played by DNA glycosylases. Here we present the novel approach for the real-time measurement of uracil DNA glycosylase activity, which employs selected oligonucleotides immobilized on the surface of magnetic nanoparticles and Förster resonance energy transfer. We also showed that the approach can be performed by surface plasmon resonance sensor technology. The developed method is suitable for the determination of enzyme activity both in defined solutions and in cellular lysates.

We also showed using the developed approach that the glycosylase activity is markedly higher in human cancer cells as compared to diploid cells. Our data further indicated that glycosylase activity and UNG level are decreased after the drug-induced senescence of HeLa cells. Surprisingly, only a very slight decrease of glycosylase activity and even increase of UNG2 level was observed in senescent human fibroblasts. Finally, our data showed that the 5-bromo-2'-deoxyuridine, a modified mucleoside inducing replicative senescence, is removed neither by bacterial UNG nor by DNA glycosylases in nuclear lysates under conditions when uracil is efficiently excised.

Acknowledgment

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Radiolabelled siderophores for the diagnosis of Burkholderia cepacia complex infections

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Abstract

Burkholderia cepacia complex (BCC) is a group of bacteria, that is especially dangerous for immunocompromised patients. Due to the broad resistance of BCC to antibiotics, it is particularly important to diagnose the pathogen quickly and initiate treatment. In this study, we present the use of various radiolabelled siderophores, for specific imaging of Burkholderia infection by means of positron emission tomography (PET).

A total of 11 microbial siderophores were labelled with gallium-68 with high radiochemical purity and in vitro uptake in BCC was tested. Overall, the most promising results were achieved with gallium-68 labelled Ornibactin, which is a siderophore specifically produced by BCC. 68Ga-Ornibactin showed favorable stability in human plasma and in a solution with neutral pH, demonstrated low protein binding values and hydrophilic properties. It achieved high uptake in bacteria from BCC and low uptake in majority of other tested respiratory pathogens. In healthy mice, it displayed optimal pharmacokinetics with rapid urinary excretion and in vivo accumulation in the site of infection in mouse BCC infection model. Thus, 68Ga-Ornibactin seems to be a promising tracer for BCC infection imaging.

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Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer

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Abstract

Introduction: One of the targets in fight with the prostate cancer is so called prostate specific membrane antigen (PSMA). Specific proteins (such as PSMA-11) binding PSMA could be radiolabeled and used as diagnostic or therapeutic radiopharmaceuticals. Current approach in this field is to employ alpha emitters; such approach is called target alpha therapy (TAT). This study presents results of the biological evaluation of four new 225Ac-labeled PSMA ligands for TAT.

Methods: The compounds (FR54, FR55, FR94 and FR96) were tested in mice model using human prostate cell line LNCaP. Ex vivo biodistribution was evaluated in various time points (1, 4, 24, 48, 72 and 120 h p.i.) by dissecting the animals and measuring radioactivity in 12 different organs. The liver, kidneys and tumors were then examined by means of histology (H&E staining, PSMA, gamma H2AX and Ki67).

Results: The principal organs accumulating tested PSMA ligands were tumor and kidneys with vastly higher uptake compared to other evaluated organs (up to 120 %ID/g). Tumor-to-blood ratio was 1 490 in case of FR94. Histology showed necrotic lesions in the tumors, high PSMA expression in tumor tissue, DNA damage in FR94/96 treated tumors and higher cellular proliferation in untreated tumors. Conclusion: Biodistribution study revealed favorable biodistribution of all tested PSMA ligands with extremely high tumor-to-blood ratios. The histology confirmed promising properties of these new potential TAT compounds.

Acknowledgment

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Tau aggregation and seeding as a therapeutic target in tauopathies

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Abstract

Accumulation of tau aggregates in the brain is a neuropathological hallmark of tauopathies. Dysfunction in signaling pathways that regulate tau phosphorylation results in the tau hyperphosphorylation, misfolding and aggregation that contribute to the neurodegeneration in tauopathies. 'Prion-like seeding' is defined by the propagation of misfolded tau aggregates between cells, across synapses and within brain networks that drive the progression of tauopathies. Therefore, targeting tau aggregation and seeding is a potential therapeutic strategy to prevent pathological tau-mediated neurodegeneration and to limit the progression of tauopathies into advanced stages. With this goal in mind, we have established the in vitro tau aggregation assay, cell models of tau phosphorylation and aggregation to study the molecular mechanisms of tau aggregation and seeding and also for the identification of potential tau aggregation inhibitors.

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Diamonds Are Forever: Development and application of ultra-bright 200-nm fluorescent nanodiamonds for the sentinel lymph nodes visualisation

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Abstract

In the past decade, the sentinel lymph node(s) mapping became standard procedure used in cancer diagnostics. However, agents commonly used in these applications, mostly molecular dyes and radiotracers, still have several disadvantages. Fluorescent nanoprobes currently show the most promising results as potential alternatives. Fluorescent nanodiamond (FND) is a biocompatible material which exhibit unique optical properties such as emission maximum of approximately 700 nm and resistance towards photobleaching. These properties make FND a promising candidate for bioimaging applications.

Narrow size-distributed (~200-nm), electron irradiated and colloidally stable FNDs were prepared. We developed modification of polymer periphery leading to a polyvalent display of mannose on the particle interface. In vitro experiments showed no cytotoxicity and confirmed positive effect of polyvalent mannosylation on FNDs uptake. We detected significant difference between uptake of non-mannosylated (FND-p) and mannosylated (FND-p-Man) in J774A.1 cells due to mannosylation confirmed by competitive and blocking assays. The retention of FNDs is ligand specific and it is caused by selective targeting to CD206 receptor. We also demonstrated that mannose interface has a beneficial effect on the retention in vivo in mice popliteal lymph nodes. Based on the results obtained, we believe that FND-p-Man could be a promising tracer for endoscopic/robotic fluoresce-guided surgery in biomedical applications.

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High-throughput screening of chemical compound libraries in Nematodes

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Abstract

Nematodes, also known as roundworms, are a diverse animal phylum from the ecdysozoa clade. They inhabit a wide spectrum of environments and ecological niches ranging from free-living Caenorhabditis elegans to parasites, such as Haemonchus contortus, Teladorsagia circumcincta and Ditylenchus destructor. All of the afore mentioned organisms have their place in research.

While screening chemical compounds on cell lines provides invaluable information regarding toxicity and primary effect of the compound, it doesn't reveal how it may react in a diverse organism. C. elegans provides a convenient stepping stone between testing chemical compounds on cell lines and mice. Because of its fortunate phylogenic placement, it has most organ systems analogous to mammals, short developmental cycle and relatively simple cultivation. Due to these factors C. elegans has been extensively researched and used as a model organism.

The three previously mentioned parasites significantly contribute to economic losses in agriculture. To complicate things these parasites often quickly develop resistances to commonly used antihelmentics. This incentivizes development of new effective antihelmentic compounds.

Taking both mentioned points into consideration a new system of screening chemical compounds in nematodes is required. We have managed to design a robust system of measuring and analyzing a large scale of chemical compounds for antihelmentic activity. To achieve this, we employ the use of automated microscopy and neural network-based analysis. Our work will further focus on optimizing and upscaling said system.

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Adaptative memory cell responses against SARS-CoV-2 virus

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Abstract

We evaluated samples from 60 adult individuals (30 from CR and 30 from Venezuela) who were: 1) not exposed to the virus (according to their knowledge), 2) suffered the infection, 3) were vaccinated and no Covid, 4) vaccinated and Covid. We monitored, CD154/IFNg (CD4 memory), CD8/CD107a (CD8 memory), CD79a (memory B cells), CD314 (NKG2D). Whole blood, 0.5 ml, viral peptides or 10 pfu of inactivated virus, and incubated for 18 hr. Positiveness was considered when the values were> 2 % as compared to the negative control. In young non-vaccinated individuals, good memory responses 30 % CD8, 20 % CD4, and 20 % B. Positive correlation CD314 memory CD8 response. Post-Covid, n=10, all memory responses were low in 3 patients even after 60 days after infection. The vaccinated group no Covid: good memory responses CD4 85 %, CD8 70 % of the individuals, B cell 50 %. A subgroup (10) compensated memory response with NK or NKT cells. CD314 decreased with time after vaccination, but CD8 memory did not fall as fast as CD4. Two individuals did not have good CD8 responses. Post-Covid and vaccinated n=4, low normal CD4 memory and low CD8 and B cell memory responses, $p < 0.01$ as compared to the vaccinated ones. Conclusions: Memory CD4 and CD8 responses can be efficiently achieved by vaccination in most cases, and protective memory responses can be observed in non-vaccinated populations. Memory responses post-Covid were poor, and vaccination may partially actívate immune cell memory.

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Citation

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Anti-SARS-CoV-2 properties of one anti-inflammatory drug

Ermin Schadich[1], Juan Bautista De Sanctis[1], Tomáš Ožd'an[1], Hana Jaworek[1], Petr Džubák[1] and Marián Hajdúch[1]

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) that spreads rapidly globally. In this study, one selected anti-inflammatory drug was tested for anti-SARS-CoV-2 activity and effects on ceramide profiles of SARS-CoV-2 infected Vero cells. In antiviral assay, Vero cells were infected by SARS-CoV2 at M.O.I of 0.02 and treated by the anti-inflammatory drug and reference drug remdesivir for 72 h. Infected and non-infected control cells were treated by corresponding concentrations of DMSO. The level of viral infection was monitored by measuring cytopathic effects and viral load using MTT assay and qRT-PCR, respectively. In separate assays, Vero cells were infected by SARS-Cov2 and treated by the anti-inflammatory drug. Their total lipids were extracted and used for mass-spectrometry analysis of ceramide profiles. The ceramide profiles of treated cells were compared with those of infected and non-infected control cells. The existing anti-inflammatory drug inhibited the SARS-Cov2 infection of Vero cells. Its C50 against SARS-Cov2 virus was 4.50 μM. It also modulated the distinct ceramide profiles of the SARS-Cov2 infected Vero cells. The existing anti-inflammatory drug has the marked property to inhibit SARS-Cov2 in infected Vero cells. It also has property to modulate the ceramide profiles of the SARS-Cov2 infected Vero cells, and this is intriguing as such property might preclude the overrated inflammatory responses.

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In silico design of synthetically feasible compounds

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Abstract

The chemical universe is extremely large that makes systematic enumeration of compounds and their virtual screening not efficient. De novo design proposes a reasonable alternative. These approaches adaptively explore chemical space by generating compounds which satisfy given activity/property constrains. The main issue of computationally generated compounds is their synthetic accessibility. Reaction-based generation approaches explicitly address this issue, but they have limited coverage of chemical space and less flexibility in making moves within this space. Fragment-based approaches provide greater flexibility to structural modifications (growing, mutation or linking of molecules) but suffer from difficulties to control synthetic feasibility of generated compounds. We developed and implemented the framework of chemically reasonable mutations (CReM) which makes structural changes taking into account chemical context of fragments. This results in always chemically valid structures and greatly increases control over synthetic feasibility of generated compounds. We will compare CReM with other approaches and demonstrate its applicability to i) enumeration of analog series for exploration of local SAR, ii) scaffold decoration, iii) lead optimization and iv) de novo design.

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Mining structural patterns relevant for anticancer activity of small molecules

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Abstract

Anticancer drug design is a challenging task. One way to approach it is to analyse molecules that show anticancer activity together with molecules that don't, and try to find patterns that cause the difference. These patterns may look like atoms, fragments or scaffolds critical for the activity. They can be used to synthesize new potential drugs. We searched for those patterns in this study using QSAR and docking. The results revealed known patterns, for instance, CDK inhibitor scaffold, along with several new potential ones. We found that thiophene linked with pyrrolopyrimidine moiety was a characteristic pattern of K562 cell line growth inhibition. Docking modelling helped to establish likely targets and screen out unlikely, namely, among cancer-related protein kinases it strongly suggested Abl and C-KIT. For other patterns we could not suggest targets yet; there are more studies needed, most importantly, experimental.

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The pharmacophore-based de novo design method

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Abstract

According to the estimated calculations, it is possible to generate up to 1060 small molecules in total. This means that the chemical space is huge, and it contains molecules that have not been studied. It is assumed that there are small molecules in the world that can be ideal drugs – to cause the desired therapeutic effect with a small dose, without causing side effects. However, this chemical space is so large that the implementation of virtual screening of all molecules is an impossible task in our time. Therefore, chemoinformatics set aside the idea of virtual screening of the entire chemical space in favor of generating a part of the chemical space of molecules with the desired properties. This branch in chemoinformatics is called de novo design.

Two projects on de novo design are being implemented in our laboratory. In the cream-pharm project, the generation of structures is carried out on the basis of fragments, which generates molecules containing a given pharmacophore. The idea is that the molecules containing the pharmacophore pattern are potentially active molecules. The peculiarity of this project is that it takes into account the local context of fragments when selecting molecular pairs, that is, the tool determines interchangeable fragments. This allows to create synthetically accessible molecules. Diversity of generated molecules will directly depend on the input molecules or the fragment database. Another approach is the deep learning model – an autoencoder. The autoencoder encodes pharmacophores into a latent space and generates 2D structures of molecules from this latent space. To do this, the autoencoder learns latent features of molecules by their pharmacophores. This approach does not use a particular fragment database that's why the generated molecule space can be larger.

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Lead optimization of the tubulin inhibitor

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Abstract

Tubulin inhibitors which binds in the colchicine's site inhibit microtubule formation and mitosis progression what is useful as an anticancer therapy. Some perspective highly-specific and effective inhibitors were found in the IMTM compounds library (IC50 ~0.03-0.09 mM for some cancer lines against of >50 mM for normal human fibroblasts). In this study in silico optimization of the lead was performed to improve affinity and lipophilicity. Part of the received in silico results were experimentally validated and discussed.

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Microthermal-induced subcellular protein damage reveals a two-phase HSP70-p97 cellular response

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Abstract

Despite proteotoxic stress and heat shock response are implicated in diverse pathologies, currently no methodology to inflict defined, subcellular thermal damage exists. Recently, we developed such a single-cell method compatible with laser-scanning microscopes, adopting the plasmon resonance principle. Dose-defined heat causes protein damage in subcellular compartments, rapid heat-shock chaperone recruitment, and ensuing engagement of the ubiquitin-proteasome system, providing unprecedented insights into spatiotemporal response to protein damage relevant for degenerative diseases. Using this versatile method, we discovered so-far unsuspected involvement of p97(VCP) translocase in the processing of heat-damaged proteins and its compensatory interplay with HSP70 chaperone during the two-wave cellular response.

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The Circulating Tumor Cells detection in Solid Tumors using CytoTrack TM11

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Abstract

The circulating tumor cells (CTCs) are tumor cells released into the peripheral blood able to migrate to distant organs. The CTCs presence is thought to be related to the tumor dissemination and recurrence. The CTCs detection using a liquid biopsy per se seems to be a very helpful noninvasive diagnostic tool. It can reveal the tumor dissemination in early stages of the disease and can improve the prognosis and therapeutic decision making. In the recent years, many different approaches for the CTCs detection have evolved more or less overcoming obstacles such as CTCs heterogeneity, rarity or cell phenotype changes due to the epithelial to mesenchymal transition. Here, we demonstrate the CTCs detection using CytoTrack device based on the EpCAM immunofluorescence and preenrichment independent CTCs capture from the whole blood samples. Following our previous blood collecting tubes validation we have focused on the CTCs stability, detection specifity and subsequent clinically relevant implementation.

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Deep amplicon sequencing of predictive markers for fast and effective tumor diagnostics

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Abstract

CuET is a copper-containing disulfiram metabolite with anticancer activity. Screening for chemical modulators of CuET activity identified cannabidiol (CBD), belonging to the cannabinoid family, as a compound significantly decreasing the cytotoxic effects of CuET. Cannabinoids are often used as supportive therapy by oncological patients. Thus, such information by itself is of high importance as concomitant CBD/CuET treatment can negatively affect the outcome.

Mechanistic insight into this rescuing effect revealed that CBD does not alter the CuET uptake. Instead, it increases the transcription of genes involved in the metallothionein pathway. Metallothioneins are proteins involved in many cellular processes, including homeostasis of biogenic metals. A very high affinity of metallothioneins for metals or metal-containing substances also protects the cell from the negative impact of heavy metals. Here we show that the overexpression of metallothioneins such as MT2A, which the CBD treatment increases levels, reduces the toxic effect of CuET significantly. Vice versa, inhibition of metallothionein pathway renders the cells hypersensitive to CuET.

Acknowledgment

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CRISPR/Cas9 technology - creating cellular models for human genetic disorders

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated enzyme (Cas) is a naturally occurring genome editing tool adopted from the prokaryotic adaptive immune system. Nowadays is this robust genome editing tool frequently used to create genetic models for studying and treating human genetic disorders, particularly diseases associated with point mutations. This versatile technology offers also another utilization. Just small modifications of this tool enabled us to regulate the transcription of many genes in just one experiment. For this application Cas9 enzyme was modified in two aspects. Firstly, the active site of this nuclease was mutated in such a way to gain inactive enzyme. Secondly, an adaptor was linked to dCas9 for enhancing transcription modulation activity. dCas9 is programmed with a guide RNA (gRNA) that directs it to a DNA locus of interest via sequence complementarity. This can be used for the elucidation of the mechanism of action of small molecules with therapeutic potential. We will conduct a primary genome-wide screen using an ultra-complex gRNA library. Fraction of the infected cell population will be subjected to a selection in the presence of a particular drug. The frequencies of gRNA-encoding cassettes in the selected population and an unselected control population will be determined by deep sequencing. From these data, hit genes and gRNAs that effectively target them will be identified.

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Clonal somatic variants in hematopoietic cells in relation to age and stroke

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Abstract

Introduction: It was recently discovered that one of the hallmarks of aging is the accumulation of clonal variants within the cells of the hematopoietic system without the presence of malignant transformation. This phenomenon is also known as clonal hematopoiesis of indeterminate potential (CHIP). Interestingly, the presence of CHIP correlates with the risk of cardiovascular system diseases.

Materials/Methods: In this study, we are detecting somatic mutations of blood cells in 4 cohorts of patients aged >65 years (presence/absence of carotid atherosclerosis or stroke). Samples of patients were compared with a control group of elderly people (>85 years) and healthy donors (<30 years). In 8 patients, DNA was isolated also from carotid plaques. All CHIP mutations were identified by the method of massive parallel sequencing using a targeted DNA custom panel (Qiagen) containing 38 CHIP-related genes.

Results and conclusions: It was shown that ~75 % of all patients (n=104) are positive, with mutations observed most often in genes DNMT3A and TET2 (~50 %), as expected. In the control group of elderly people (n=24), 96 % of individuals were positive and no mutations were detected in young donors (n=24). The presence of CHIP mutations was also confirmed in patient samples of plaques. As a next step, a mutational analysis will be performed on endothelial progenitor cells isolated from blood by FACS. A panel of antibodies for EPC sorting was designed based on the recent literature.

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