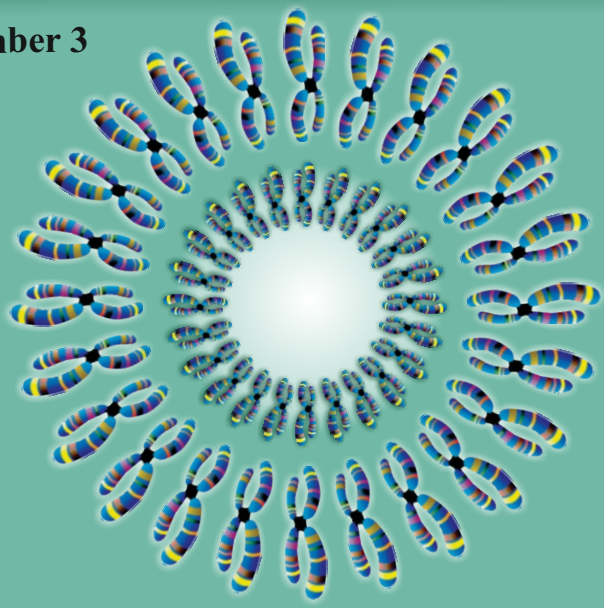
UNIVERSITATEA „ALEXANDRU IOAN CUZA” DIN IAȘI

JOURNAL OF EXPERIMENTAL AND MOLECULAR BIOLOGY

Tome XX, Number 3
2019

ISSN 2601 – 6974
ISSN-L 2601 - 6974



Editura Universității „Alexandru Ioan Cuza” din Iași

UNIVERSITATEA „ALEXANDRU IOAN CUZA”
DIN IAȘI

JOURNAL OF EXPERIMENTAL AND MOLECULAR BIOLOGY

TOME XX, Number 3 **2019**

Editura Universității „ALEXANDRU IOAN CUZA” din Iași

EDITORIAL OFFICE

Universitatea „Alexandru Ioan Cuza" din Iași, Facultatea de BIOLOGIE
Laboratorul de Biochimie și Biologie Moleculară
Bulevardul Carol I, Nr. 20A, 700506, Iași, România
www.jemb.bio.uaic.ro / gbmpapers@yahoo.com

Current volume gathers the abstracts of oral presentations and posters from

FACULTY of BIOLOGY · FACULTY of CHEMISTRY · ALEXANDRU IOAN CUZA UNIVERSITY of IAȘI · ROMANIAN SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY · Regional Institute of Oncology Iași

ANNUAL INTERNATIONAL CONFERENCE of the ROMANIAN SOCIETY of BIOCHEMISTRY and MOLECULAR BIOLOGY Iași, 26-27th of September 2019

which will be held according to the following program

Thursday, 26.09.2019				
8:00 – 9:00	**Registration, The** Hall of the Echoing Footsteps, Alexandru Ioan Cuza University of Iasi, Building A, Bulevardul Carol I, Nr.11, 700506, Iași, România			
9:00 – 9:15	**Welcome Speech – Iasi or SRBBM / quick facts about the upcoming event,** Aula Magna „Mihai Eminescu" Alexandru Ioan Cuza University of Iasi, Building A, Bulevardul Carol I, Nr.11, 700506, Iași, România			
Section PB: **Proteins biochemistry: proteomics, structure and function**				
9:15 – 10:00	PB_O_06	Minh	David	ABSOLUTE BINDING FREE ENERGIES FROM BINDING FREE ENERGIES TO MULTIPLE RIGID RECEPTOR CONFORMATIONS
10:00 – 10:30	SFEG_O_02	Petrescu	Andrei-Jose	COMPUTATIONAL ASSISTED WORK IN STRUCTURAL IMMUNOBIOLOGY
10:30 – 10:50	PB_O_05	Spiridon	Laurentiu	GMOLMODEL - A METHOD TO OVERCOME CONFORMATIONAL SAMPLING HURDLES USING ROBOTICS
10:50 – 11:10	**Coffee Break**			
11:10 – 11:40	PB_O_04	Petrescu	Stefana-Maria	ENDOPLASMIC RETICULUM QUALITY CONTROL AND PROTEIN MISFOLDING
11:40 – 12:00	PB_O_01	Silaghi	Radu	REDOX REACTIVITY IN GLOBINS: MODULATION BY COVALENT AND NON-COVALENT MODIFICATIONS WITH BIOMEDICAL RELEVANCE
12:00 – 12:20	PB_O_07	Tu	Le	PROTEINS AND ENZYMES DYNAMICS IN DIFFERENT ENVIRONMENTS
12:30– 14:30	**Lunch Break and Poster session, posters PB_P; SFEG_P; BN_P**			
Section BN: **Bio and nanotechnologies for biomedical and environmental research**				
14:30 – 15:15	BN_O_04	Farcasanu	Ileana Cornelia	HEAVY METAL ACCUMULATION BY YEAST CELLS ARMED WITH METAL-BINDING PEPTIDES TARGETED TO THE INNER FACE OF PLASMA MEMBRANE

15:15 – 15:35	BN_O_01	Licarete	Emilia	TARGETING MELANOMA MICROENVIRONMENT WITH LIPOSOMAL PREDNISOLONE IMPROVED THE THERAPEUTIC OUTCOME OF LIPOSOMAL DOXORUBICIN
15:35 – 15:55	BN_O_02	Patras	Laura	DUAL ROLE OF CANCER-DERIVED EXTRACELLULAR VESICLES IN THE MODULATION OF TUMOR MICROENVIRONMENT
16:00 - 16:20	**Coffee Break**			
16:20 – 16:40	PB_O_03	Gradinaru	Vasile Robert	PRODUCTION AND CHARACTERIZATION OF A NEW FUNGAL EXTRACELLULAR LIPASE
16:40 - 17:00	BN_O_04	Trif	Mihaela	CHARACTERIZATION AND IN VITRO BIOCOMPATIBILITY EVALUATION OF POLYMER NANOPARTICLES POLY (D, L-LACTIDE-CO-GLYCOLIDE) (PLGA) -AS DRUG DELIVERY DEVICES
Section SFEG: **Structural, functional and evolutionary genomics**				
17:00 – 17:20	SFEG_O_01	Bunu	Gabriela	SYNERGISM AND ANTAGONISM IN AGING
17:20 – 17:40	SFEG_O_02	Crăciun	Adrian	IMPROVING THE ACCURACY AND REPRODUCIBILITY OF MICROBIOME MEASUREMENTS ACROSS LABS
19:00	**Gala dinner** – Panoramic Hotel Unirea, Piața Unirii nr. 5, Iași 700056			

Friday, 27.07.2019				
Section MCB: **Molecular and Cellular Biology, including medical applications**				
9:00 – 9:30	MCB_O_01	Zamfir	Alina	DEVELOPMENT OF ADVANCED MASS SPECTROMETRIC PLATFORMS FOR GLYCOCONJUGATE MAPPING, BIOMARKER DISCOVERY AND STUDY OF THEIR FUNCTIONAL INTERACTIONS
9:30 – 9:50	MCB_O_11	Chiritoiu	Marioara	GRASP55 REGULATES IRE1ACTIVITY WHICH IN MACROPHAGES CONTROLS INTERLEUKIN-1 SECRETION AND AGGREGATION
9:50 – 10:10	MCB_O_03	Rauca	Valentin-Florian	SHIFTING THE BALANCE TOWARDS AN ANTI-TUMORIGENIC MELANOMA MICROENVIRONMENT VIA CO-ADMINISTRATION OF LIPOSOME-ENCAPSULATED SIMVASTATIN AND DMXAA IN VIVO
10:10 – 10:30	MCB_O_06	Cruceriu	Daniel	THE DUAL ROLE OF TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN 3D BREAST CANCER CELL MIGRATION

10:30 - 11:00	**Coffee Break**			
11:00 – 11:30	MCB_O_07	Sima	Livia	ROLE OF TISSUE TRANSGLUTAMINASE IN ANTI-TUMOR IMMUNE RESPONSE
11:30 – 11:50	MCB_O_05	Popa	Ioana	HIGH-THROUGHPUT SCREENING TO IDENTIFY RAGE INHIBITORS FOR CANCER THERAPY
11:50 – 12:10	MCB_O_13	Jitaru	Daniela	TUMORICID POTENTIAL OF CATIONIC PEPTIDES TESTED ON TUMOR CELL LINES
12:10 - 12:30	PB_O_02	Mihasan	Marius	NICOTINE-INDUCED PROTEOME OF SURPRISINGLY USEFUL MICROORGANISM: PAENARTHAROBACTER NICOTINOVORANS PAO1
12:30 – 14:30	**Lunch Break and Poster session, posters MCB_P**			
14:30 – 14:50	MCB-O-14	Petre	Brindusa Alina	ENZYMATIC DIAGNOSIS OF LYSOSOMAL STORAGE DISORDERS USING SUBSTRATES FOR FLUORIMETRY AND MRM-MASS SPECTROMETRY
14:50 – 15:10	MCB_O_08	Stefan	Marius	NOVEL SYNTHETIC FLAVONOIDS WITH POTENT ANTIMICROBIAL PROPERTIES: A POTENTIAL SOLUTION TO FIGHT ANTIBIOTIC RESISTANCE
15:10 – 15:30	MCB_O_09	Banica	Alexandra-Maria	FUNCTIONAL INTERACTION BETWEEN THE TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL MEMBER 8 (TRPM8) AND THE PROSTACYCLIN RECEPTOR (PTGIR)
15:30 - 16:00	**Coffee Break**			
16:00 - 16:20	MCB_O_12	Boiangiu	Razvan Stefan	ANTI-ACETYLCHOLINESTERASE AND PRO-COGNITIVE PROFILE OF COTININE AND 6-HYDROXY-L-NICOTINE IN AN AMYLOID BETA 25-35-INDUCED RAT MODEL OF ALZHEIMER DISEASE
16:20 - 16:30	MCB_P_06	Orobeti	Stefana	OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS UNDER SIMULATED MICROGRAVITY
16:30 - 16:50	MCB_O_10	Popescu	Costin-Ioan	IDENTIFICATION OF PHOSPHOINOSITIDE METABOLIZING ENZYMES AS NEW HOST FACTORS INVOLVED IN HCV LIFE CYCLE
17:00 – 17:30	**Closing Ceremony,** Aula Magna „Mihai Eminescu" Alexandru Ioan Cuza University of Iasi, Building A, Bulevardul Carol I, Nr.11, 700506, Iași, România			

J. Exp. Molec. Biol. 2019, Tome XX, No 3

REDOX REACTIVITY IN GLOBINS: MODULATION BY COVALENT AND NON-COVALENT MODIFICATIONS WITH BIOMEDICAL RELEVANCE

RADU SILAGHI-DUMITRESCU[1]*

Keywords: hemoglobin, myoglobin, oxidative stress, redox, blood

Abstract: Redox reactivity in globins includes the well-known physiologically-relevant autooxidation, as well as a range of other reactions, some biomedically-relevant and some only observed in vitro. These include the nitric oxide dioxygenase reaction, the nitrite reductase reaction, the nitrite oxygenase reaction, the peroxidase reactions (all of which held biomedical relevance) as well as reactions with more exotic ligands such as hypochlorite or sulfide, also of possible biological relevance. Our recent work has shown that some anticancer drugs can modulate the redox reactivity of globins, in some cases manifested as far as an effect observable on the red blood cells. Antioxidants can alleviate or exacerbate these effects, partly due to their redox properties and partly due to an apparent unusually high affinity for globins.
Covalent modifications of globins, such as the polymerization/condensation/reticulation with bifunctional agents such as glutaraldehyde or with monofunctional agents such as polyethylene glycol with the purpose of producing efficient hemoglobin-based oxygen carriers (HBOC), also has a distinct effect on the redox reactivity as well as on the performance of the HBOC in transfusion experiments.
In non-vertebrate globins, such as those from plants or from bacteria, redox reactivity is even more central, as for some such globins it is proposed to be involved in the main physiological function rather than being a side-reaction. Here again, interaction with antioxidants appears to strongly modulate redox reactivity – and not only by direct electron exchange.
Assays for antioxidant as well as for prooxidant reactivity of organic compounds or of natural extracts have been proposed based on reactivities such as described above, using globins as key reagents [1-12].

Acknowledgements: Funding from the Romanian Ministry for Education and Research (grant PN-III-P4-ID-PCE-2016-0089) is gratefully acknowledged.

1. Surducan, M.; S. V Makarov; R. Silaghi-Dumitrescu. O-S bond activation in structures isoelectronic to ferric-peroxide species known in O-O-activating enzymes: relevance for sulfide activation and sulfite reductase, *Eur. J. Inorg. Chem.*, **2014**, 34, 5827–5837.
2. Toma, V. A.; A. D. Farcas; I. Roman; B. Sevastre; D. Hathazi; F. Scurtu; G. Damian; R. Silaghi-Dumitrescu. Comparative In Vivo Effects of Hemoglobin-Based Oxygen Carriers (HBOC) with Varying Prooxidant and Physiological Reactivity, *PLoS One*, **2016**, 11, e0153909.
3. Makarov, S. V. V; A. K. Horvath; R. Silaghi-Dumitrescu; Q. Gao; A. K. Horváth; R. Silaghi-Dumitrescu; Q. Gao. Recent developments in the chemistry of thiourea oxides, **2014**, 20, 14164–14176.
4. Farcas, A. D.; A. C. Mot; C. Zagrean-Tuza; V. Toma; C. Cimpoiu; A. Hosu; M. Parvu; I. Roman; R. Silaghi-Dumitrescu. Chemo-mapping and biochemical-modulatory and antioxidant/prooxidant effect of galium verum extract during acute restraint and dark stress in female rats, *PLoS One*, **2018**, 13.
5. Dunne, J.; A. Caron; P. Menu; A. I. I. Alayash; P. W. W. Buehler; M. T. T. Wilson; R. Silaghi-Dumitrescu; B. Faivre; C. E. E. Cooper. Ascorbate removes key precursors to oxidative damage by cell-free haemoglobin in vitro and in vivo, *Biochem. J.*, **2006**, 399, 513–524.
6. Bischin, C.; A. Mot; A. Stefancu; N. Leopold; D. Hathazi; G. Damian; R. Silaghi-Dumitrescu. Chlorite reactivity with myoglobin: Analogy with peroxide and nitrite chemistry?, *J. Inorg. Biochem.*, **2017**, 172.
7. Svistunenko, D. A. A. *et al.* Reaction of Aplysia limacina metmyoglobin with hydrogen peroxide, *Dalt. Trans.*, **2007**, 840–850.
8. Hathazi, D. *et al.* Involvement of ferryl in the reaction between nitrite and the oxy forms of globins, *J Biol Inorg Chem*, **2014**, 19, 1233–1239.
9. Mot, A. C. C.; C. Bischin; G. Damian; R. Silaghi-Dumitrescu. Antioxidant activity evaluation involving

hemoglobin- related free radical reactivity, *Methods Mol. Biol.*, **2015**, 1208, 247–255.
10. Miclea, P.; C. Bischin; G. Leția Naumova; A. Moț; S. Dorneanu; R. Silaghi-Dumitrescu. Class 1, 2 and 3 hemoglobins from Arabidopsis thaliana: overexpression and autooxidation, *PLoS One*, **2018**, submitted.
11. Puscas, C.; A. Mircea; M. Raiu; M. Mic; A. A. A. Attia; R. Silaghi-Dumitrescu. Affinity and effect of anticancer drugs on the redox reactivity of hemoglobin, *Chem. Res. Toxicol.*, **2019**, in press.
12. Moț, A. C. *et al.* An assay for pro-oxidant reactivity based on phenoxyl radicals generated by laccase, *Food Chem*, **2014**, 143, 214–222.

[1] Department of Chemistry, Babes-Bolyai University, 1 Mihail Kogalniceanu street, Cluj-Napoca 400084, Romania.

*Corresponding author: E-mail:rsilaghi@chem.ubbcluj.ro, phone +40264593833, fax +40264590818; Department of Chemistry, Babes-Bolyai University, 1 Mihail Kogalniceanu street, Cluj-Napoca 400084, Romania.

NICOTINE-INDUCED PROTEOME OF SURPRINSINGLY USEFULL MICROORGANISM: PAENARTHAROBACTER NICOTINOVORANS pAO1

MARIUS MIHĂȘAN[1,2*], CORNELIA BABII[1], DEVIKA CHANNAVEERAPPA[2], ROSHANAK ASLEBAGH[2], EMMALYN DUPREE[2], COSTEL C. DARIE[2]

Keywords: nicotine, green-chemicals, proteome, nanoLC-MS/MS

Abstract: 6-hidroxy-L-nicotine is a metabolic intermediate found in the nicotine catabolic pathway encoded by the pAO1 megaplasmid of *Paenarthrobacter nicotinovorans*. Extensive work has shown that 6-hidroxy-L-nicotine has shown a great potential as a neuroprotective drug. Thereby, a biotechnology based on *Paenarthrobacter nicotinovorans* is currently under development for the conversion of nicotine containing waste 6-hidroxy-L-nicotine and other green chemicals. Using *P. nicotinovorans* for biotechnological applications is nevertheless hindered by the lack of information on how the cells cope with the accumulation and toxicity of the resulting nicotine metabolic by-products. In order to address this issue at the protein level, we performed a proteomics study using reversed phase nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS) was performed. *P. nicotinovorans* was grown on different carbon sources, including nicotine and the cells were harvested at 3 different time intervals: 7, 10 and 24 hours post inoculation. The cells were lyzed, cell free extracts were prepared the proteins were identified using a gel-based proteomics approach employing a NanoAcquity UPLC (Waters, Milford, MA, USA) coupled to a Q-TOF Xevo G2 MS (Waters). Data analysis was performed using Mascot v.2.5.1 (Matrix Science, London, UK) and Scaffold (v.4.8.2, Proteome Software Inc., Portland, OR, USA). This approach allowed us to produce two different sets of proteomics data: PXD008751 (ProteomeXchange Consortium) describing the bacterial proteome on different carbon sources and containing 792 non-redundant proteins as well as PXD012577 (PRIDE) describing a time-based evolution of the nicotine-related proteome that contains 584 non-redundant proteins with an FDR of 0.3%. The differences in protein abundance in the different growth conditions showed that deamination is preferred in the nicotine pathway when citrate can be used as a carbon source. Several putative genes from the pAO1 megaplasmid have been shown to have a nicotine-dependent expression, including a hypothetical polyketide cyclase. This data provides insights into bacterial cells adaptation to the nicotine metabolic intermediates that are known to be toxic and to accumulate in the growth medium.

This work was supported by a grant of CNCS-UEFISCDI, project number PN-III-P1-1.1-TE-2016-0367, within PNCDI III.

[1] Alexandru Ioan Cuza University, Faculty of Biology, 20A Carol I Bd., 700506 Iasi, Romania

[2] Clarkson University, Department of Chemistry & Biomolecular Science, Clarkson Ave. 13699-5810, Potsdam, NY, USA

*Corresponding author: marius.mihasan@uaic.ro,+40740602434

PRODUCTION AND CHARACTERIZATION OF A NEW FUNGAL EXTRACELLULAR LIPASE

LAMYA EL AAMRI[1,2], MAJIDA HAFIDI[2], CATALINA IONICA CIOBANU[3], MANUELA RUSS[4], MICHAEL O. GLOCKER[4], BRINDUȘA ALINA PETRE[1], VASILE ROBERT GRADINARU[1]*

Keywords: fungi, lipase, chromatography, spectrometry

Abstract. This is the first report about the characterization of *Rhodosporidium babjevae* LE.154 extracellular lipase. Lipase production was carried out in yeast extract medium supplemented with olive oil, for 5 days at 28 ^{0}C and pH 7.5. The lipase was identified in the extracellular fraction and concentrated by ammonium sulfate or Polyethylene Glycol 2000 precipitation methods. The dialyzed enzyme was purified using two chromatographic steps: anion exchange and gel filtration chromatography. Lipase activity was screened using p-nitrophenyl palmitate as a substrate. Kinetic parameters of newly purified lipase were determined for both p-nitrophenyl- and 4-methylumbelliferyl palmitate substrates. The progress of lipolysis of tripalmitin using purified enzyme was monitored in a biphasic aqueous-chloroform system by ^{1}H-NMR. Mono- and diacylglycerides were identified by this technique suggesting a broad lipase specificity. The purity of lipase was confirmed by 15% SDS-PAGE. Lipase displays a band with a molecular weight around 15 KDa. In-Gel Digestion of the lipase in the SDS Gel Band followed by Nano LC-ESI HDMSE analysis were performed. The investigated lipase sequence from *Rodosporidium babjevae* LE.154 culture shares the partial common features with that of the protein from *Rhodotorula graminis* (strain WP1).

[1] Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Department of Chemistry, 11 Carol I, 700506 Iasi, Romania;

[2] Moulay Ismail University, Faculty of Sciences, Department of Biology, B.P. 11201 Zitoune, Meknes, Morocco;

[3] Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Research Department, 11 Carol I, 700506 Iasi, Romania;

[4] Proteome Center Rostock, University of Rostock, Schillingallee I, No. 69, 18057 Rostock, Germany

*Corresponding author: Vasile Robert Gradinaru, Email address: robert.gradinaru@uaic.ro; Phone: +40-232-201138; FAX: +40-232-201313; 11 Carol I, 700506 Iasi, Romania

ENDOPLASMIC RETICULUM QUALITY CONTROL AND PROTEIN MISFOLDING

ȘTEFANA M. PETRESCU[1,*]

Keywords: endoplasmic reticulum, ERAD, ER quality control, protein folding, EDEM, melanoma, insulin, proteome

Abstract: Endoplasmic reticulum (ER) is the site where newly synthesized proteins mature undergoing chaperone-assisted folding and post-translational modifications. All organelle and cell membrane proteins and all cell secretory proteins are hosted by the ER lumen during their folding and transport to the Golgi. To maintain the protein homeostasis, the folding processes are regulated by the ER quality control and the misfolded polypeptide chains are removed by the ER associated degradation pathway- ERAD. Tremendous progress has been made in the recent years regarding the molecular events accompanying the retrotranslocation of the ubiquitinated misfolded ERAD substrates and their proteasomal degradation. The molecular details of the substrate recognition are still under intense investigation. In this context we have addressed the role of EDEM protein family in the timely disposal of the ERAD substrates. EDEMs are proteins with mannosidase enzymatic activity that generate signal glycans attached to the misfolded proteins for ERAD and promote ERAD glycosylated and non-glycosylated proteins. Considering these multiple functions of EDEMs we have characterized in details the mechanisms of protein recognition and degradation in the presence of EDEM1, EDEM2 and EDEM3. Our data give mechanistic insights in the EDEMs- ERAD substrate interactions, as well as in the complexes engaging EDEMs in the ER degradation pathways and support the involvement of the intrinsically disordered regions of EDEMs in the substrate recognition pathway.

Acknowledgements: This work was financed by the Romanian Academy and PNCDI-III-PCCDI-2018-1

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy

*Corresponding author: Stefana M. Petrescu, stefana.petrescu@biochim.ro; Phone/Fax: (+4).021.223.90.69/(+4).021.223.90.68; Institute of Biochemistry of the Romanian Academy, 296 Splaiul Independenței, sector 6, Bucharest, Romania, 060031

GMOLMODEL - A METHOD TO OVERCOME CONFORMATIONAL SAMPLING HURDLES USING ROBOTICS

LAURENTIU SPIRIDON[1,*], DAVID MINH

Keywords: molecular simulation, robot mechanics

Abstract: Equilibrium constants are one of the most important measures that characterize chemical processes. These are defined as functions which can be interpreted as probability ratios. One important example is the probability of a drug to be in a bound state to its target in solution. Finding this probability however requires the knowledge of its sample space which consists of all the possible configurations of the drug and of the protein when bound together and when unbound.
Unfortunately, the configurational space of even the smallest of biological relevant structures is too large to be covered entirely by the molecular simulation methods available today or to be dealt with analytically in terms of the partition functions. This leads to the usage of sampling techniques which aim to get sufficiently relevant samples from the configurational space needed to describe the distribution as a whole.
One class of such methods is constrained dynamics using generalized coordinates. These methods take advantage of the special structure of biological molecules and use only certain degrees of freedom to sample the conformational space. For example, bond and angle movement can be eliminated to only exploit the high mobility of dihedral angles as in torsional dynamics. However, this comes at the cost of distorting their proper Boltzmann probability distribution. To recover the proper distribution additional Fixman terms must be used. Even so, the constrained degrees of freedom will not be sampled. Gmolmodel method solves this problem of constrained dynamics using Gibbs sampling. Namely, it mixes arbitrary constraint parametrizations with fully flexible dynamics. The arbitrary parametrizations are made possible by the usage of robot mechanics. This method gives the user a high degree of flexibility in choosing the parameters, hence its remarkable applicability.

References:

1.Spiridon L, Minh DDL (2017) Hamiltonian Monte Carlo with Constrained Molecular Dynamics as Gibbs Sampling. Journal of Chemical Theory and Computation 13: 4649-4659.

[1] Institute of Biochemistry of the Romanian Academy, Romania

[2] Illinois Institute of Technology, USA

*Corresponding author: spiridon.laurentiu@gmail.com

ABSOLUTE BINDING FREE ENERGIES FROM BINDING FREE ENERGIES TO MULTIPLE RIGID RECEPTOR CONFORMATIONS

DAVID MINH

Abstract: Implicit ligand theory (ILT) provides a way to calculate noncovalent binding free energies between flexible partners through an exponential average of the binding potential of mean force (BPMF) - the binding free energy between a flexible ligand and rigid receptor [4,10]. Receptor configurations must be drawn from or reweighed to an apo [10] or holo [4] ensemble. Computing binding free energies based on multiple BPMFs has several advantages over simulations with a flexible receptor: the ensemble of receptor configurations can be thoroughly sampled once and recycled for a large chemical library of ligands; accuracy can be progressively increased by computing BPMFs for more receptor snapshots [6]; the task of computing multiple BPMFs is trivially parallel; and BPMFs can be much faster to compute and more scalable than binding free energies to flexible proteins. In the last several years, my group derived ILT [4,10], developed tools to compute protein-ligand BPMFs based on replica exchange [9] and the fast Fourier transform [3], and applied them to the binding of small hydrophobic molecules to T4 lysozyme [6] and in the D3R grand challenge [1]. Along the way, we have contributed methodological advances that have implications beyond protein-ligand binding: to better understanding replica exchange efficiency [8], implemented ergodic Boltzmann sampling based on constrained dynamics [7], improved the interpolation of interaction energy grids [5], and developed a strategy to evaluate ensemble reduction algorithms for docking and BPMF calculations [2].

[1] Xie, B.; Minh, D. D. L. J. Computer-Aided Molecular Design 2019, 33 (1), 61–69.
[2] Xie, B.; Clark, J. D.; Minh, D. D. L. J Chem. Inf. Model. 2018, 58 (9), 1915–1925.
[3] Nguyen, T. H.; Zhou, H.-X.; Minh, D. D. L. J. Comput. Chem. 2018, 39, 621–636.
[4] Nguyen, T. H.; Minh, D. D. L. J. Chem. Phys. 2018, 148 (10), 104114.
[5] Minh, D. D. L. J. Comput. Chem. 2018, 39 (19), 1200–1207.
[6] Xie, B.; Nguyen, T. H.; Minh, D. D. L. J. Chem. Theory Comput. 2017, 13 (6), 2930–2944.
[7] Spiridon, L.; Minh, D. D. L. J. Chem. Theory Comput. 2017, 13 (10), 4649–4659.
[8] Nguyen, T. H.; Minh, D. D. L. J. Chem. Theory Comput. 2016, 12 (5), 2154–2161.
[9] Minh, D. D. L. arXiv:1507.03703v1.
[10] Minh, D. D. L. J. Chem. Phys. 2012, 137 (10), 104106.

THE ROLE OF PHOSPHATIDYLINOSITOL (PTDINS) PHOSPHATASE SAC1 IN THE HBV LIFE CYCLE

MIRELA ANDREEA POPESCU [1], DAVID-SEBASTIAN PATRICHE[1], IOAN C. POPESCU[1], NORICA NICHITA[1,*]

Abstract: Sac1 is a 65kDa membrane protein localized at the endoplasmic reticulum (ER) and Golgi membranes in mammalian cells (Nemoto et al.,2000). It has been previously shown that Sac1 is a major phosphatidylinositol (PtdIns) phosphatase that downregulates the phosphoinositide phosphatases (PIPs) signaling (Whitters et al.,1993) as it catalyzes the dephosphorylation of PtdIns-3-P, PtdIns-4-P and PtdIns-3,5-P2 to PtdIns. PtdIns-4-P is the most abundant PIP derivative in mammalian cells and plays a crucial role in the transport of secretory proteins from the Golgi system to the plasma membrane, in apoptosis, metabolism, cell proliferation and cell growth (Frumanet al.,1998). Hepatitis B virus (HBV) is a small DNA virus, member of the Hepadnaviridae family. It is estimated that around 400 million people chronically infected with HBV are at risk of developing liver diseases. One stage of the viral particle maturation consists in budding of preformed cytoplasmic nucleocapsids into the three viral envelope proteins (L, M, and S) in the ER. The presence of SAC1 at the ER and Golgi membranes and its implication in PIPs signaling and trafficking event has prompted the investigation of this protein in the HBV life cycle. To modulate Sac1 expression in HBV replication-permissive Huh7 cells we employed both transient siRNA silencing and CRISPR/Cas9 technology. Efficient Sac1 gene silencing was confirmed by Western blot. Our preliminary results show a significan reduction of both viral and subviral particles from Huh7 cells with reduced Sac1 expression, as measured by PCR and ELISA. The result suggests that SAC1 may play a role in the late stages of the virus assembly/trafficking. Our future studies aim to understand the mechanism of this inhibition and clarify whether Sac1 can be a novel target for antiviral therapy.

References:

1. Nemoto, Y., Kearns, B.G., Wenk, M.R., Chen, H., Mori, K., Alb, J., De Camilli, P., and Bankaitis, V.A. (2000). Functional characterization of a mammalian SacI and mutants exhibiting substrate specific defects in phosphoinositide phosphatase activity. J. Biol. Chem. 275, 34293– 343051

2. Whitters, E.A., Cleves, A.E., McGee, T.P., Skinner, H.B. and Bankaitis, V.A., 1993. SAC1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. *The Journal of cell biology*, *122*(1), pp.79-94

[1] Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy

*Corresponding author: nichita@biochim.ro, Institute of Biochemistry, 296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031, Tel: (+4).021.223.90.69, FAX: (+4).021.223.90.68

THE COMPLEX STUDY OF SCHINUS POLYGAMUS OIL EFFECTS ON THE OXIDATIVE STATUS OF THE HIPPOCAMPUS OF A DEMENTIA ANIMAL MODEL

TEODORA RUXANDRA CRAESCU[1,*] LUCIAN HRITCU[1]

Keywords: Schinus Polygamus, Dementia, Parkinson disease, Oxidative status

Abstract: Parkinson's disease was first described in the form of a neurological syndrome by James Parkinson. He was particularly well-known due to the studies from "An Essay on the Shaking Palsy". However, information about the disease has been found in much older writings. Subsequently, anatomical, biochemical and physiological studies have identified pharmacological and neurological markers that have allowed the development of new therapies designed to diminish the effects of Parkinson's disease, which is still considered to be incurable. The purpose of this paper was to determine the influence of *Schinus Polygamus* oil on the oxidative status of the hippocampus of a dementia animal model. The studies of its effects were performed with the aid of enzymatic and non-enzymatic specific markers. The experiments in the present study were realised on 20 male Wistar rats, weighing 350 ± 10 g at the start of the experiment. The rats were treated in accordance with the institutional rules for experimental laboratory testing and all experimental procedures are in accordance with the European Council Directive of 24 November 1986 (86/609 / EEC).
Of the pathological markers of the disease, the most important were tested in this study. Among them we mention:

- SOD (Superoxidedismutase) – an enzyme produced by O2 metabolism, and if its mechanism of production is not regulated, cellular damage occurs.
- GPX (Glutathioneperoxidase) – an enzyme involved in the protection of organisms against oxidative stress.
- MDA (Malondialdehyde) – an organic compound, highly reactive that occurs as the enol. It occurs naturally, has no color, and is a marker for oxidative stress.

The use of the plant came to support its antioxidant effects, in order to reduce the symptomatology of the disease and even eliminate it.

[1] Alexandru Ioan Cuza University of Iasi, Bulevardul Carol I, nr.11 700506

*Corresponding author: Teodora Ruxandra Crăescu, ruxandra.craescu@gmail.com, 0745220461; Faculty of Biology, Biology Department, Alexandru Ioan Cuza University of Iasi, Iasi, Romania

NEW MECHANISTIC INSIGHTS FROM STUDIES OF EDEM3 CONFORMATIONAL DOMAINS

GEORGIANA MĂNICA[1], CRISTIAN BUTNARU[2], SIMONA GHENEA[1], MARIOARA CHIRIȚOIU[1], IOANA MARIN[1], ȘTEFANA M. PETRESCU[1]*

Abstract: Despite being considered a redundant member of the glycosyl hydrolase 47 family, more and more studies reveal surprising implications of the Endoplasmic Reticulum (ER) degradation-enhancing alpha-mannosidase-like protein 3 (EDEM3) in a variety of processes. Recognized as an active mannosidase, EDEM3 was proposed to have a role in cancer cell viability and cell response to hypoxia or to be used as a biomarker for peripheral blood mononuclear cell quality.

In order to get insights into the molecular mechanistics underlying these unique properties, we investigated the behavior of a series of truncated mutants of EDEM3 in different cell lines. These constructs presented distinct outcomes regarding their cellular expression and co-localization with ER-resident proteins. Also, they affect the degradation and demannosilation of some well-established Endoplasmic Reticulum Associated-Degradation (ERAD) substrates in particular ways. Moreover, when exposed to hypoxia, the accumulation of the hypoxia-inducible factor 1-alpha (HIF-1-α) is modified in the presence of EDEM3.

Togheter, our results propose individual functions for the main conformational domains of EDEM3. This characterization defines EDEM3 as an important part of the canonical ERAD pathway, but also opens the path for exploring its undiscovered activities.

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independenței 296, 060031, Bucharest, Romania

[2] Photonic Investigations Laboratory, Center for Advanced Laser Technologies, INFLPR, Atomistilor 409, 077125, Magurele, Ilfov, Romania

*Corresponding author: stefana_petrescu@yahoo.com; tel: (+4).021.223.90.69; fax: (+4).021.223.90.68

DETERMINING THE SENSITIVITY OF INFLUENZA VIRUS TO ANTIVIRALS

MARIA ELENA MIHAI [1, 2], CARMEN MARIA CHERCIU[1], GRIGORE MIHĂESCU[2], MIHAELA LAZĂR [1]

Keywords: influenza virus neuraminidase, phenotypic method, antivirals

Abstract: Neuraminidase inhibitors like oseltamivir are currently the only antiviral use in treatment of respiratory infections caused by influenza viruses. National Influenza Center of Cantacuzino Institute serve as the focal point and provide support for isolation, antigenic and genetic characterization, antiviral susceptibility testing.
Monitoring the sensitivity of influenza viruses to oseltamivir in Romania.
Influenza strains; NA-Fluor™ Influenza Neuraminidase Assay (Applied Biosystems); neuraminidase inhibitor oseltamivir carboxylate (Hoffmann-La Roche Ltd); reference strains.
The antiviral inhibition of influenza virus neuraminidase by a phenotypic test is a functional assay based on a substrate similar to the natural substrate (sialic acid).The antiviral concentrations required for 50% inhibition of enzyme activity (IC50) are calculated for each strain, the IC50 is expressed in nM oseltamivir.
Between 2016-2019, in Romania, 105 influenza strains isolated were tested phenotypically. All strains showed a normal inhibition of neuraminidase activity to oseltamivir with the exception of a strain, A/Covasna/2018, subtype H1N1pdm09, which had an IC50 value greater than the wild type mean.
The gene sequencing of some influenza viruses identified mutations did not revealed the presence of substitutions associated with reduced antiviral susceptibility, with one exception , influenza virus strain A/Covasna /2018. It presented the substitution of S247N, which was reported in the literature as associated with antiviral resistance.
According to WHO criteria categories(1), none of the test results indicated reduced or highly reduced inhibition to NAIs oseltamivir, but further monitoring and more sequencing studies are required to track possible mutations causing resistance.
Influenza isolate with mean IC50 value greater than the mean for wild type reference was considered outliers.

Reference. Weekly epidemiological record, No. 39, 2012.
Acknowledgment. This study is based upon the financial stuy – IMOVE (Monitoring vaccine effectiveness during seasonal influenza in the European Union) for isolation and phenotipycal assay, and national research project PN-III-P1-1.1-PD-2016-1726 for genetic sequencing of neuraminidase gene; Oseltamivir carboxylate was kindly provided by Hoffman-La Roche Inc.; microsoft excel- point-by-point templat provide by dr.A. Lackenby, PHE London, UK.

[1] "Cantacuzino" National Medico-Military Institute for Research and Development

[2] Faculty of Biology, University of Bucharest

METABOAGE: A DATABASE OF METABOLITE CHANGES DURING AGING

TEODORA BUCACIUC[1*], CORINA VIOLETA MORARU[2], ANCA ANGHEL[2], CATALIN FLORENTIN ION[1], ROBI TACUTU[1], ANDREI LAZAR GLIGOR[2]

Abstract: Aging and age-related pathologies are one of the biggest challenges of our society, yet the underlying processes are not fully understood. In the last years, research in the field increased significantly and a lot of relevant data, including metabolomic data, is now available. In order to structure this information and make it accessible for further research, we developed a database that contains information about human metabolites with variations during aging. The data is extracted from scientific articles and is manually curated. Metabolite variations are included in the database based on the following criteria: 1) only studies in which a direct comparison between at least two age groups is shown (eg: young vs old) are considered, 2) only metabolites that show significant changes with age are included, 3) metabolites whose concentrations in the body are affected by supplements taken prior to the experiment are excluded. Up to date, chemical and molecular biology/biochemistry data was recorded for 233 metabolites, including ID, exact mass, and other chemical formula cross-referenced with other databases (The Human Metabolome Database, KEGG, Pubchem etc). In addition, body location was integrated into MetaboAge using publicly available ontologies. Data regarding sex- and age-related variation of metabolites was curated for 318 entries (over 150 metabolites). The MetaboAge website allows to surf aging-related metabolomics data, as well as associated chemical, pathway and ontology information.

[1] Institute of Biochemistry of the Romanian Academy, Bucharest

[2] Reverse Senescence Biotechnologies S.R.L, Campia Turzii

*Corresponding author: teo.bucaciuc@gmail.com; phone number: +40 730126917; Institute of Biochemistry of the Romanian Academy, Spl. Independentei 296, Bucharest 060031

DEEP PROTEOMICS OF A HUMAN MELANOMA CELL LINE USING OFFLINE 2D-HPLC FRACTIONATION AND CONCATENATION

CRISTIAN V.A. MUNTEANU[1*], GABRIELA N. CHIRIȚOIU[2], ANDREI-JOSE PETRESCU[1], ȘTEFANA M. PETRESCU[2]

Keywords: melanoma, proteome, mass spectrometry, HPLC, biomarkers

Abstract: One of the aims in discovery proteomics is the characterization of the human proteome in order to denote disease-specific potential biomarkers that could translate in the clinics. This would be of special interest, particularly in a pathologic context like melanoma in which recent epidemiologic studies show a global rise in incidence and mortality. To increase proteome coverage current methodologies, focus on two main fields: one approach is the development and advancement of instrumentation by increasing the speed of proteome data acquisition, dynamic range and sensitivity and another alternative is the development of proteome separation strategies orthogonal to the LC-MS/MS frequently used.
Here we describe the proteome characterization of A375 melanoma cells, using offline reversed-phase HPLC separation with fraction concatenation and nanoLC-MS/MS analysis. In order to obtain orthogonality between the two separation methods we combined high-ph reverse-phase separation with low-ph nanoLC-MS/MS analysis. We obtained over 1 000 000 MS/MS spectra by combining all the HPLC fractions and their technical replicates which resulted in over 250 000 peptide spectrum matches and assembled in over 5 000 proteins. This is one the largest proteome coverage reported for A375 melanoma cell line and future work will provide possible new melanoma biomarkers by comparing with other normal and cancer cell lines.

Acknowledgements: This work was financed by CCCDI-UEFISCDI Grant PN-III-P1-1.1-PD-2016-1528, PNCDI-III-PCCDI-2018-1 and the Romanian Academy

[1]Department of Bioinformatics & Structural Biochemistry, Institute of Biochemistry of the Romanian Academy

[2]Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy

*Corresponding authosr: Cristian V.A. Munteanu, cristian.munteanu@biochim.ro, Phone/Fax: (+4).021.223.90.69/(+4).021.223.90.68; Institute of Biochemistry of the Romanian Academy (IBRA), 296 Splaiul Independenței, sector 6, Bucharest, Romania, 060031

IDENTIFICATION OF TWO PROTEIN TYROSINE PHOSPHATASES SPECIFICALLY ACTING ON PLCγ2

RODICA BADEA[1,*], AURA IONESCU[1], STEFAN SZEDLACSEK[1]

Keywords: PLCγ2, difluoro phosphonomethyl phenylalanine, pulldown, protein tyrosine phosphatase

Abstract: Phospholipase Cγ2 (PLCγ2) plays a critical role in B cells differentiation by acting downstream BCR-mediated cell signaling. After binding of antigen to the B cell receptor (BCR), PLCγ2 is translocated to the cell membrane where it forms a signaling protein complex with the immunoglobulin α (Igα) and Igβ subunits of BCR, the protein tyrosine kinases Lyn, Syk and Btk and the adaptor protein B cell linker (BLNK). In the complex, PLCγ2 docks on BLNK and is activated by phosphorylation of Y^{759} by Btk kinase. PLCγ2 tyrosine-phosphorylation enables the phospholipase active site to gain access to and to hydrolyze its membrane-located substrate, phosphatidylinsositol 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) to trigger calcium release from intra-cellular stores. Although the activation of PLCg2 by tyrosine-phosphorylation is well understood, the converse process of PLCγ2 dephosphorylation and the protein tyrosine phosphatase(s) (PTPs) responsible for that are not yet clarified. In this work, we used a straightforward method, recently developed by us, for identification of PTPs that specifically dephosphorylates a given protein phosphotyrosine site. Therefore, we used a synthetic phosphopeptide containing the phosphotyrosine Y^{759} of PLCγ2 replaced by difluoro-phosphonomethyl phenyl alanine (F2pmF) to pulldown PTPs from cell lysates where PLCγ2 is endogenously expressed (e.g. Raji and HEK293T cells). Finally, the captured PTPs were identified by nanoLC-MS/MS. Analysis of the mass spectrometry results revealed two PTPs identified with high confidence: PTPN2 (TC-PTP) and PTPN1(PTP1B). Next, we found by *in vitro* tests that the identified PTPs directly dephosphorylated PLCγ2 co-expressed with Btk kinase in cell lysates. In addition, each of D/A and/or C/S trapping mutants of both PTPs were able to pulldown PLCγ2 when they were co-expressed in HEK293T cells. In summary, our results suggest that TC-PTP and PTP1B are specific PTPs for PLCγ2 dephosphorylation.

[1] Institute of Biochemistry of the Romanian Academy, Department of Enzymology, Splaiul Independentei 296, Bucharest, Romania

*Corresponding author: rdobre@biochim.ro, rodicabadea_2004@yahoo.com, Phone: +4021-2239069, FAX: +4021-2239068.

A MASS-SPECTROMETRY APPROACH FOR THE IDENTIFICATION OF THE NPC1 INTERACTOM

ADRIANA-ALINA RUS[1], CRISTIAN V.A. MUNTEANU[1], ȘTEFANA M. PETRESCU[1*]

Abstract: The lysosomal storage disorder Niemann-Pick type C1 is a rare progressive genetic disease characterized by the abnormal accumulation of the cholesterol and other lipids. The deficiency of NPC1 protein results in the inability of the body to transport lipids from the endosomal–lysosomal system of the cells. It is a matter of common knowledge that the NPC1 is involved in the transport of cholesterol but the detailed molecular mechanism and the interactors are not yet elucidated.

The first step towards the study of NPC1 interactors by a proteomic approach was the generation of a stable NPC1-knockout cell line. We used the CRISPR-Cas9 system to disrupt the NPC1 gene which provides the Niemann-Pick type C1 phenotype in HeLa cells. To this aim we have designed three gRNA sequences to uniquely target the NPC1 gene within the human genome. Each gRNA was cloned in the spCAS9 vector. HeLa cells were transfected with the sgRNA/Cas9 co-expression plasmid and a primary selection of the cells was performed by adding puromycin to cells media. Further, single cell clones were isolated by serial dilution. The cell clones were then characterized in terms of cholesterol subcellular distribution by filipin staining. Further, NPC1 expression level was detected by immunoblotitng and the lysosomal volume was measured by flow cytometry.

For the analysis of the NPC1 interactom we have identified by LC-MS/MS- the proteins co-immunoprecipitated with NPC1. The HeLa and CHO cells WT vs KO were lysed in 1% digitonin and immunoprecipitated with αNPC1-antibody. The proteins were eluted by Soft Elution Buffer (SEB) and LB, separated by SDS-PAGE, stained with Coomassie Brilliant Blue and the resulting bands were excised. The samples were reduced and alkylated. Further, the digestion was made in gel with trypsin and the resulting peptides were analyzed by LC-MS/MS. From these analyses we have identified some possible interactors of NPC1 protein that are presented and their role in the NPC1 function is further discussed.

Acknowledgement: This project has been supported by Lysomod programme, European Union's Horizon 2020 under the Marie Skłodowska-Curie grant agreement No 734825.

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independenței 296, 060031, Bucharest, Romania

*Corresponding author: Stefana M. Petrescu, stefana.petrescu@biochim.ro; Phone/Fax: (+4).021.223.90.69/(+4).021.223.90.68; Institute of Biochemistry of the Romanian Academy, 296 Splaiul Independenței, sector 6, Bucharest, Romania, 060031

OPTIMIZING SILAC LABELING PROTEOMICS OF A HUMAN MELANOMA CELL LINE

GABRIELA N. CHIRIȚOIU[1*], CRISTIAN V.A. MUNTEANU[2], ANDREI-JOSE PETRESCU[2], ȘTEFANA M. PETRESCU[1]

Keywords: melanoma, proteome, mass spectrometry, SILAC, isotopes

Abstract: Melanoma is a highly aggressive form of skin cancer, with a high rate of recurrence. Currently, the research in this field is centered on finding biomarkers that can help, diagnostic, prognostic and treatment of this cancer. Quantitative proteomics is a key-method in deciphering proteome changes during malignant transformation and SILAC (Stable Isotope Labeling by Amino acids in Cell culture) is currently considered the gold standard regarding quantitative accuracy. A key-aspect in SILAC experiments is related to the efficiency of the labeling process of cells in culture. The most frequent amino acids labeled in SILAC experiments are Arginine and Lysine, in order to have each peptide labeled after trypsin digestion.

While lysine is an essential amino acid, arginine can also be obtained by metabolic conversions and a frequently problem in cell culture labeling experiments is the Arg to Pro interconversion. Here we have optimized several aspects of melanoma cells SILAC labeling, by establishing optimal concentrations of the supplemented amino acids and compared at the proteome level labeled and unlabeled cells. We have observed, that particularly for A375 melanoma cells the arginine concentration is important and that supplementing the cell culture medium with Proline increases the labeling efficiency. System wide analysis uncovered quantitative proteome transformation during cell culture labeling, mapping possible affected biological pathways. Our results will further help in the future for quantifying proteome changes in melanoma cells induced by various conditions.

Acknowledgements: This work was financed by CCCDI-UEFISCDI Grant PN-III-P1-1.1-PD-2016-1528, PNCDI-III-PCCDI-2018-1 and the Romanian Academy

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy

[2] Department of Bioinformatics & Structural Biochemistry, Institute of Biochemistry of the Romanian Academy

*Corresponding author: Gabriela N. Chirițoiu , gabic@biochim.ro; Phone/Fax: (+4).021.223.90.69/(+4).021.223.90.68; Institute of Biochemistry of the Romanian Academy (IBRA); 296 Splaiul Independentei sector 6, Bucharest, Romania, 060031

A PROTEOMIC STUDY OF THE RELATIONSHIP BETWEEN HYPOXIA AND BRAF INHIBITORS TREATED MELANOMA CELLS

IOANA MILITARU[1], CRISTIAN V.A. MUNTEANU[1], ȘTEFANA M. PETRESCU[1*]

Abstract: Melanoma remains the most aggressive type of skin cancer, due to multiple pathways involved in metastasis, proliferation and resistance. Single therapy may be ineffective nowadays and can lead to resistance to most available medications. Continuous efforts are made in order to diminish the resistance and combinations of several medications are widely used to reduce the unwanted resistance. In order to study the molecular mechanisms involved in resistance we used mass spectrometry (MS)-based proteomics. Differences in expression levels of melanoma biomarkers determined by MS were further investigated. Using several medications we developped resistant cell lines, among them to vemurafenib, one of the most used therapy in those BRAF mutant melanoma. Another approach was the characterization of the changes occurring in melanoma cell lines subjected to different O2 concentration. Hypoxia may give new insights in the way in which proteome derived from several cell lines is changed due to low oxygen conditions. A comparative study of the proteome derived from cells exposed to 1% O2 and those grown in normoxia for 1 or 3 days is presented. In this study we are trying to bring light in the mechanisms and pathways involved in acquired resistance to therapy, and compare them to those involved in survival of cells in hypoxia condition.

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independenței 296, 060031, Bucharest, Romania

*Corresponding author: stefana_petrescu@yahoo.com; tel: (+4).021.223.90.69; fax: (+4).021.223.90.68

CALCULATION OF TYR-YMD/HLA-I/TCR COMPLEXES BINDING FREE ENERGY

TEODOR ASVADUR SULEA[1,*], CRISTIAN V.A. MUNTEANU[1], GABRIELA N. CHIRITOIU[2], STEFANA M. PETRESCU[2], ANDREI-J PETRESCU[1], LAURENTIU SPIRIDON[1]

Keywords: melanoma, HLA, free energy calculation, binding energy

Abstract: Melanoma is the most dangerous form of cancer, hence the designing of efficient vaccines is a chief step in fighting this disease. Vaccines based on peptidic epitopes from melanoma cells have been shown to have promising effects. One such epitope is the YMD peptide (aa: 369 - 377) of tyrosinase which is overexpressed in melanoma. Recent experiments indicate that YMD is often oxidized and show higher antigenicity (1).
Here we use two types of computational methods to evaluate the binding free energy (ΔG) of various YMD oxidized forms to Human Leukocyte Antigen (HLA) and/or T-Cell receptor (TCR) in both binary and tertiary complexes. On one hand the thermodynamic integration (TI) calculates ΔG using a nonphysical coupling parameter. It is based on integrating an ensemble averaged quantity along the thermodynamic path between the two end points with respect to the coupling parameter. By contrast MM/PBSA and MM/GBSA (MM: Molecular Mechanics / PB: Poisson-Boltzmann / GB: Generalized Born / SA: Surface Area) are end-point implicit solvation methods and therefore less accurate than TI but considerably faster. Results from both type of methods are in good agreement with the experiment, but in addition they can be used to select the most antigenic YMD peptide diastereomer given that the experimental data was obtained only on YMD mixtures.

References:

Chiritoiu GN, Jandus C, Munteanu CV, Ghenea S, Gannon PO, Romero P, Petrescu SM. "*Epitope located N-glycans impair the MHC-I epitope generation and presentation*.", ***Electrophoresis***. **37(11)**,1448-60 (2016)

[1] Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

[2] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

*Corresponding author: e-mail: teo.sulea@gmail.com

PLANT R-PROTEIN STRUCTURE - MODEL vs. CRYO-EM COMPARISON

ELIZA C MARTIN[1], LAURENTIU N SPIRIDON[1], OCTAV CALDARARU[1], ANDREI-J PETRESCU[1*]

Abstract: CC-NBS-LRR resistance proteins are central to plants innate immune system, as they act as pathogen sensors and initiate the HR signal. Over the past decade, in order to compensate the complete lack of structural experimental data on R-plant proteins we developed a computational assisted experimental approach in which by using remote and ab-initio modeling techniques bound by experimental and bioinformatics derived constraints we generated increasingly reliable models of R-protein modules that proved instrumental in understanding their mechanisms and interaction networks.
The recently reported first global plant-resistosome cryo-EM structures[1,7] bring bolder horizons in the field and allowed us to compare here a decade of computational work with cryo-EM data. The predictive remote homology and ab-initio 3D models of various R proteins, such as RX1, GPA2, LR10, NRC1, PM3, etc[2,3,4,5,6] show an overall 3-5Å RMSD deviation and 1-3Å on individual subdomains, both in auto-inhibited ADP-bound and the internally rearranged ATP-activated conformation. Moreover, the Rx1 NBS-LRR docking model built using experimental mutation constraints[5], was also validated by the cryo-EM to which it superimposes within ~5Å RMSD given a 24% sequence identity on NBS-LRR. These results confirm the power of predictive computational methods in assisting biological problems research.

Financial support: UEFISCDI grant PN-III-ID-PCE-2016-0650.

1 Science, 364, eaav5868,2019
2 Plant Physiol. 178,2018
3 New Phytol. 208, 210-223,2015
4 Mol.Plant-Microbe Interact. 27, 835-845,2014
5 Plant Physiol.162, 1510–1528,2013
6 Mol. Plant Pathol, 13, 276–287,2012
7 Science,364, eaav5870,2019

[1] Department of Bioinformatics, Institute of Biochemistry of the Romanian Academy, Romania

*Corresponding author: email: ap@biochim.ro; phone: +40212239069; address: 296 Splaiul Independentei, sec 6, Bucharest, Romania, 060031

TARGETING MELANOMA MICROENVIRONMENT WITH LIPOSOMAL PREDNISOLONE IMPROVED THE THERAPEUTIC OUTCOME OF LIPOSOMAL DOXORUBICIN

EMILIA LICARETE[1,2*], DENISE-MINERVA DROTAR[1], IOANA STEJEREAN [1], LAURA PATRAS [1,2], VALENTIN-FLORIAN RAUCA [1,2], LAVINIA LUPUT [1,2], ALINA SESARMAN [1,2], MANUELA BANCIU [1,2]

Keywords: melanoma;angiogenesis; tumor associate macrophages

Abstract: Recent research in the melanoma field has contributed to a better understanding of the molecular mechanisms responsible for tumor growth and metastasis as well as drug resistance and led to approval of several novel therapies such as immune check point inhibitors and targeted therapy with Braf and Mek inhibitors. Although a survival benefit was obtained after the use of these novel biological agents, the resistance to treatment remains a major problem. To overcome this limitation, combination therapies have been developed but, despite to clinical efficacy, they are associated with serious adverse effects and are restricted to molecularly defined subsets of patients. In many types of cancer, including melanoma, TAMs are polarized to M2 phenotype supporting tumor growth, inflammation, angiogenesis, immunosuppression, and metastasis. Additionally, TAMs were associated with a lower efficacy of several cytotoxic drugs. In this context and based on our reported results, the aim of the present study was to develop a combination therapy based on liposomal prednisolone (LCL-PLP) as a tumor microenvironment modulator and liposomal doxorubicin (LCL-DOX) as a cytotoxic drug for melanoma cells. To this aim, 10 mg/kg of PLP and 5 mg/kg of DOX in long circulating liposomes (LCL) were administered simultaneously as well as separately to B16F10 tumor bearing mice. Our result demonstrated that the combined therapy induced a stronger inhibition of the tumor growth compared to both formulations administered separately. The molecular mechanisms underlying this antitumor effect of the combined therapy are based on the inhibition of C-Jun and MMP-2 activation. Also, the combined therapy induced a stronger inhibition of two important proangiogenic proteins: VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor).
In conclusion, our data indicated that LCL-PLP enhanced the therapeutic outcome of LCL-DOX and this therapy could be promising for the treatment of metastatic melanoma.

[1] Department of Molecular Biology and Biotechnology, Babes-Bolyai University, Cluj-Napoca, Romania;

[2] Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences, Babes-Bolyai University, Cluj-Napoca, Romania;

*Corresponding author: Emilia Licarete, PhD, E-mail: emilia_licarete@yahoo.com; Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, 5-7 Clinicilor Street, 400006, Cluj-Napoca, Romania.

DUAL ROLE OF CANCER-DERIVED EXTRACELLULAR VESICLES IN THE MODULATION OF TUMOR MICROENVIRONMENT

LAURA PATRAS[1*], ALINA SESARMAN[1], ALINA PORFIRE[2], VALENTIN FLORIAN RAUCA[1], RENATA HAJDU[1], ANDREEA KOSA[1], LAVINIA LUPUT[1], EMILIA LICARETE[1], MANUELA BANCIU[1]

Keywords: extracellular vesicles, tumor microenvironment, drug resistance, macrophages, drug delivery systems

Abstract: Recent findings have suggested that cancer cell-derived extracellular vesicles (CEVs) mediated bidirectional transfer of functional molecules between cancer cells and tumor microenvironment (TME) cells and strongly contributed to the reinforcement of tumor development, mainly by supporting protumor processes such as angiogenesis, inflammation, invasiveness, and evasion of immune surveillance. Therefore, we investigated the roles of CEVs derived from doxorubicin (DOX)-treated cancer cells in the modulation of TME cells as well as other recipient cancer cells. Our data suggested that CEVs released from DOX-treated cells (B16.F10 murine melanoma cells and C26 murin colon carcinoma cells) modulated the phenotype of different subpopulations of tumor-infiltrated macrophages to create neoplastic TME. Thus, screening for the expression of different macrophage markers via ELISA and qRT-PCR indicated that not only the M1 phenotype of antitumor macrophages was changed into M2 protumor phenotype of this cell type but also protumor function of M2 macrophages was exacerbated. Moreover, DOX-elicited CAVs modulated the response of stromal cells to chemotherapy by favoring the settlement of resistance of cancer cells to this cytotoxic drug as the tumor cell expression of the anti-apoptotic protein Bcl-xL was enhanced.
Besides CEVs functions in the creation of favorable tumor milieu for cancer development, there is increasing evidence regarding their use as anticancer drug delivery systems. Based on these recent data our studies investigated whether CEVs might be exploited for the development of DOX delivery systems to target cancer cells. Thus, besides their analogy to liposomes CEVs being produced by the tumor cells themselves, they can mimic the cancer cell membrane in an extent even greater than liposomes and therefore, CEVs possess the ability to be taken up by recipient cells more efficiently than liposomes. Our preliminary data suggested that polyethylene glycol (PEG)-decorated CEVs containing DOX administered in combination TAMs-targeted liposomal simvastatin favored the M1 antitumor phenotype of macrophages and exerted strong synergistic cytotoxic action on cancer cells *in vitro*.

This work was supported by CNCS – UEFISCDI: project PN-III-P4-ID-PCE-2016-0342, contract 91/2017 within PNCDI-III.

[1] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babeş-Bolyai University, Cluj-Napoca, Romania; e-mail: patras.laura88@yahoo.com, tel.0040264431691, fax 0040264431858

[2] Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

*Corresponding author: patras.laura88@yahoo.com

COLD ACTIVE ALDEHYDE DEHYDROGENASE-BASED BIOSENSORS

GEORGIANA NECULA-PETRAREANU[1*], PARIS LAVIN[2], ALINA VASILESCU[3], CRISTINA PURCAREA[1]

Keywords: aldehyde dehydrogenase, psychrophilic enzyme, kinetics, biosensor

Abstract: Aldehyde dehydrogenases (ALDHs) are important biotechnological enzymes that transform aldehydes present in food, beverage or cosmetics in their corresponding carboxylic acids by reducing the $NAD(P)^+$ to NAD(P)H. The recent utilization of cold adapted enzymes in biotechnologies and biosensing could lead to significant energy savings and cost reductions. This study presents the isolation, cloning, expression, purification, characterization and utilization of a recombinant ALDH from the psychrophilic bacterial strain *Flavobacterium* PL002 isolated from Antarctic sea water. This aldehyde dehydrogenase (F-ALDH) was cloned in pHAT2 vector and expressed in *E. coli* BL21(DE3). The enzyme was purified to homogeneity in one step by affinity chromatography. This homo-tetrameric ALDH was NAD^+/$NADP^+$ dependent and catalyzed the oxidation of a series of aliphatic and aromatic aldehydes. Although originating from a psychrophilic microorganism, F-ALDH was highly stable at temperatures up 60°C. Kinetic parameters showed a higher catalytic efficiency for aliphatic substrates independent of the cofactor, with lower K_M values when using $NADP^+$. This extremozyme was immobilized and used to construct a biosensor for aldehyde detection that proved to be efficient in detection of aldehydes used in winery and fungicides detection.

[1] Institute of Biology Bucharest, 296 Splaiul Independentei, 060031, Bucharest, Romania

[2] Laboratorio de Complejidad Microbiana y Ecología Funcional, Instituto Antofagasta, Universidad de Antofagasta, 601 Angamos Av., Antofagasta, Chile

[3] International Center of Biodynamics, Intrarea Portocalelor Street, Nr.1B, 060101, Bucharest, Romania

* Corresponding author: Georgiana Necula-Petrareanu, georgiana.petrareanu@ibiol.ro, Institute of Biology Bucharest, 296 Splaiul Independentei, 060031, Bucharest, Romania

HEAVY METAL ACCUMULATION BY YEAST CELLS ARMED WITH METAL-BINDING PEPTIDES TARGETED TO THE INNER FACE OF PLASMA MEMBRANE

ILEANA C. FARCASANU[1*]

Keywords: heavy metal; accumulation; *Saccaromyces cerevisiae*; metal-bining peptides.

Heavy metal pollution represents a threat to water supplies, agriculture soils, human and animal health, whereas the deficiency is considered equally deleterious for life or for important human activities, such as agriculture. Heavy metals are challenging pollutants as they are natural components of the earth's crust, they are persistent in the environment and they are non-degradable. Under such circumstances, removal of contaminating metals by means of (micro)organisms is often regarded as the only eco-friendly alternative, and obtaining resistant species which accumulate heavy metals from contaminated sites represents a pre-requisite for bioremediation approaches. In our laboratory we obtained heavy metal hyperaccumulating *Saccharomyces cerevisiae* cells designed primarily for metal-related bioremediation and bioextraction actions. In this study we present the possibility to engineer yeast cells for heavy metal accumulation by targeting heavy metal-binding oligopeptides to the inner face of the plasma membrane. We obtained a collection of DNA plasmids harboring sequences which encode artificial metal-binding oligopeptides (MeBPep) fused to myrGFP. The *myrGFP* casette introduces a myristoylation site, allowing both directional targeting to the inner face of the plasma membrane and monitoring of the intracellular localization. To estimate and to control the potential toxicity of the constructs, the expression of *myrGFP-MeBSeq* was monitored by placing the chimeric DNA under the inducible *GAL1* yeast promoter. We generated a collection of yeast strains which (over)express artificial metal-binding oligopeptides (6-30 residues) fused to myrGFP. This collection was investigated against an array of heavy metals in terms of metabolic changes, growth defects and heavy metal (hyper)accumulation.

[1] Department of Organic Chemistry, Biochemistry and Catalysis, Faculty of Chemistry, University of Bucharest, Sos. Panduri 90-92, Bucharest, Romania.

*Corresponding author: ileana.farcasanu@chimie.unibuc.ro

CHARACTERIZATION AND *IN VITRO* BIOCOMPATIBILITY EVALUATION OF POLYMER NANOPARTICLES – POLY (D, L-LACTIDE-CO-GLYCOLIDE) (PLGA) -AS DRUG DELIVERY DEVICES

PAULA E. FLORIAN[1], MAGDALENA MOISEI[1], MADALINA ICRIVERZI[1], LIVIA E. SIMA[1], GABRIEL SOCOL[2], ANCA ROSEANU[1], MIHAELA TRIF[1*]

Abstract: Polymer nanoparticles (PNPs), one of the most innovative non-invasive approaches for drug delivery applications has the capacity to convey the therapeutic molecule directly into the target organ or tissue. Poly(D,L-lactide-co-glycolide) (PLGA) is a synthetic polymer used in biological applications, approved by the Food and Drug Administration. PLGA posess the ability to efficiently entrap hydrophobic and hydrophilic drugs and have been shown to enhance the cellular uptake of entrapped bioactive compounds improving the drug efficacy. In our studies PLGA nanoparticles containing anti-inflammatory/antitumoral drugs were optimized for their physico-chemical properties in term of size and polidispersity index. Biocompatibility and intracellular uptake of FITC-PLGA were studied in different cell types (mouse embryonic fibroblasts - K41, mouse melanona cell - B16-F10, human hepatoma cell- HepaRG and epithelial cells from bovine kidney-MDBK) to develope a novel drug delivery systems usefull for anti-inflammatory/antitumoral therapy. PNPs interaction with mammalian cells was assessed by cytotoxicity measurement, fluorescence microscopy investigation, and flow cytometry quantitation. PLGA based NPs showed extremely low cytotoxicity only when delivered in high concentrations (over 2mg/mL). PLGA nanoparticles have the capacity to pass membrane barriers and showed increased tendency to aggregate, which could interfere with their potential function as drug carriers. Our studies revealed the conditions required for PLGA based NPs interactions with the cells and their intracellular uptake. The presented data are significant for the potential applications of PLGA-based NPs formulation as drug delivery vehicle.

Acknowledgements: This research received funding from Romanian National Authority for Scientific Research (CNCS–UEFISCDI), projects TERAMED 63PCCDI/2018 and Structural and Functional Proteomics Research Program of the Institute of Biochemistry of the Romanian Academy.

[1] Institute of Biochemistry, Romanian Academy, 296, Spl. Independentei, 060031, Bucharest 17, Romania

[2] National Institute for Lasers, Plasma and Radiation Physics, 409 Atomistilor Street, 077125, Magurele, Romania

*Corresponding author: E-mail: mtrif2000@yahoo.com; Tel/Fax: 0040 21 223 90 69/ 68; Address: Institute of Biochemistry, Romanian Academy, 296, Spl. Independentei, 060031, Bucharest 17, Romania

CRISPR-CAS SYSTEM IN *PSEUDOMONAS SP.* - STRUCTURE AND ROLE IN HORIZONTAL GENE TRANSFER OF ANTIMICROBIAL RESISTANCE GENES

DUMITRANA FIȚ [1,2*], ANCA FARKAS [2], ANCA BUTIUC-KEUL [2]

Keywords: *Pseudomonas*, antimicrobial resistance, CRISPR-Cas

Abstract: Bacteria and Archaea have developed several defense strategies against foreign nucleic acids, such as viral genomes and plasmids. These strategies include the CRISPR-Cas system, consisting of the CRISPR sequence (**c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats), along with associated genes, named Cas (**C**RISPR **as**sociated). CRISPR system provides protection against previously encountered foreign elements, possessing an "immunological memory" and being the only system involved in adaptive immunity of bacteria. The bacteria's natural pathogens are viruses, which are called bacteriophages. Normally, following an infection, the viral DNA may integrate into the host genome and replicate, possibly destroying the host cell, but if the bacterial cell has a CRISPR system, it will provide immunity.
CRISPR recognizes the foreign DNA, but does not distinguish between structures: viruses, plasmids, integrons, etc. Thus, it was assumed that the system could be an impediment to the Horizontal Gene Transfer (HGT) – the phenomenon responsible for the spread of antibiotic resistance, therefore bacteria that have CRISPR systems should own less antibiotic resistance genes. Through our study realized on 263 isolates of *Pseudomonas* from different sources we have concluded that CRISPR-Cas system does not stand in the way of HGT – isolates with CRISPR and those without having a similar percentage of antibiotic resistance genes.

[1] College for Advanced Performance Studies, Babeș-Bolyai University, 400084 Cluj-Napoca, Romania
[2] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeș-Bolyai University, 400084 Cluj-Napoca, Romania

*Corresponding author: e-mail: dumitranafit@gmail.com; phone: +40 752 217 958

TRYPTOPHAN – ASSISTED FLUORESCENT SILVER NANOPARTICLES SYNTHESIS

MARIANA VOICESCU[1*], VALENTIN S. TEODORESCU[2], SORANA IONESCU[3], JOSE M. CALDERON-MORENO[1], MIHAI ANASTASESCU[1], DANIELA C. CULITA[1]

Keywords: fluorescence spectroscopy, silver nanoparticles, tryptophan, riboflavin.

Abstract. Nano-size and shape of fluorescent silver nanostructures are important for a wide range of bio-applications, especially as drug delivery systems, imaging and sensing [1]. Riboflavin (Vitamin B_2) (RF) belongs to flavoenzymes which catalyzes redox-reactions in human beings essential for cell growth and development [2, 3]. This work described tryptophan – assisted fluorescent silver nanoparticles ($SNPs^{FL}$) synthesis followed by their analysis by UV-Vis Absorption, TEM, SEM, DLS, AFM, fluorescence spectroscopy. Multi-twined $SNPs^{FL}$, with the size within 15-40 nm, were obtained. $SNPs^{FL}$ functionalized with RF was performed. Riboflavin thermal destruction into RF/$SNPs^{FL}$ system by fluorescence spectroscopy was studied as well. RF is reduced at the $SNPs^{FL}$ surface, $\lambda_{abs} \sim 570$ nm, and its fluorescence behaviour in the RF/$SNPs^{FL}$ system does not undergo major structural changes. As temperature increases (25-80°C range) RF maintains its high fluorescence at $\lambda_{em} = 530$ nm. No aggregation but rather a dissociation of RF molecule in the $SNPs^{FL}$/RF system occurs. The results are relevant for various pharmaceutical formulations like drug release and especially RF containing drugs which must be thermally treated for a precise purpose.

[1]. N.L. Rosi, C.A. Mirkin, *Nanostructures in Biodiagnostics*, Chem. Rev., 105, 1547-1562, 2005.
[2]. R.V. Bennsasson, E.J. Land, T. G. Truscott, *Flash photolysis and pulse radiolysis in biology and medicine*, Pergamon Press, Oxford, 1983.
[3]. R. Miura, *Versatility and specificity in flavoenzymes: control mechanisms of flavin reactivity*, Chem. Rec., 1, 183-194, 2001.

[1] Institute of Physical Chemistry "Ilie Murgulescu" of the Romanian Academy, Splaiul Independentei 202, 060021 Bucharest, Romania
[2] Institute of Atomic Physics, National Institute of Materials Physics, Magurele 077125, Romania
[3] Department of Physical Chemistry, University of Bucharest, Bd. Regina Elisabeta 4-12, Bucharest 030018, Romania

*Corresponding author: m.voicescu@yahoo.com

NEW COMPOSITE COATINGS FOR TOPICAL TREATMENT

MADALINA ICRIVERZI[1], PAULA FLORIAN[1], MAGDALENA MOISEI[1], OANA GHERASIM[2,3],GIANINA POPESCU-PELIN[2], MIHAELA TRIF[1], GABRIEL SOCOL[2], ANCA ROSEANU[1*]

Keywords: PLGA coatings, biomedical device, topical treatment

Abstract: Different medical devices for cutaneous wound healing such as patches and hydrogels containing biologically active principles were developed over time. In our study we proposed composite coatings based on PLGA (poly (lactic-co-glycolic acid)) embedded with Ibuprofen (IBU) as potential dressings for topical wounds management. The PLGA:IBU materials were evaluated by performing long-term studies under biologically-simulated dynamic conditions and assessed *in vitro* for biological effects. IBU-loaded composite coatings, PLGA:IBU (2:1 and 10:1 wt. %) were obtained by combining dip-coating and drop-cast methods. The uniform PLGA:IBU (2:1) coatings resulted in slowly and progressive release of the drug and emphasized a pronounced IBU release starting from day 10 of dynamic evaluation. These observations were accompanied by noticeable mass variations and significant morphological modifications of the coatings. The viability and proliferation of the cells are not affected, irrespective of composite surface type or time of incubation. Cell adhesion and morphology investigations performed by fluorescence microscopy showed a predominantly round shape for THP-1 cells cultured on control material. On composite materials, the macrophages presented a modified morphology adapted to substrate content, and an increased cell shape with more evident actin filaments. All materials per se do not induce an inflammatory effect as revealed by TNF-α release measurements based on ELISA assay.
Physical-chemical and biological characteristics of PLGA:IBU composite coatings revealed that these materials could represent useful medical devices in topical treatment of skin injures.
Acknowledgement: This research received funding from Romanian National Authority for Scientific Research (CNCS–UEFISCDI), under the projects TERAMED 63PCCDI/2018 and Structural and Functional Proteomics Research Program of the Institute of Biochemistry of the Romanian Academy

[1] Institute of Biochemistry, Romanian Academy, 296, 060031, Spl. Independentei, Bucharest 17, Romania

[2] National Institute for Lasers, Plasma and Radiation Physics, 077125, 409 Atomistilor Street, Magurele, Romania

[3] Politehnica University of Bucharest, 011061, 1-7 Gheorghe Polizu Street, Bucharest, Romania

*Corresponding author: roseanua@gmail.com Tel/FAX. 0040212239069/68; Institute of Biochemistry, Romanian Academy, 296, 060031, Spl. Independentei, Bucharest 17, Romania

IDENTIFICATION OF FREE AMINO ACIDS IN BREWER'S YEAST AFTER HEAVY METALS BIOSORPTION

ANDREEA STĂNILĂ[1,*], ZORIŢA DIACONEASA[1], FLORICUŢA RANGA[1], FLORINELA FETEA[1]

Keywords: Brewer yeast, amino acids, biosorption, heavy metals

Abstract. The aim of this study was to identify if the free amino acids present in brewer yeast are involved in metal biosorption due to their capacity to coordinate metal ions. Yeasts of genera *Saccharomyces* are efficient biosorbents for heavy metal ions. As biosorbent was used living brewer's yeast type *Saccharomices cerevisae.* Copper, lead and zinc solution of 1mg/L concentrations were prepared using their salts. The experiments were conducted at pH=5,5 and pH=8. The amino acids were identified by HPLC-DAD/-ESI-MS chromatography. The experiments were conducted by mixing metals solution with yeast and shaken at a constant speed of 120 rpm at 20^0C for 120 minute. The samples were centrifuged at 2500 rpm for 15 minute and the supernantant was analysed for amino acids identification. The HPLC analysis were performed on a Agilent 1200 system equipped with a binary pump delivery system LC-20 AT, a degasser DGU-20 A3, diode array SPD-M20 A, UV–VIS detector (DAD). Amino acids were identified using an EEZ:Faast Kit for free amino acids, The amino acids identified by HPLC method were glycine, glutamic acid, leucine, isoleucine, ornithine, lysine, histidine, homophenylalanine, tyrosine, glutamine in control brewer yeast (before biosorption) and their profile differs according with the metal ions types and pH medium. According with the peaks area there are differences in the presence of the amino acids due to the possible coordination with copper, lead and zinc ions.

[1] Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, 400372, 3-5, Mănăştur St., Cluj-Napoca, Romania

* Corresponding author: andreea.stanila@usamvcluj.roTel: 0264596384/213

EFFECTS OF *ROSMARINUS OFFICINALIS* ESSENTIAL OIL IN MEMORY FORMATION AND RELIEVING BRAIN OXIDATIVE STRESS IN ZEBRAFISH MODEL

LUMINITA CAPATINA[1], ELENA TODIRASCU-CIORNEA [1], EDOARDO NAPOLI[2], LUCIAN HRITCU[1]

Abstract: This study was done to assess the anxiolytic, anti-amnesic and antioxidant potential of *Rosmarinus officinalis* essential oil, in three concentrations 25, 150 and 300 ml / L.
Anxious behavior and memory performance were assessed by NTT (new tank diving test) and Y and T mazes, and scopolamine (100 μM) was administered by immersion 30 minutes before behavioral testing to induce anxiety and a mild dementia.
Our data has shown that essential oil of rosmarin has diminished anxious behavior and amnesia induced by the use of scopolamine in parallel with the decrease in oxidative stress in the zebra fish brain.
The essential oil of rosmarin is known for its anti-inflammatory effects due to its compounds - α-pinene, camphor, eucalyptol - the chemical composition being identified by GC-MS. At the same time, literature mention the anti - amnesic and anti - acetylcholinesterase effects of rosemary essential oil in a scopolamine - induced Wistar rat animal model.
The results obtained from the behavior of zebrafish behavior in the three behavioral tests show a significant difference between the activity of scopolamine-treated animals and those exposed to rosmarin oil, especially in the concentration of 25 μl / L. In conclusion, the essential oil studied could be considered a good candidate for improving memory by inhibiting acetylcholinesterase and reducing oxidative stress in the brain.

[1] *Alexandru Ioan Cuza University of Iasi, Iasi, Romania*

[2] *CNR Instituto di Chimica Biomolecolare, Catania, Italy*

Author's contacts: Luminița Căpățînă- capatina.luminita@yahoo.com; Elena Todirașcu-Ciornea- ciornea@uaic.ro; Edoardo Napoli- edoardo.napoli@icb.cnr.it; Lucian Hrițcu- hritcu@uaic.ro

DIVERSITY AND ANTIBIOTIC RESISTANCE OF HETEROTROPHIC BACTERIA ISOLATED FROM MUIERILOR CAVE (ROMANIA)

DIANA FELICIA BOGDAN [1*], ADORJÁN CRISTEA [1], DORIANA-MĂDĂLINA BUDA[1], ANDREEA BARICZ [2], MIHAELA IONESCU [3], ALEXANDRA CRĂCIUN [3], OANA TEODORA MOLDOVAN [4], HORIA LEONARD BANCIU [1]

Keywords: heterotrophic bacteria, caves, antibiotic resistance, opportunistic bacteria

Abstract: Currently, there is sparse information on components and functioning of cave microbiomes. Here we aimed at assesing the culturable diversity and antibiotic resistance of the aerobic heterotrophic bacteria associated to Muierilor Cave, a cave located in south-western Romania. Soil, clay, guano and water were sampled from touristic and non-touristic parts of the cave. Samples were plated on R2A Agar, DIFCO 2216 and PCA solid-media at room temperature (20°-25°C), and at 37°C, respectively. The retrieved colonies were subsequently purified by successively plating. For molecular identification of isolates, genomic DNA from each isolate was subjected to 16S rRNA gene amplification using primer pair 27FB-1492R. The resulting amplicons were sequenced by the Sanger method. In total, 62 partial 16S rRNA gene sequences were obtained and analysed using MEGA X software. Our results indicate that the bacterial isolates are affiliated to Proteobacteria (39% sequences), Firmicutes (51%) and Actinobacteria (10%) phyla. The strains isolated from soil samples were closely related to *Glutamicibacter nicotianae*, *Ralstonia solanaracearum*, *Kocuria rizophila*, *Brevibacterium frigoritolerans*, *Stenotrophomonas maltophilia*, and *Bacillus sp*., while isolates from water samples were assigned to *Acinetobacter* sp., *Sphingomonas* sp., uncultured *Masillia* sp. and *Pseudomonas chlororaphis*. A violacein-producing bacterium, *Janthinobacterium lividum*, was isolated from fossilized guano while *Staphylococcus hominis* and *Paracoccus marinus* were retrieved from beech wood debris outside the cave. The antibiotic testing was performed by convenational diffusimetric method according to CLSI performance standards. Preliminary results confirmed the presence of intrinsic antibiotic resistance of *Bacillus mycoides* to penicillin, ampicillin, and cefoxitin; of *Stenotrophomonas maltophilia* to carbapenems; *Sphingomonas* sp. and *Acinetobacter lwofii* showed a small diameter of inhibition to colistin.
In conclusion, cave-associated micro-habitats are populated by active heterotrophic communities and our work might bring insights about the touristic impact on the antibiotic resistance of some bacterial strains.

This work was supported by a grant of Ministry of Research and Innovation, CNCS – UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0016, within PNCDI II

Acknowledgements: Erika Levei and Marin Șenilă (INOE 2000 INCD, ICIA Cluj-Napoca) for their support for chemical analyses.

[1] Babeș-Bolyai Univesity, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street, Cluj-Napoca, Romania

[2] National Institute of Research and Development in Biological Sciences, Institute of Biological Research, 46 Republicii Street, Cluj-Napoca, Romania

[3] Iuliu Hatieganu University of Medicine and Pharmacy, Faculty of Medicine, Department of Microbiology and Departament of Biochemistry, 4, Louis Pasteur Street, Cluj-Napoca, Romania

[4] *Emil Racoviță* Institute of Speleology, 5, Clincilor Street, Cluj-Napoca, Romania

*Corresponding author: email: dianafelicia.bogdan@gmail.com; phone number: 0724 414 749; 5-7, Clinicilor Street, Faculty of Biology and Geology, Departament of Biology and Biotechnology, Biophysics and Biochemistry laboratoratory, 400006, Cluj-Napoca, Romania.

THE CHARACTERISATION OF *CANNABIS SATIVA* LEAVES EXTRACTS AND THEIR SILVER NANOPARTICLES PRODUCTS FOR POTENTIAL BIOLOGICAL APPLICATIONS

LUCIAN COPOLOVICI [1], ADRIANA CSAKVARI [2], LEONARD MARIAN OLARIU [1], GEORGETA POP [3], DORINA CHAMBRE [1], DANA COPOLOVICI [1*]

Keywords: *Cannabis sativa* extracts, chemical composition, silver nanoparticles, GC-MS, HPLC-MS

Abstract: *Cannabis* spp. is a plant native in Central and South Asia being known to the human population at least from 3700 B.C. In the 1st century A.C., in the Chinese pharmacy, the medicinal properties of the plant were already known. Among several species of Cannabis, *Cannabis sativa* (hemp) is used for centuries, both for industrial applications and as phsyhotropic medicine.
The aim of our study was to obtain silver nanoparticles by using *Cannabis sativa* leaves extracts for potential biological applications. Firstly, we obtained methanolic and aqueous extracts of leaves of *Cannabis sativa* (two monoicous and one dioicous varieties) harvested in western part of Romania. Then, we determined the chemical composition using a gas-chromatograph and also a liquid-chromatograph coupled with mass spectrometer detectors. We identified compounds from diverse chemical classes contained in the extracts: esters, alcohols, organic acids, and aldehydes. The determination of the antioxidant activity of the obtained extracts by DPPH assay reveiled their mild antioxidant potential compared with other medicinal plants, which is in good correlation with the total polyphenolic content determined by Folin-Ciocalteu assay. Thereafter, silver nanoparticles with less than 30 nm, as revealed by SEM, were obtained by using $AgNO_3$ solution and *Cannabis sativa* leaves extracts.

[1]"Aurel Vlaicu" University of Arad, Faculty of Food Engineering, Tourism and Environmental Protection, and Institute of Research, Development, Innovation in Technical and Natural Sciences, 2 Elena Dragoi St., 310330, Arad, Romania;

[2] University of Oradea, Biomedical Sciences Doctoral School, 1 University St., 410087, Oradea, Romania;

[3] Banat University of Agricultural Sciences and Veterinary Medicine King Michael 1st of Romania from Timisoara, 119 Calea Aradului St., Timisoara, Romania;

Corresponding author: Dana Copolovici, E-mail: dana.copolovici@uav.ro, Tel: 0748564459; "Aurel Vlaicu" University of Arad, Faculty of Food Engineering, Tourism and Environmental Protection, 2 Elena Dragoi St., 310330, L. 31, Arad, Romania;*

ENTRAPMENT OF *N*-HYDROXYPHTHALIMIDE CARBON DOTS IN DIFFERENT TOPICAL GEL FORMULATIONS: NEW COMPOSITES WITH ANTICANCER ACTIVITY

MIHAIL BUTURA[1], CORINA-LENUTA SAVIN [2], CRINA TIRON [1,*], EUGEN CARASEVICI [1], CORNELIU S. STAN [2], SORIN ALEXANDRU IBANESCU [3], BOGDAN C. SIMIONESCU [2,3], CATALINA A. PEPTU [2]

Abstract: The antitumoral potential of three gel formulations loaded with carbon dots prepared from *N*-hydroxyphthalimide (CD-NHF) was examined and the influence of the gels on two types of skin melanoma cell lines and two types of breast cancer cell lines in 2D (cultured cells in normal plastic plates) and 3D (Matrigel) models was investigated. Antitumoral gels based on sodium alginate (AS), carboxymethyl cellulose (CMC), and the carbomer Ultrez 10 (CARB) loaded with CD-NHF were developed according to an adapted method reported by Hellerbach. Cell proliferation, apoptosis, and mitochondrial activity were analyzed according to basic methods used to evaluate modulatory activities of putative anticancer agents, in both classic 2D and 3D cultures. The presence of CD-NHF within the gels induces a slight decrease of the dynamic moduli, indicating a flexible gel structure. 3D cell cultures displayed visibly larger structure of tumor cells with less active phenotype appearance. The in vitro results for tested CD-NHF-loaded gel formulations revealed that the new composites are able to affect the number, size, and cellular organization of spheroids and impact individual tumor cell ability to proliferate and aggregate in spheroids.

Acknowledgment: This work was supported by a grant of the Romanian Ministry of Research and Innovation, PCCDI-UEFISCDI, project number PN-III-Pr-l.2.PCCDI-2017.0083contract nr 3TPCCDI din 01/03/2018.

[1] Regional Institute of Oncology, 2-4, General Henri Mathias Berthelot Street, 700483 Iasi, Romania;

[2] Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi, 700050 Iasi, Romania;

[3] "Petru Poni" Institute of Macromolecular Chemistry, 41A Aleea Grigore Ghica Vodă street, 700487 Iasi, Romania

*Corresponding author: crinatiron2003@yahoo.com; 0765677356,

IMIDE DERIVED CARBON DOTS – A NEW PROMISING APPROACH IN CANCER TREATMENT

GABRIEL LUTA[1], CRINA TIRON[1], CORNELIU S. STAN[2], ADRIAN TIRON[1], CATALINA A. PEPTU[2], FLORIN E. ZUGUN[1], BOGDAN C. SIMIONESCU[2, 3]

Abstract: Cancer represents a major health problem all over the world. Multiple and heterogeneous intrinsic molecular defects account for malignancy aggressive features. Distorted and inappropriate control of fundamental cell biology programs such as cell survival, cell suicide, cell differentiation, cell tissular architectural integration stand at the core of tumor development. Subsequently, one major goal in cancer therapy is to target either lezional, causative molecules or the proximal biochemical interactions at the origin of these defects. We speculate that the investigated imide derived Carbon Dots might amplify their biochemical interactions and confer new beneficial abilities relevant to cancer control. The effect of imide derived Carbon Dots were investigated in several cell culture types, both normal and neoplasic, and the results indicate promising antitumoral effects.

Acknowledgment: This work was supported by a grant of the Romanian Ministry of Research and Innovation, PCCDI-UEFISCDI, project number PN-III-Pr-l.2.PCCDI-2017.0083contract nr 3TPCCDI din 01/03/2018.

[1] Regional Institute of Oncology, Iasi Romania
[2] Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iaşi, Romania
[3]I nstitute of Macromolecular Chemistry "Petru Poni" Iasi, Romania

*Corresponding author: Crina Tiron, 0765677356, crinatiron2003@yahoo.com.

MOLECULAR OUTLINE/PROFILE OF HEAVY METAL RESISTANT BACTERIA ISOLATED FROM CONTAMINATED SOILS

BOGDAN HAȚEGAN[1], DORINA PODAR[1]

Keywords: Contaminated soil, Bacteria, Bioremediation, Heavy Metal Resistance, rRNA 16S.

Abstract: Microorganisms are most likely to adapt to environmental changes since the anthropic factors, like industrialization or mining, often result in unbalanced chemical composition of soil, water or air leading, in many cases, to high pollution. Understanding their resistance or tolerance mechanisms represent a step forward to environmental remediation employ resistant colony.
The aim of the study was to identify heavy metal resistant/tolerant/modifying bacteria from heavy metal contaminated soils in Transylvania, Romania. Bacteria isolated from soil samples, collected from contaminated sites, were tested for their ability to grow on nutrient agar media supplemented with different metals. Bacterial isolates able to withstand growth on heavy metals selective media were selected and identified based on rRNA 16S sequence. Presence of genes encoding for known enzymes that allows resistance/tolarance of heavy metals was assessed by PCR. Sequenced of 16S fragments were used for identification-based phylogeny using BLAST-NCBI.The result showed that predominant bacteria, isolated from contaminated soils, displaying heavy metals resistance are *Bacillus, Pseudomonas, Sphingobacterium* and *Arthrobacter* based on rRNA 16S PCR analysis. Presence of resistance gene was confirmed in heavy metal resistant bacterial isolates such as presence of MerA genes, was confirmed in certain strains.The MerA enzyme reduce Hg^{+2} to Hg^{0} which is less toxic for living cell. For this reason they could have a potential application in remediation of heavy metals contamintated soil.

[1] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania,

*Corresponding author: *dorina.podar@ubbcluj.ro*

BIOCOMPATIBILITY ASSESSMENT OF A HEMA/AMPS/LDH MATERIAL FOR SOFT TISSUE ENGINEERING APPLICATIONS

SIMONA-REBECA IGNAT [1], SORINA DINESCU [1,2], MIRELA ŞERBAN [1], IONUT RADU [3], CĂTĂLIN ZAHARIA [3], MARIETA COSTACHE [1,2*]

Keywords: tissue engineering, human adipose-derived stem cells, nanomaterials

Abstract: Soft tissue engineering involves carefully selecting the type of cells and scaffold's material to achieve the best outcome. In regard of choosing the biomaterial, copolymerization of two different monomers usually brings more advantages to the final material. In our study, we used (2-hydroxyethyl methacrylate) (HEMA) and 2-Acrylamido-2-methylpropanoesulfonic acid (AMPS). During recent years, some nanomaterials also gained interest for tissue engineering applications such as layered double hydroxides (LDH). The aim of this study was to evaluate the biocompatibility of HEMA/AMPS/LDH materials and select the ones with the greatest potential for future soft tissue engineering applications. Human stem cells isolated from adipose tissue (hASCs) were seeded in 3D materials with different concentrations of HEMA (95/97% wt.), AMPS (3/5% wt.) and LDH (1/2% wt.). Their biocompatibility was evaluated by quantitative and qualitative tests after 3 and 6 days. Cell viability and proliferation rate were evaluated by MTT test. Cytotoxicity was determined by the LDH assay. The LiveDead assay allowed simultaneous visualisation of dead and live cells by fluorescence microscopy. The MTT test showed the highest cell viability on the 95% HEMA 5% AMPS 2% LDH material after 3 days post-seeding hASCs in the materials. After 6 days, the same material showed the best results, suggesting a good copolymerization of HEMA with AMPS in 95% - 5% concentrations. The LDH assay showed that 97% HEMA 3% AMPS 1% LDH material induced the highest citotoxity. The Live/Dead assay confirmed the results from the quantitative tests and showed less live cells in the material with 97% HEMA 3% AMPS than in the materials with 95% HEMA 5% AMPS.
All of the materials evaluated proved to be biocompatible. The 95% HEMA 5% AMPS 2% LDH material showed the best results that recommend it for future soft tissue engineering applications.

Acknowledgements: This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0782/REGMED, within PNCDI III.

[1] Biochemistry and Molecular Biology Department, Faculty of Biology, University of Bucharest

[2] Research Institute of University of Bucharest

[3] Advanced Polymer Materials Group, University Politehnica of Bucharest

* Corresponding author: marieta.costache@bio.unibuc.ro, +40213181575, Blvd. Mihail Kogălniceanu, no. 36-46, 50107, Bucharest,

SYNERGISM AND ANTAGONISM IN AGING

GABRIELA BUNU[1*], CATALIN ION[1], TOREN D[1], SARGHIE L[1], TACUTU R[1]

Keywords: Synergism, Genetic mutants, Longevity-associated genes, Systems biology, Database

Abstract: Genetic interventions can modulate aging and determine lifespan in model organisms. This type of experiments have shown lifespan extension of up to 50% in mice and even up to ten times in nematodes. Currently, more than 2000 longevity-associated genes (LAGs) are known, however, when combining two or more genetic interventions, the effect is rarely additive, as genes can be epistatic and interact in nonlinear ways.

In order to evaluate the synergism and antagonism of LAGs, we developed a database with gene combinations that affect lifespan. Based on this data, our next aim was to use network analysis and characterize the synergism between genes with regard to their impact on longevity.

SynergyAge contains manually curated data from scientific articles with experimentally validated results, describing at least one long- or short-lived genetic model with at least two interventions (mutations, knockout, overexpression or RNA interference). We classify interactions as synergistic, almost additive, dependent or antagonistic and we included in the analysis only lifespan assays that include the double mutant, the two corresponding single mutants and the wild type in the same conditions.

The SynergyAge database contains almost 1000 combinations of genes and more than 3000 lifespan values for various single/double/triple mutants in *C. elegans*, *D. melanogaster* and *M. musculus*. The data is freely available and can be easily visualized through a user-friendly web interface. Here we also present the analysis of synergistic and antagonistic longevity-associated genes.

This work is supported by the Competitiveness Operational Programme 2014-2020, POC-A.1-A.1.1.4-E-2015.

[1] Computational Biology of Aging Group, Institute of Biochemistry, Romanian Academy, Bucharest, Romania

*Corresponding author: E-mail: gbunu@biochim.ro; Phone: 0735469333; Address: Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, Bucharest, Romania

COMPUTATIONAL ASSISTED WORK IN STRUCTURAL IMMUNOBIOLOGY

ANDREI-J. PETRESCU[1,*]

Abstract: Host deffence against pathogens in multicellular organisms rely on complex mechanisms of non-self molecular recognition. In turn these trigger the response of the system through either a wide range of innate immune pathways or, in the case of higher vertebrates, through the deployment of the adaptative immune arsenal - MHC, IG, TCR etc - which has at its core the receptor diversification by V(D)J recombination. Over the past decade we have successfully used in this field a computational assisted experimental strategy based on molecular modeling and simulation. Intertwining computation with experiment proved by this work to be twofold valuable. On one hand it allowed in the absence of stuctural data to generating increasingly accurate models of the structure, interactions and dynamics of these large immune machineries, based on sparse experimental and bioinformatics constraints. On the other hand the iteratively improved models were instrumental in elaborating working hypotheses regarding the functioning of these molecular systems; hypotheses that were further tested experimentally. We illustrate this approach by several results obtained on resistance R-gene families in plants, in the case of innate immunity (1,2,3); and on the recombination activating genes 1 and 2 machinery, in the case of adaptative immunity (4,5,6,7).

1. Maekawa T, Cheng W, Spiridon LN, Töller A, Lukasik E, Saijo Y, Liu P, Shen Q-H, Micluta MA, Somssich IE, Takken FLW, Petrescu A-J, Chai J, Schulze-Lefert P, "*Coiled-coil domain-dependent homodimerization of intracellular MLA immune receptors defines a minimal functional module for triggering cell death*", ***Cell Host-Microbe***, **9(3)**: 187-199 (2011)

2. Slootweg EJ, Spiridon LN, Roosien J, Butterbach P, Pomp R, Westerhof L, Wilbers R, Bakker E, Bakker J, Petrescu A-J, Smant G, Goverse A "*Structural Determinants at the Interface of the ARC2 and LRR Domains Control the Activation of the NB-LRR Plant Immune Receptors Rx1 and Gpa2.*", ***Plant Physiol.***, **161(3)**, 1510-1528 (2013)

3. Wróblewski T, Spiridon L, Martin EC, Petrescu AJ, Cavanaugh K, Jose-Truco M, Xu H, Gozdowski D, Pawłowski K, Michelmore RW, Takken FLW.. "*Genome-wide functional analyses of plant coiled–coil NLR-type pathogen receptors reveal essential roles of their N-terminal domain in oligomerization, networking, and immunity.*" ***PLOS Biology*** **16(12)**: e2005821 (2018)

4. Ciubotaru M, Trexler AJ, Spiridon LN, Surleac MD, Rhoades E, Petrescu A-J, Schatz DG. "*RAG and HMGB1 create a large bend in the 23RSS in the V(D)J recombination synaptic complexes.*", ***Nucl.Acid.Res.***, **41(4)**, 2437-2425 (2013)

5. Zhang YH, Shetty K, Surleac MD, Petrescu A-J, Schatz DG. "*Mapping and Quantitation of the Interaction between the Recombination Activating Gene Proteins RAG1 and RAG2.*", ***J.Biol.Chem***. **290(19)**, 11802-17. (2015)

6. Ciubotaru M, Surleac MD, Metskas LA, Koo P, Rhoades E, Petrescu A-J, Schatz DG., "*The architecture of the 12RSS in V(D)J recombination signal and synaptic complexes*" ***Nucleic Acid Res***, **43(2)**, 917–931 (2015)

7. Zhang Y, Cheng TC, Huang G, Lu Q, Surleac MD, Mandell JD, Pontarotti P, Petrescu AJ, Xu A, Xiong Y, Schatz DG. "Transposon molecular domestication and the evolution of the RAG recombinase.", ***Nature***. **569**:79-84 (2019)

[1] Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031 Bucharest 17, Romania

*Corresponding author: ap@biochim.ro

IMPROVING THE ACCURACY AND REPRODUCIBILITY OF MICROBIOME MEASUREMENTS ACROSS LABS

R. KEMP[1], S. TANG[1], E. BECKET[1], M. MAGER[1], M. VAN EDEN[1], S. FORMAN[1], E. KIRCHER[1], J. SHERMAN[1], L. BASILLIO[1], M. THAI[1], S. WONG[1], AND L. JIA[1]

PRESENTING AUTHOR: ADRIAN CRACIUN[2]

Abstract: The field of microbiomics has developed rapidly in the past several years. However, there are concerns due to poor data reproducibility across labs. To objectively assess the performance of different microbiomics workflows, it is essential to have accessible, well-defined, and accurately characterized mock microbial community standards to serve as reference materials for optimization, validation and controls of microbiomic workflows. Acknowledging this deficit, we have created well-characterized standards to be used as reference material for microbiome measurements. Using these, we assessed the performance of several of the most cited DNA extraction protocols used in the Microbiomics field and the effect of various library preparation techniques for 16S and shotgun sequencing. We found that the most commonly used protocols in this field for DNA extraction, including the HMP fecal DNA extraction protocol, are significantly biased. Most protocols over-represent the abundance of easy-to-lyse organisms, such as *Bacteroidetes*. Using the standards, we were also able to assess bias in the library preparation steps, such as GC bias in shotgun sequencing and PCR chimera in 16S sequencing. Thus, improving all steps involved from sample collection and DNA extraction to sequencing and bioinformatics will harmonize the data generated in this very fast expanding field of research.

[1] Zymo Research Corporation, Irvine, California*, US*

[2] Bio Zyme, Romania

COMPARATIVE TRANSCRIPTOMICS OF LONGEVITY: INSIGHTS FROM CROSS-SPECIES RNA-SEQ ANALYSIS

URSU EUGEN[1*], KULAGA ANTON[1], TOREN DMITRI[1], GUINEA RODRIGO[2], TACUTU ROBI[1]

Keywords: lifespan variability, bioinformatics, cross-species analysis, RNA-Seq

Abstract: Various species have different lifespans. This is in part due to genome differences, however particularities in the transcriptional patterns can also have an important role in ensuring long lifespan and resistance to diseases. The main goal of this work is to investigate whether long-lived species share common gene expression patterns that determine their exceptional longevity.

Investigating transcriptional patterns requires comprehensive transcriptomics analyses. While for comparative genomics established methods based on multiple sequence alignments and phylogenetics exist, the methods for comparative transcriptomics are still in their infancy and analyses are more challenging to carry out. As such, comparison of expression levels for different species involves many degrees of uncertainty and potential errors, including sample heterogeneity issues, differences in sequencing equipment and in sample preparation protocols, low quality of many *de novo* transcriptome and genome assemblies, errors in alignments and transcriptome assemblies. To address the above-mentioned challenges, we developed a novel analysis methodology based on the combination of Sparse PCA, Elastic Net regression, and modular subnetwork analyses.

To gain new insights into the expression patterns of long-lived animals, we applied the above-mentioned methodology to RNA-Seq samples for 5 tissues and 19 mammalian species with diverse lifespans. The results revealed a subset of genes which explain most of the variability and differences in species' lifespan. These genes were then divided into gene modules and functionally analyzed. Gene set enrichment analysis identified several biological processes and pathways which were not previously linked to lifespan variability.

Overall, the results obtained advance our understanding of the evolution of longevity transcriptomics and put emphasis on novel components that could determine species' lifespan.

[1]Institute of Biochemistry of Romanian Academy, Bucharest, Romania

[2]Pontifical Catholic University of Peru, Peru

*Corresponding author: Ursu Eugen, ursu_eugen@hotmail.com; +40731394355

DEVELOPMENT OF ADVANCED MASS SPECTROMETRIC PLATFORMS FOR GLYCOCONJUGATE MAPPING, BIOMARKER DISCOVERY AND STUDY OF THEIR FUNCTIONAL INTERACTIONS

ALINA D. ZAMFIR [1,*], MIRELA SARBU [1], ALINA PETRUT [1], RALUCA ICA [1], CRISTIAN V.A. MUNTEANU [2], ANCA SIMULESCU [3], ANDREI J. PETRESCU [2]

Keywords: mass spectrometric platforms; glycoconjugates; biomarker discovery; functional interaction.

Abstract: The last years have witnessed remarkable technical achievements among which, the development of high resolution mass spectrometry (HR MS) using orbital trap analyzers (Orbitrap MS), ion mobility separation incorporated into MS (IMS MS) and microfluidics-based systems for electrospray ionization (ESI). Since their first introduction, all these techniques demonstrated a high potential to discover novel biomolecular species and assess their structural and functional interactions due to the efficient ionization, high resolution and mass accuracy, minimization of the in-source fragmentation of labile groups and elevated sensitivity. In combination with multistage fragmentation of a chosen precursor ions, structural elucidation of even minor species in highly complex mixtures extracted from biological matrices, could be achieved as well.

The present study is focused on the implementation and optimization of these ultramodern methods for the identification, structural analysis and study of the noncovalent interactions of gangliosides (GGs) expressed in normal adult and fetal brain as well as of those species associated to severe brain pathologies. Hence, fully automated chip-based ESI, IMS MS, HR MS and multistage MS on Orbitrap approaches were developed by our group for mapping and fragmentation of GGs in native mixtures extracted from different normal and diseased (neurodegeneration, primary and secondary brain tumors brain) regions. The purpose of the study was the discovery of novel biomarkers, their detailed structure elucidation and determination of the interactions of gangliosides with proteins, which might play a significant biological role at the level of human central nervous system.

Our findings and the structural data collected upon novel species and their interactions indicate that the advanced MS has real perspectives to become a routine method for early diagnosis of brain afflictions, based on the determination of ganglioside fingerprints.

Supported by the Romanian National Authority for Scientific Research, UEFISCDI through projects PN-III-P4-ID-PCE-2016-0073, PN-III-P1-1.2-PCCDI-2017-0046 granted to ADZ and PN-III-P1-1.1-PD-2016-0256 granted to MS.

[1] National Institute for R&D in Electrochemistry and Condensed Matter, Timisoara, Romania

[2] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

[3] University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

* Correspondence author: Prof. Dr. Alina D. Zamfir, Phone 0040-728615639, Plautius Andronescu Str. 1, RO-300224, alina.zamfir@uav.ro

SHIFTING THE BALANCE TOWARDS AN ANTI-TUMORIGENIC MELANOMA MICROENVIRONMENT VIA CO-ADMINISTRATION OF LIPOSOME-ENCAPSULATED SIMVASTATIN AND DMXAA *IN VIVO*

VALENTIN-FLORIAN RAUCA [1,2,*], LAURA PATRAS[1,2], LAVINIA LUPUT[1,2], ALINA SESARMAN[1,2], EMILIA LICARETE[1,2], VLAD TOMA[1], AUGUSTIN C. MOT[3], MANUELA BANCIU[1,2]

Keywords: melanoma, microenvironment, macrophages, angiogenesis, targeted therapy

Abstract: The aberrant activation of multiple interconnected signaling pathways poses a big challenge to scientists trying to pinpoint the specific mechanisms of melanoma progress and development need to overcome. On the other hand, as an exponent of reconfigured signaling pathways, melanoma is an attractive option for targeted drug development [1] and microenvironment-targeted therapies. The ability of co-administered simvastatin and DMXAA (5, 6-dimethylxanthenone-4-acetic acid) to suppress the aggressive phenotype of B16.F10 melanoma cells co-cultured with tumor associated macrophages under hypoxia-mimicking conditions was already demonstrated by our *in vitro* studies [2]. Therefore, our next aim was to encapsulate the two therapeutic agents in efficient delivery systems represented by long circulating liposomes and to test them on a more complex *in vivo* murine melanoma model. Consistent with our previous findings, the combined liposomal drug therapy inhibited the expression/production levels of several key molecules involved in promoting the invasive capacity of tumor cells (HIF-1α, pAP1-cJun, MMP-2, MMP-9, IL-1β). The immunohistochemical examination of tumor tissues revealed a decrease in tumor angiogenesis and an increase in macrophage infiltration. Decreased expression of ARG-1 and iNOS in the context of an abundant macrophage infiltration suggests an immunomodulatory effect of the liposomal combined therapy which might overcome drug resistance. In conclusion, this novel targeted therapy holds the potential to shift the balance towards an anti-tumorigenic melanoma microenvironment.

[1] Lopez-Bergami P. et al., Photochem Photobiol 84(2): 289–306, 2008
[2] Rauca V.F. et al., PLoS One 13(8): e0202827, 2018.

Acknowledgement: This work was supported by grants of the Romanian Ministery of Research and Innovation, CNCS–UEFISCDI,
project number PN-III-P4-ID-PCE-2016-0342, contract number 91/2017 within PNCDI-III.

[1] Department of Molecular Biology and Biotechnology of Babes-Bolyai University, Cluj-Napoca, Romania;

[2] Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences of Babes-Bolyai University, Cluj-Napoca, Romania;

[3] Department of Biochemistry and Biochemical Engineering of Babes-Bolyai University, Cluj-Napoca, Romania;

*Corresponding author: PhD student Rauca Valentin-Florian, valirauca@yahoo.co.uk; +40748224469; Babes-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street 400006, Cluj-Napoca Romania

HIGH-THROUGHPUT SCREENING TO IDENTIFY RAGE INHIBITORS FOR CANCER THERAPY

IOANA POPA*, CARMEN ALEXANDRA TANASE*, FLORIN PASTRAMA

Abstract: The receptor for advanced glycation end products (RAGE) is a multiligand receptor, as it may bind a variety of pro-inflammatory endogenous, as well as pathogen-derived molecules, including members of S100 protein family, high mobility group box protein-1, amyloid peptides and fibrils, lipopolysaccharide or F-protein from respiratory syncytial virus. RAGE-ligand interactions have been intensively studied in relation with their contribution to pathological alterations in inflammatory disorders, diabetes, neurodegeneration and cancer, and the possibility to be targeted for therapeutic interventions. Of these interactions, S100P-RAGE axis was shown to be involved in pancreatic and colon cancers. Our goal is to find new compounds to block the oncogenic pathways driven by S100P-dependent RAGE activation.

In this study we describe two fluorescence-based methods which we set up to measure RAGE-S100P binding and be rapidly and effectively used in high-throughput screening for compounds that can interfere with the binding. One method relies on the specific binding of a EGFP fusion of S100P to soluble RAGE, pre-adsorbed in 96-well plates. The second method employs a time-resolved FRET pair using S100P-EGFP and an antibody conjugated with Terbium chelate directed against the His-tag of the soluble RAGE.

We discuss the advantages of each method, their use for competitive binding assays and the results of a natural product library screening which led us to the identification of several molecules that can block RAGE-S100P interaction. The evaluation of the newly found inhibitors in cell culture cancer models is currently undertaken.

In conclusion, we designed and developed two reliable, rapid and inexpensive methods to be used for high-throughput screening of compounds, and identified several putative S100P-RAGE inhibitors.

This work was supported by Grant No 159/2017, PN-III-P2-2.1-PED-2016-2000, funded by the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), Romania.

[1] Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, Bucharest 060031, tel/fax:0212239069/68, *contributed equally

*Corresponding author: ipopa@biochim.ro, ctanase@biochim.ro

THE DUAL ROLE OF TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN 3D BREAST CANCER CELL MIGRATION

DANIEL CRUCERIU [1,2*], OANA BALDAȘICI [1,3], LOREDANA BĂLĂCESCU [1], OVIDIU BĂLĂCESCU [1,4], DANIEL IRIMIA [5], OANA TUDORAN [1]

Keywords: breast cancer, TNF-α, 3D cell migration, microfluidic technology.

Abstract. TNF-α is one of the most important pro-inflammatory cytokines found in the breast tumor microenvironment, strongly influencing the fate of the tumor. It can trigger signals for cell proliferation, survival and invasion, but also for apoptosis, depending on the specific cellular context and the molecular traits that characterize each cell line or tumor. However, the significance of TNF-α signaling in breast cancer (BC) metastasis remains unclear, due to its dual role in shaping the malignant phenotype. Therefore, we evaluated the physiological and molecular effects related to BC cell migration in response to TNF-α. The migration capacity of seven BC cell lines was evaluated in microfluidic devices and their migration speed was correlated with their molecular signature. The gene expression of 493 genes was correlated (rho $> \pm 0.8$; $p < 0.05$) with the migratory phenotype. TNF-α was found to be one of the most important upstream regulators of the signaling networks in which these 493 genes participate, suggesting its involvement in cell migration. In order to assess the impact of TNF-α signaling on the migration capacity, the TNF-α-receptor TNFR1 was silenced by siRNA in three BC cell lines (T47D, MDA-MB-468, MDA-MB-231). After TNFR1 silencing, cell migration velocity was evaluated in microfluidic devices, while the molecular effects triggered by TNFR1 inhibition were monitored by RT-qPCR. According to our results, TNF-α stimulates cell migration in mesenchymal-like breast cancer cell lines, while it inhibits the migration of epithelial-like cancer cells. Several genes involved in cell motility, such as *RUNX2, PLAUR* and *VEGFA* were down-regulated in all TNFR1-siRNA treated cell lines, while *JAG1* was inhibited only in the mesenchymal-like MDA-MB-231 cell line. Corroborating all these results, TNF-α seems to play a dual role in BC cell migration, with JAG1 being a possible decisive molecule in the acquisition of the TNF-α-induced migratory phenotype.

[1] Department of Functional Genomics and Experimental Pathology, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania.

[2] Department of Molecular Biology and Biotechnology, "Babes-Bolyai" University, Cluj-Napoca, Romania

[3] Department of Farmaceutical Technology and Biofarmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[4] 11th Department of Medical Oncology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[5] Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, Shriners Hospital for Children, Boston, Massachusetts, USA.

*Corresponding author: Daniel Cruceriu, dani_cruceriu@yahoo.com, Tel: +40-740015058 Fax: +40-264431878;"Babes-Bolyai" University, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street, Cluj-Napoca 400006, Romania

ROLE OF TISSUE TRANSGLUTAMINASE IN ANTI-TUMOR IMMUNE RESPONSE

LIVIA E. SIMA[1#*], HORACIO CARDENAS[1], SIQI CHEN[2], YINU WANG[1], HAO HUANG[1], GUANYUAN ZHAO[1], BIN ZHANG[2], DANIELA MATEI[1]

Keywords: ovarian cancer, tissue transglutaminase, immune response, ID8 cells, tumor microenvironment

Abstract: Tissue transglutaminase (TG2) is a multifunctional protein - it can perform enzymatic (transglutaminase, isopeptidase, protein disulfide isomerase), GTPase and extracellular matrix (ECM) scaffold activities. TG2 is overexpressed in cancer and is involved in metastasis, resistance to chemotherapy, and cancer stem cell signaling to the tumor niche. The functions of TG2 in cancer cell lines have been already described for several types of solid tumors. However, little is known about the role of TG2 in the host in cancer models. We hypothesized that by knocking-out TG2 in the host ($TG2^{-/-}$) the OC tumor progression will be altered. We used the Roby model of murine ID8 ovarian cancer cells injected *ip* to investigate tumor progression and anti-tumor immune response in wild-type (WT) and $TG2^{-/-}$ mice.

We observed a significant decrease in tumor burden in $TG2^{-/-}$ *vs.* WT mice as evidenced by less ascites accumulation, increased median survival, and decreased number of cancer cells in ascites. This phenotype was accompanied by significant changes in anti-tumor immunity, as revealed by flow cytometry examination of major immune cell subsets in spleens and ascites from tumor-bearing animals. $CD8^+$ cells recovered from ascites or spleens of $TG2^{-/-}$ mice expressed higher levels of PD-1. *Ex vivo* stimulation of T cells from ascites revealed a significant increase in responsive IFNγ-secreting $CD8^+$ and $CD4^+$ cells in $TG2^{-/-}$ compared with WT mice. Interestingly, $TG2^{-/-}$ mice ascites contained an increased number of effector/memory $CD8^+$ T cells. Concomitantly, we found an increase of pStat3 levels in $TG2^{-/-}$ memory $CD8^+$ T cells. Expression of PD-L1 was decreased on all myeloid subsets as well as on $EpCAM^+$ cells in $TG2^{-/-}$ ascites.

Collectively, our data suggest decreased tumor burden concurrently with increased activation and effector functions of T cells, and loss of immunosuppressive signals in the tumor microenvironment resulting in development of an anti-tumorigenic phenotype in $TG2^{-/-}$ mice.

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, USA

[2] Department of Medicine-Division of Hematology/Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, 60611, USA

[#]Current affiliation: Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, 060031, Romania

*Corresponding author: livia_e_sima@yahoo.com; lsima@biochim.ro; Phone: +4021-2239069; Fax: +4021-2239068; Institute of Biochemistry, 296 Spl. Independentei, 060031, Bucharest, Romania

NOVEL SYNTHETIC FLAVONOIDS WITH POTENT ANTIMICROBIAL PROPERTIES: A POTENTIAL SOLUTION TO FIGHT ANTIBIOTIC RESISTANCE.

CORNELIA BABII [1], ANCA NEAGU [1], IRINA GOSTIN [1], MARIUS MIHASAN [1], COSMIN TEODOR MIHAI [2], LUCIAN BARSA[1], MARIUS STEFAN[1,*]

Abstract: The emergence of pathogenic multidrug resistant microorganisms demands new approaches in finding effective antimicrobial agents. Synthetic flavonoids could be a reliable solution due to their important antibacterial and antifungal activity. We report here the potent *in vitro* antimicrobial activity of a new class of synthetic sulfur tricyclic flavonoids. The antimicrobial effects were tested using the minimum inhibitory concentration (MIC), time kill and biofilm formation assays. Fluorescence microscopy and scanning electron microscopy were employed to study the mechanism of action. MTT test was used to assess the cytotoxicity. Our results showed that Gram positive bacteria were more sensitive (MIC = 0.24 μg/mL) to flavonoids compared to the Gram negative ones (MIC = 3.9 μg/mL). Also the tested flavonoids showed a very good anti-Candida activity (MIC values as low as 15.62 μg/mL). We found that our compound showed significantly enhanced antibacterial activities, 32 to 72 folds more active than other synthetic flavonoids. Our flavonoids showed a bactericidal and fungicidal activity at concentrations ranging from 0.48 to 31.25 μg/mL. At twice the MIC, all *Escherichia coli* and *Klebsiella pneumoniae* cells were killed within 1 h. Also the flavonoids presented a good anti-biofilm activity. The mechanism of action is related to the impairment of the cell membrane integrity. No or very low cytotoxicity was evidenced at effective concentrations against Vero cells. Based on the strong antibacterial activity and cytotoxicity assessment, our synthetic flavonoids have a good potential for the design of new antimicrobial agents.

[1] University Alexandru Ioan Cuza of Iasi, Iasi, Romania

[2] University of Medicine and Pharmacy Grigore T Popa, Iasi, Romania

* Correspondence author: stefanm@uaic.ro; phone – 0232201524; postal address - University Alexandru Ioan Cuza of Iasi, Bld Carol I, nr 11, Iasi, jud. Iasi, Romania

FUNCTIONAL INTERRACTION BETWEEN THE TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL MEMBER 8 (TRPM8) AND THE PROSTACYCLIN RECEPTOR (PTGIR)

ALEXANDRA-M. BANICA[1*], ALEXANDRU BABES[2], STEFAN SZEDLACSEK[1], SORIN TUNARU[1]

Keywords: GPCR, ion channels, signal transduction

Abstract: Transient Receptor Potential Cation Channel Member 8 (TRPM8) belongs to a superfamily of ion channels known as Transient Receptor Potential (TRP). TRPM8 is involved in the detection of sensations such as coolness, being activated by temperatures lower than 25˚C. Menthol and icilin, among others, function as agonistic modulators of TRPM8 activity. TRPM8 is a cation channel, being permeable to monovalent and bivalent cations, such as sodium, potassium and calcium.

In humans, TRPM8 is highly expressed in the liver, smooth muscle cells, prostate, pancreatic-β cells and periferic neurons. The biological role of TRPM8 in these anatomical entities remains unclear.

Prostacyclin receptor (PTGIR) belongs to the superfamily of 7-transmembrane spanning receptors also known as G-protein coupled receptors (GPCRs). PTGIR is activated by endogenous ligands known as prostanoids, which are arachidonic acid-derived eicosanoids.

Physiologically, PTGIR is activated by prostaglandin I2 (PGI2) to induce mainly vascular effects such as vasorelaxation of high-resistance arteries. In addition, PGI2 has antithrombotic effects by inhibiting platelets aggregation. All these effects are mediated by the property of PTGIR to couple to the intracellular Gs proteins which in turn activate membrane-bound adenylyl-cyclases (ACs) leading to the elevation of intracellular cAMP concentrations.

TRPM8 and PTGIR have been shown to be co-expressed in the smooth muscle cells of arteries.

Here we show that the activation of PTGIR by a synthetic agonist cicaprost impairs icilin-induced TRPM8 activation in HEK293T cells in a concentration- and time-dependent manner, although cicaprost does not function as an agonist or antagonist of TRPM8. Moreover, the inhibitory effect of cicaprost on TRPM8 functionality does not depend on the accumulation of intracellular cAMP, as demonstrated by experiments with forskolin and cholera toxin (adenilyl cyclase activators). Conversely, the activation of TRPM8 with icilin seems to also reduce the efficacy of cicaprost at PTGIR.

In conclusion, we have found a functional interaction between an ion channel (TRPM8) and a GPCR (PTGIR) in HEK293T cells. Future work will determine the relevance of this interaction *in vivo*.

[1] Institute of Biochemistry of the Romanian Academy, Dept. of Enzymology, 060031, Bucharest, Romania

[2] Department of Anatomy, Physiology and Biophysics, Faculty of Biology, University of Bucharest, 050095 Romania.

*Corresponding author: alexandrabnc29@gmail.com; Phone: +4021-2239069; Fax: +4021-2239068 Adress: Institute of Biochemistry, 296 Spl. Independentei, 060031, Bucharest, Romania

IDENTIFICATION OF PHOSPHOINOSITIDE METABOLIZING ENZYMES AS NEW HOST FACTORS INVOLVED IN HCV LIFE CYCLE

DAVID PATRICHE[1], MUNTEANU CRISTIAN [1] , OVIDIU VLAICU[2], PASTRAMA FLORIN [1], BANICA LEONTINA [2], PARASCHIV SIMONA [2], OTELEA DAN [2], ROUILLES YVES [3], DUBUISSON JEAN [3], POPESCU COSTIN-IOAN [1*]

Keywords: Hepatitis C Virus, phosphoinositide, virus-host interaction, proteomics

Abstract: Hepatitis C Virus (HCV) represents a global health problem with 71 million people being infected worldwide. HCV infection becomes chronic in 70% of the infected patients inflicting liver pathology raging from steatosis to hepatocellular carcinoma. The host factors involved in the progression of the liver disease induced by HCV are not completely understood. To identify new host factors involved in HCV life cycle and possibly in the disease progression, we characterized the endogenous interactome of viral proteins involved in different steps of HCV life cycle. Since lipid metabolism is deeply connected to HCV life cycle and liver pathology, we focused our analysis on several phosphoinositide (PtdIn) metabolizing enzymes (PME). The expression of the different host factors was modulated by CRISPR/Cas9 genome editing. Functional assays specific for each step in the viral life cycle showed that PMEs are involved in both early and late steps in HCV life cycle. Direct protein-protein interactions were confirmed by co-immunoprecipitation in infected cells and heterologous systems. Subcellular co-localization experiments were also performed using confocal fluorescence microscopy. Mutant rescue experiments are in progress to characterize the structural determinants of PMEs which are involved in their function in the virus life cycle. The present work may extend our understanding of the role of PtdIns in HCV life cycle and the intracellular membrane traffic.

[1] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

[2] National Institute of Infectious Disease „Matei Bals", Bucharest, Romania

[3] Hepatitis C Laboratory, Center for Infection and Immunity of Lille, University Lille Nord de France, CNRS-UMR8204, Inserm-U1019, Pasteur Institute of Lille, Lille, France

*Corresponding author: Costin-Ioan Popescu, pop@biochim.ro; Tel/Fax: 0040212239069/8, Splaiul Independentei 296, 060031, Bucharest

GRASP55 REGULATES IRE1α ACTIVITY WHICH IN MACROPHAGES CONTROLS INTERLEUKIN-1β SECRETION AND AGGREGATION

MARIOARA CHIRITOIU[1,3*], NATHALIE BROUWERS[1], GABRIELE TURACCHIO[2], MARINELLA PIROZZI[2], STEFANA M. PETRESCU[3], VIVEK MALHOTRA[1]

Abstract: Interleukin (IL)-1β, a major pro-inflammatory cytokine, is produced as response to tissue damage and infections by cells of the immune system, primarily tissue resident or circulating macrophages and monocytes. It is synthesized as inactive form in the cytoplasm (*pro*IL-1β) and it is cleaved by caspase-1 to mature *m*IL-1β, which is secreted to the extracellular space without entering the endoplasmic reticulum-Golgi pathway. We report that processing and export of endogenous IL-1β from macrophages is sensitive to inhibitors of the unfolded protein response (UPR). PERK inhibition affects caspase-1 proteolytic activity thereby controlling the amount of *m*IL-1β generated. On the contrary, inhibition of IRE1α RNase activity impairs *m*IL-1β secretion, which assembles into intracellular aggregates. Moreover, we found that GRASP55 deletion impairs IRE1α mediated *XBP-1* mRNA splicing, without affecting the PERK pathway and enhances endogenous *m*IL-1β aggregation. We propose GRASP55 as novel regulator of IRE1α/XBP-1 signaling pathway, which in turn controls the secretion and aggregation of IL-1β in macrophages.

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute for Science and Technology, Barcelona, Spain

[2] Institute of Protein Biochemistry, National Research Council, Naples, Italy

[3] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

*Corresponding author: Marioara Chiritoiu, mari.chiritoiu@biochim.ro; Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independentei, 296, 060031, Bucharest, Romania

ANTI-ACETYLCHOLINESTERASE AND PRO-COGNITIVE PROFILE OF COTININE AND 6-HYDROXY-L-NICOTINE IN AN Aβ$_{25-35}$-INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

RAZVAN STEFAN BOIANGIU[1], MARIUS MIHASAN[1], LUCIAN HRITCU[1*]

Keywords: nicotine, cotinine, 6-hydroxy-L-nicotine, Alzheimer's Disease, β-amyloid 25-35 peptide

Abstract: Alzheimer's disease (AD) is the most common and severe form of dementia. Worldwide, it is estimated that 46.8 million people suffer from dementia and by 2050, this number is expected to reach approximately 131.5 million people due to increasing numbers of elderly people. One of the neuropathological features of AD is represented by the degeneration of cholinergic neurons in the basal forebrain. At the cognitive level, the main hallmark of AD is memory decline. Nicotinic acetylcholine receptors (nAChRs) modulate the neurobiological processes underlying hippocampal learning and memory. Nicotine stimulates nAChRs thus improving the attention, memory and learning. However, nicotine's cardiovascular and addictive side-effects have limited its therapeutic use in AD but remain a strong scaffold for developing new drugs for AD. Cotinine (COT) and 6-hydroxy-L-nicotine (6HLN), two nicotine derivatives that are structurally similar, were found to possess antioxidant and cognitive-enhancing properties without showing the side-effects of their precursor. In this study, we used *in silico* tools to evaluate and compare the binding potential of COT and 6HLN in two different allosteric binding sites (α4-α4 and α4-β2) of human α4β2 nAChRs (PDB ID 6CNK). We have also performed a series of *in vivo* tasks to assess the effects of COT and 6HLN on memory impairment in a rat model of AD induced by brain infusion of Aβ$_{25-35}$ peptide. The acetylcholinesterase (AChE) activity was also measured. Our results showed that COT and 6HLN bind preferentially and with higher energy than nicotine to α4-β2 compared to α4-α4 interface of α4β2 nAChRs. COT and 6HLN administration mitigate the spatial and recognition memory deficits and decreased the specific activity of AChE in the hippocampus of Aβ$_{25-35}$-treated rats. These results suggest that COT and 6HLN might represent new therapeutic agents in AD by modulating cholinergic activity.

This work was supported by a grant of CNCS-UEFISCDI, project number PN-III-P1-1.1-TE-2016-0367, within PNCDI III.

[1] Alexandru Ioan Cuza University, Faculty of Biology, 20A Carol I Bd., 700506 Iasi, Romania

*Corresponding author: hritcu@uaic.ro, +40740601303

CYTOTOXIC ANTIMICROBIAL PEPTIDES TESTED ON TUMOR CELL LINES FOR TUMORICIDAL EFFECT

DANIELA JITARU[1], LOREDANA MIHAIELA DRAGOS[1]

Keywords: tumor cell line, cationic peptides.

Abstract: There are studies showing that some cationic peptides like dermaseptin and lycotoxin known to have antimicrobial cytotoxic effect have also tumoricidal potential for tumor cell lines.
Tumor cell lines were incubated for 48 hours with dermaseptin and lycotoxin peptides. Cell viability was tested by using two techniques: MTT and flow cytometry. Gene expression of molecular targets involved in the molecular pathways of survival, growth, proliferation and apoptosis was determined in the presence or absence of the studied peptides by optimized molecular biology techniques.
Dermaseptin activated tumor cell apoptosis, especially for the HT29 line (colorectal carcinoma), but also for A549 (pulmonary alveolar carcinoma), by increasing gene expression of *CHOP* and by lowering *BCL2* gene expression. Oxidative stress determined the increase in gene expression of *XBP*, which directly influenced *CHOP*. The decrease in *NRF2* gene expression highlighted the inhibition of cell proliferation, while the decrease in *HIF1alpha* gene expression evidenced the decrease in cell survival. The variation of *HIF1alpha* was correlated with the increase in IRE 1alpha gene expression, as a result to cellular oxidative stress under the action of the peptide. The tumoricidal properties of lycotoxin were analyzed using the same panel of genes: *CHOP*, *BCL2* (involved in apoptosis's activation), *AKT* (role in inhibition of protein synthesis) and *NRF2* (important role in tumor proliferation and invasion).
Studies have shown that dermaseptin and lycotoxin have a tumoricidal potential, with close interdependence between the peptide dose and the magnitude of the effect and type of tumor cell.

[1] Regional Institute of Oncology, Iasi

*Corresponding author: danielajitaru@yahoo.com

ENZYMATIC DIAGNOSIS OF LYSOSOMAL STORAGE DISORDERS USING SUBSTRATES FOR FLUORIMETRY AND MRM-MASS SPECTROMETRY

LAURA ION[1,2], CRISTINA DIMITRIU[4], MICHAEL PRZYBYLSKI[2], BRINDUSA ALINA PETRE[1,2,3]

Abstract: The activity loss or lack of specific lysosomal enzyme is a characteristic for a group of genetic metabolic disorders called lysosomal storage disorders (LSDs). The majority of LSDs (e.g., neuronal ceroid lipofuscinoses, mucopolysaccharidosis, Fabry's Disease; Gaucher's Disease, etc.) result from defective lysosomal acid hydrolysis of endogenous macromolecules and their consequent accumulation leading to multiple organ failure (e.g., skeletal malformations, pulmonary deficits, short stature, retarded growth etc.) and finally death. Therefore, an early diagnosis represents a critical step during the patient's clinical stage.

Currently, LSDs diagnostic uses in first stage biochemical tests, such as enzymatic determinations by fluorimetry or by mass spectrometry (limited cases) with the aid of dry blood spots (DBSs) based on different substrates-structures.

Here, we describe highly specific and sensitive diagnostics on DBSs for (i) molecular determinations of LSDs, particularly mucopolysaccaridoses (MPSs) and neuronal ceroid lipofuscinoses (NCLs), by simultaneous fluorimetric and mass spectrometric analysis using newly synthesized and standard derivatives; (ii) clinical diagnostics of LSDs by multiplex-MS-MRM analysis using specific substrate. The enzymatic activity levels in DBSs were determinate by fluorimetry or MRM-MS in the presence of an internal standard (4-ethylumbelliferone) showing a good statistical correlation in singles assays. Moreover, we developed duplex and triplex assays for the diagnosis of different LSDs from the muccopolysacharidoses family using modified substrates based on different coumarin derivates obtained through Pechmann condensation. The data obtained suggest that the new mass spectrometric assay is fast, reliable and can be successfully used in clinical trials for quantification of umbelliferone derivatives as products of enzymatic reactions either by fluorimetry or mass spectrometry.

In conclusion, the established method for enzyme activity determinations can be easily validated and used for neonatal screening in specialized laboratories, leading for a genetic confirmation only for the cases with low enzyme activity.

[1] Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi, Romania

[2] Steinbeis Centre for Biopolymer Analysis and Biomedical Mass Spectrometry, Ruesselsheim, Germany

[3] TRANSCEND - Regional Institute of Oncology, Iasi, Romania

[4] Department of Biochemistry, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania

*Corresponding author: brindusa.petre@uaic.ro

DETERMINING THE ASSOCIATION BETWEEN THE METABOLIC SYNDROME AND TWO GENES APOA5 AND ACE BY THE ANOVA ONE WAY MATHEMATICAL ALGORITHM

ALEXANDRA ALINA STANISLAV[1,2] *, TATIANA VASSU-DIMOV[1]

Keywords: genes, APOA5, ACE, Metabolic Syndrome.

Abstract: The APOA5 (rs662799 and rs3135506) gene is associated with the metabolic syndrome (MS), and the ACE ID polymorphism is associated with diabetes mellitus, high blood pressure, and obesity. The aim of this study was to identify the high risk of developing MS in the gene APOA5(rs662799 and rs31315506) and gene ACE DD(RS1799752) on the basis of clinical data and biochemical laboratory investigations, in patients, by using the ANOVA One Way mathematical algorithm.

Both genes were sequenced by the Advance NGx-GWAS test, at 126 subjects for MS at Giurgiu County Emergency Hospital; on the basis of these parameters: BMI, HBP, cholesterol, triglycerides, HDL-cholesterol, glucose, LDL-cholesterol and uric acid were prelucrated with Graph Pad Prism 7, Matlab R2009b and Quattro Pro X3.

The results obtained by the bioinformatics programs were the same: for gene APOA5 rs662799, BMI, Cholesterol, HTA, HDL, cholesterol, LDL, uric acid; and HBP are associated with MS; and glucose, triglycerides are not significantly associated. For gene APOA5 rs3135506, the results are not significantly associated with MS. For gene ACE RS1799752 the BMI, HDL, cholesterol, LDL, uric acid, HBP is associated with MS and; glucose, triglycerides are not significantly associated with MS.

Results of this two genes APOA5 and ACE present a high risk of developing the metabolic syndrome.

[1] University of Bucharest, Faculty of Biology, Department of Genetics, Bucharest, Intrarea Portocalelor, nr. 1-3

[2] Clinical Laboratory, Giurgiu County Emergency Hospital, Giurgiu, str. București no. 82

* Corresponding author: SAalexy@yahoo.com, +40 723213564, Clinical Laboratory, Giurgiu County Emergency Hospital, Giurgiu, str. București no. 82

SCREENING AND SEQUENCING OF LIPID-LINKED CARBOHYDRATES IN HUMAN CAUDATE NUCLEUS BY NANOELECTROSPRAY IONIZATION HIGH RESOLUTION TANDEM MASS SPECTROMETRY

ALINA PETRUT [1]*, CRISTIAN V.A. MUNTEANU [2], MIRELA SARBU [1], RALUCA ICA [1], ALINA D. ZAMFIR [1], ANDREI J. PETRESCU [2]

Keywords: high resolution mass spectrometry; gangliosides; caudate nucleus; nanoelectrospray ionization.

Abstract: Gangliosides (GGs), sialylated glycosphingolipids are involved in many molecular and cellular brain functions. Compositional and structural elucidation of GGs in mixtures extracted from human brain is essential for correlating their profile with the specialized function of each brain area in health and disease.
As a part of our ongoing studies on GG expression, structure and role in various regions of the human brain in health and disease, we developed and applied here an advanced mass spectrometry (MS) method based on high resolution Orbitrap MS and CID tandem MS for the investigation of GGs in a specimen of normal human caudate nucleus (CN). Optimized chip-nanoESI MS method made possible the identification in CN of a large number of glycoforms exhibiting high heterogeneity in their sugar core structure and lipid composition in only 2 min of signal acquisition. Though the native GG mixture from CN was found dominated by mono-, di- and trisialylated GGs, several highly sialylated species were also identified. Moreover, several glycoforms modified by fucosyl (Fuc), *O*-acetyl (*O*-Ac) were also discovered. Therefore, this atypical incidence of polysialylated, fucosylated and *O*-acetylated GGs and in particular of those exhibiting long-chain fatty acids in their Cer portion could be strongly correlated with the specific functions of CN. By CID tandem MS, atypical species could be confirmed and structurally characterized. Finally, by using this approach, a more realistic representation of GG heterogeneity in human brain, when compared with the ganglioside pattern obtained by convention analytical methodologies, can be achieved. Such findings provide more information on the role of rare structures in the ethiopathogenesis of CN-related neurodegenerative disorders.

Supported by the Romanian National Authority for Scientific Research, UEFISCDI through projects PN-III-P4-ID-PCE-2016-0073, PN-III-P1-1.2-PCCDI-2017-0046 granted to ADZ and PN-III-P1-1.1-PD-2016-0256 granted to MS.

[1] National Institute for R&D in Electrochemistry and Condensed Matter, Timisoara, Romania

[2] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

* Correspondence author: Alina Petrut, alina_mld@yahoo.com; Phone 0040-723851984, Plautius Andronescu Str. 1, RO-300224,

GLYCOSPHINGOLIPIDOMICS OF HUMAN CEREBELLUM BY ORBITRAP MULTISTAGE MASS SPECTROMETRY

RALUCA ICA [1], CRISTIAN V.A. MUNTEANU [2], ALINA PETRUT [1], MIRELA SARBU [1], ANDREI J. PETRESCU [2], ALINA D. ZAMFIR [1]

Keywords: glycosphingolipids; human cerebellum; nanoelectrospray ionization; mass spectrometry

Abstract: Gangliosides (GGs) are present and concentrated on cell surfaces, with the ceramide moiety embedded in the plasma membrane and the oligosaccharide chain located on the extracellular surface, where they constitute points of recognition for extracellular molecules or surfaces of neighbouring cells. Moreover, gangliosides are involved in a series of biological and pathological processes.
The introduction of electrospray (ESI) ion sources into biological mass spectrometry (MS) addressed the fundamental issue of how to analyze minute amounts of complex biological samples. A strategy to characterize and monitor the changes with age of the GG profile in different developmental stages of human cerebellum, by ESI Orbitrap MS and tandem MS (MS^2) is presented here. Two native ganglioside mixtures originating from normal human fetal cerebellum in the 15th (Cc15) and 40th (Cc40) gestational week were subjected to Orbitrap MS and multistage MS (MS^n) by collision induced dissociation (CID) analysis under thoroughly optimized experimental conditions. After only 2 min of signal acquisition in the negative ion mode, over 100 species have been detected and identified, among them several potential biomarkers. Although a similar number of species were identified in both mixtures, the GG profile was found to be essentially different. Obtained results also indicated differences in the expression of polysialylated species in the two ganglioside mixtures, which support an earlier hypothesis regarding the direct correlation between sialylation degree and brain developmental stage.
The method provided elevated ionization efficiency, high speed of analysis, almost 100% in-run and run-to-run reproducibility at a sample consumption per experiment situated in the femtomole range.

Supported by the Romanian National Authority for Scientific Research, UEFISCDI through projects PN-III-P4-ID-PCE-2016-0073, PN-III-P1-1.2-PCCDI-2017-0046 granted to ADZ and PN-III-P1-1.1-PD-2016-0256 granted to MS.

[1] National Institute for R&D in Electrochemistry and Condensed Matter, Timisoara, Romania

[2] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

* Correspondence author: Raluca Ica, Phone 0040-773974813, Plautius Andronescu Str. 1, RO-300224, raluca.ica@gmail.com

AN ADVANCED MASS SPECTROMETRY APPROACH FOR GLYCOLIPID BIOMARKER DISCOVERY IN NEURODEVELOPMENTAL DISEASES

MIRELA SARBU [1]*, CRISTIAN V.A. MUNTEANU [2], RALUCA ICA [1], ALINA PETRUT [1], ANDREI J. PETRESCU [2], ALINA D. ZAMFIR [1]

Keywords: mass spectrometry; gangliosides; neurodevelopmental disorders; anencephaly; nanoelectrospray ionization.

Abstract: A pivotal role in the brain development is played by the cellular membrane. Since gangliosides (GGs) are the predominant components of plasma membrane, a direct correlation of GGs with crucial processes and neurological disorders exists. Therefore, GGs, formed by a ceramide moiety attached to a mono- or polysialylated oligosaccharide chain, are important biomarkers in early diagnosis of central nervous system (CNS) pathologies, being in the focus of our research as potential therapeutic targets. In this context, we have developed here an approach based on nanoESI Orbitrap MS in combination with ion fragmentation by collision induced dissociations (CID) for profile comparison and structural characterization of native GG mixtures extracted and purified from histopathologically-defined anencephalic fetal brain remnant originating from different developmental stages. The native ganglioside extracts dissolved in pure methanol were infused on a LTQ Orbitrap mass spectrometer. Based on high resolution mass spectrometry capability for a reliable determination of glycopatterns, changes in diversity and number of GGs with age were observed. Over 100 distinct ganglioside structures were identified in the all three samples of anencephalic fetal brain remnant. The high resolution of the instrument allowed not just the ionization and detection of low-abundance species, such as polysialylated GGs, but also revealed the presence of different components modified by fucosylation and acetylation. Tandem MS (MS/MS) experiments carried out in the LTQ by CID in the manual mode of ion selection and fragmentation using variable collision energy within 25-30 eV confirmed the incidence of potential biomarker species in the investigated anencephalic fetal brain remnants.

Supported by the Romanian National Authority for Scientific Research, UEFISCDI through projects PN-III-P4-ID-PCE-2016-0073, PN-III-P1-1.2-PCCDI-2017-0046 granted to ADZ and PN-III-P1-1.1-PD-2016-0256 granted to MS.

[1] National Institute for R&D in Electrochemistry and Condensed Matter, Timisoara, Romania

[2] Institute of Biochemistry of the Romanian Academy, Bucharest, Romania

* Correspondence author: Dr. Mirela Sarbu, mirela.sarbu86@yahoo.co.uk ; Phone 0040-726210626, Plautius Andronescu Str. 1, RO-300224

CLASS II PHOSPHATIDYLINOSITOL 3-KINASES REGULATE HBV LIFE CYCLE IN HEPATOMA CELL LINES

DAVID-SEBASTIAN PATRICHE[1], MIRELA ANDREEA POPESCU[1], COSTIN-IOAN POPESCU[1], NORICA BRANZA NICHITA[1*]

Abstract: Class II phosphatidylinositol 3-kinase (PI3KC2) phosphorylate the 3-hydroxy position of phosphatidylinositol PtdIns and PtdIns(4)P, resulting in PtdIns(3)P and PtdIns(3,4)P2, which are crucial secondary signaling molecules involved in apoptosis, cell proliferation and secretion of proteins. Owing to these functions, the PI3KC2 proteins may play potential roles in pathogen infection of host cells. Hepatitis B infection is a major health issue worldwide, approximately 400 million people being chronically infected, despite the availability of a vaccine. HBV is a small, enveloped DNA virus, with a unique life cycle which involves reverse transcription, nuclear stabilization of a replication form and secretion of a vast excess of subviral particles (SVPs) over mature virions. In this work we showed that PI3KC2 are well-expressed in human hepatoma cell lines . We further modulated this expression in Huh7 cells supporting HBV replication and investigated the effects on the HBV life-cycle. To achieve PI3KC2 depletion we employed siRNA silencing and complete/partial knockout via CRISPR/Cas9 and CRISPR/Cas9D10A Protein Knockdown and downregulation was confirmed by Western Blot. Analysis of intracellular and extracellular encapsidated viral DNA by qPCR and of SVP's , by ELISA indicated a significant decrease of both fractions, which strongly suggests a role of PI3KC2 in viral replication and or assembly. Further studies aim to understand the mechanism of this inhibition and clarify whether these proteins might be an antiviral target.

[1] Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy

*Corresponding author: nichita@biochim.ro, Institute of Biochemistry, 296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031, Tel: (+4).021.223.90.69, FAX: (+4).021.223.90.68

OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS UNDER SIMULATED MICROGRAVITY

ȘTEFANA OROBEȚI[1,2]*, GABRIELA CHIRIȚOIU[1], FLORIN JIPA[2], CRISTIAN BUTNARU[2], FELIX SIMA[2], LIVIA SIMA[1]

Keywords: simulated microgravity, mesenchymal stem cells, osteogenic differentiation, FGF2, microfluidic chip

Abstract: Spaceflight osteopenia is a disease that causes astronauts a significant bone mass loss of 1-1.5% each month and it is similar to the well-known osteoporosis from Earth. It has become apparent that due to the lack of mechanical loading, microgravity produces deterioration of bones and muscles. However, there is still no effective treatment that could reverse these damaging effects.
The aim of our project was to evaluate the potential of fibroblast growth factor (FGF2) to treat bone degeneration in Space explorers. For this, we have used an Airbus Random Positioning Machine (RPM) that simulates the microgravity conditions. As a result, we could identify that one-week treatment with FGF2 during osteogenic induction enhanced osteoblast differentiation of mesenchymal stem cells (MSCs) subjected to microgravity. Moreover, we could test the effect of perfusing different controlled doses of FGF2 by using a microfluidic glass chip as a miniaturized screening platform. In this way, we could establish the optimal *in vitro* dose of FGF2 and the temporal administration protocol to be provided as novel therapeutic strategies for treating spaceflight osteopenia or osteoporosis.

[1] Institute of Biochemistry of the Romanian Academy, 296 Splaiul Independentei, Bucharest 060031, Romania

[2] Center for Advanced Laser Technologies (CETAL), National Institute for Laser, Plasma and Radiation Physics (INFLPR), 409 Atomistilor, Magurele 077125, Romania

*Corresponding author: stefana.orobeti@biochim.ro, stefana.iosub21@gmail.com; Phone number: +4 021 223 90 69; Fax number: +4 021 223 90 68; Postal address: 296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031

MANIPULATING THE IMMUNE PHENOTYPE OF TUMOR MICROENVIRONMENT WITH LIPOSOMAL CURCUMIN AND DOXORUBICIN

ALINA SESARMAN[1,2,3], LUCIA TEFAS[3], BIANCA SYLVESTER[3], EMILIA LICARETE[1,2], VALENTIN RAUCA[1,2], LAVINIA LUPUT[1,2], LAURA PATRAS[1,2], SEBASTIAN PORAV[1,4], MANUELA BANCIU[1,2] AND ALINA PORFIRE[3]

Keywords: liposomes, tumor microenvironment, curcumin, doxorubicin

Abstract: The tumor microenvironment (TME) is a complex milieu in which, embedded in an extracellular matrix, cancer cells, fibroblasts, endothelial cells, smooth muscle cells, and immune cells interact and modulate different aspects of their behavior, fostering cancer progression. The goal of this study was to investigate the therapeutic efficacy of targeting the TME of colon cancer with long circulating liposomes (LCL) co-encapsulating curcumin (CURC) and doxorubicin (DOX). Thus, tumor growth and specific markers of processes sustained by pro-tumorigenic communication between cells of TME were monitored, after treatment of BALB/c mice bearing subcutaneous C26 colon carcinomas with LCL-CURC-DOX. Our results showed that LCL-CURC-DOX shifted the immune phenotype of TME towards an antineoplastic, Th1 phenotype, suppressing the tumor-associated angiogenesis, inflammation, oxidative stress, and resistance to apoptosis. These inhibitory effects may account for the enhanced tumor growth inhibitory potential of LCL-CURC-DOX, demonstrated *in vivo*. Therefore, liposomal co-administration of CURC and DOX represents a feasible strategy to manipulate the equilibrium between Th1 and Th2 cells, in order to stimulate antitumor effects within TME, offering new avenues for designing targeted therapeutic interventions for colon cancer and related malignancies.

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-0220 and PN-III-P4-ID-PCE-2016-0342.

References:

1. Gabizon, A., Papahadjopoulos D. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(18):6949-53.

2. Sesarman A, Tefas L, Sylvester B, Licarete E, Rauca V, Luput L, Patras L, Banciu M, Porfire A, Anti-angiogenic and anti-inflammatory effects of long-circulating liposomes co-encapsulating curcumin and doxorubicin on C26 murine colon cancer cells. Pharmacol Rep 70(2): 331-339, 2017.

[1] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, "Babes-Bolyai" University, 1 Mihail Kogalniceanu Str., 400006, Cluj-Napoca, Romania

[2] Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, "Babes-Bolyai" University, 42 Treboniu Laurian Str., 400271, Cluj-Napoca, Romania

[3] Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", 41 Victor Babes Str., 400012 Cluj-Napoca, Romania

[4] National Institute of R&D of Isotopic and Molecular Technologies, 67-103 Donath Str., 400293 Cluj-Napoca, Romania

*Corresponding author: Assistant professor Alina Sesarman, Email:sesarman@gmail.com; Tel:+40752075505; Babes-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street, 400006 Cluj-Napoca, Romania

COMPARATIVE TRANCRIPTOME ANALYSIS OF *HALOFERAX ALEXANDRINUS* UNDER STRESS-INDUCING SILVER LEVELS

DORIANA-MĂDĂLINA BUDA [1], PAUL-ADRIAN BULZU [1], ADRIAN ZETY [1], HORIA LEONARD BANCIU [1*]

Keywords: Archaea, halophiles, RNA-Seq, silver, transcriptome.

Abstract: In recent years, RNA-sequencing (RNA-Seq) transcriptomics emerged as the standard approach for measuring and comparing gene expression patterns across varying experimental conditions. Although the molecular and biochemical mechanisms underlying metal resistance in prokaryotes have been intensively studied, the RNA-Seq-based assessment of the metal-induced cellular responses could provide broader insights into the engagement of versatile microorganisms possessing rare metabolic capacities (i.e. metal and hypersalinity resistance) in the bioremediation of heavy metal-polluted industrial wastewaters with salinity fluctuation.
The transcriptional landscape of the extremely halophilic archaeon *Haloferax alexandrinus* DSM 27206 cultivated in the presence of increasing $AgNO_3$ concentrations was analyzed by RNA-Seq. Total RNA was extracted using the Zymo Research Direct-zol MiniPrep Kit, followed by cDNA library preparation and sequencing on Illumina Hiseq 2500 PE150 platform (performed by a commercial provider). RNA transcript quantification was performed by the pseudoalignment-based Salmon software using the *Hfx. alexandrinus* genome as reference, followed by transcript abundance normalization using the edgeR software package.
Preliminary data suggested that exposure to silver influenced transcription patterns of a large set of genes involved in basic metabolic pathways, environment-specific microbial metabolism, and biosynthesis of secondary metabolites. Genes participating in ATP synthesis, cysteine biosynthesis, as well as metallic ions transporters were overexpressed proportionally with increasing $AgNO_3$ concentrations. Cells exposed to highest silver concentrations showed abundant transcripts putatively involved in oxidative stress management, as well as in DNA replication and repair processes.
RNA-Seq profiling was employed to compare transcriptomes of silver-stressed *Hfx. alexandrinus*. We demonstrated that the investigated strain was able to elicit a tailored response to the presence of increasing concentrations of silver salts based on the differential expression of genes mainly associated with energy metabolism, oxidative stress, DNA replication and membrane transport processes.

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project number PN-III-P4-ID-PCE-2016-0303.

[1]Babeș-Bolyai Univesity, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street, Cluj-Napoca, Romania

*Corresponding author: Horia Leonard Banciu, Faculty of Biology and Geology, Babes-Bolyai University, 5-7 Clinicilor Str., 400006 Cluj-Napoca, Romania. Tel.: +40-264-431691; fax: +40-264-431858; e-mail: horia.banciu@ubbcluj.ro

ANTI-*CANDIDA* ACTIVITY OF A NEW SYNTHETIC FLAVONOID WITH HALOGENATED SUBSTITUENTS

MIHAELA SAVU[1,*], LUCIAN BARSA[2], MARIUS ȘTEFAN[1]

Keywords: antifungal activity, synthetic flavonoids, fungistatic effect, synergistic effect.

Abstract: Increased resistance to antifungal agents and their toxicity is a major concern in the therapy of diseases caused by pathogenic fungi such as *Candida* sp. Therefore, the discovery of new drugs with high efficacy and low toxicity is a priority in scientific research. A possible solution is the use in therapy of flavonoids, natural compounds known for a long time for their antifungal, antioxidant, anti-tumor and anti-inflammatory properties. In addition to natural flavonoids, synthetic flavonoids are interesting due to their higher antimicrobial activity. In this context, we investigated the antifungal activity of a tricyclic flavonoid with iodine and chlorine as halogen substituents against *Candida albicans* ATTC 10239. The influence of I-Cl flavonoid on cell growth and viability and the synergistic effect with fluconazole were assessed. The mechanism of action was investigated using biofilm formation and hyphae formation assays. I-Cl flavonoid showed a very good antifungal activity, with a minimum inhibitory concentration value of 7.8 µg/ml and a fungistatic effect recorded for more than 34 hours. In the presence of the tested flavonoid, *Candida* cells lost viability (total kill) within only 4 hours. Synergistic and additive effects were observed when the flavonoid was used in combination with fluconazole. Flavonoid I-Cl inhibited the formation of hyphae and showed anti-biofilm activity. Our results that flavonoid I-Cl has a high potential to be used as an efficient antifungal agent.

[1] Faculty of Biology, Biology Department, The Alexandru Ioan Cuza University of Iasi, Iasi, Romania

[2] Faculty of Chemistry, The Alexandru Ioan Cuza University of Iasi, Iasi, Romania

*Corresponding author: Mihaela Savu, mihaelasavu2@gmail.com, Faculty of Biology, Biology Department, The Alexandru Ioan Cuza University of Iasi, Bulevardul Carol I, Nr. 11, 700506 Iasi, Romania.

EFECTS OF DIFFERENT SEVOFLURANE CONCENTRATIONS ON AKT ISOFORMS IN NORMAL AND CANCER BREAST CELLS

PAULA ALEXANDRA POSTU[1], EMILIA PATRAȘCANU[2,3], CRINA TIRON[1*], ADRIAN TIRON[1], IRINA C. VACAREAN TRANDAFIR[1], EUGEN CARASEVICI[1], IOANA GRIGORAȘ[2,3]

Abstract: Multiple perioperative factors influence cancer patient evolution and outcome. Microenvironmental factors activate different gene programs that enable tumor cell to invade, survive and promote drug resistance and metastasis. The effects of anaesthetic drugs on cancer progression is under scrutiny, but published data are controversial and the involved mechanisms unclear. Tumour development implies PI3K/AKT pathway activation. Akt isoforms are frequently amplified in various malignant tumors and associated with malignant cell survival, proliferation and invasion. Their activation is often observed in human cancers and is associated with decreased survival rate. Sevoflurane alters tumour cell proliferation and Akt isoforms expression in a dose-dependent manner. The phenotype of 3D 2mM sevoflurane exposed cells show an increased migration capacity which indicates increased aggressivity. Sevoflurane exposure of breast cancer cells influences cell proliferation, phenotype and Akt isoform expression. Increased sevoflurane concentrations activate different Akt isoforms, putatively related to epithelial-mesenchimal transition and promotes cancer cell invasion, migration and metastasis.

[1]*Regional Institute of Oncology, Department of Molecular Biology, TRANSCEND, Iasi, Romania*

[2]*"Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania*

[3]*Regional Institute of Oncology, Anaesthesia and Intensive Care Department, Iasi, Romania*

*Corresponding author: crinatiron2003@yahoo.com, 0765677356

INCIDENCE OF SOME ANOMALIES ASSESSED BY MLPA AND FLOW CYTOMETRY AT PATIENTS WITH MULTIPLE MYELOMA

LOREDANA M. DRAGOS[1,2], IULIU C. IVANOV[1*], IRINA C. VACAREAN TRANDAFIR[1,2], OANA MARIA PINTILIE[1], MIHAELA MENTEL[1], DANIELA JITARU[1], LUCIAN GORGAN[2*]

Keywords: multiple myeloma, MLPA, flow cytometry

Abstract: Multiple myeloma, a clonal proliferation of plasma cells (PC), is a heterogeneous, stepped, multistage disease. Various recurrent cytogenetic abnormalities in MM have been identified throughout the disease, from the premalignant stage of monoclonal gammopathy of unknown significance to the end stage of MM.
The study aims to evaluate the frequency of cytogenetic anomalies investigated by MLPA in 63 patients with MM (diagnosed in IRO Iasi), in correlation with the malignant PC phenotype assessed by flow cytometry (FC). The antibody panel for MM diagnosis by FC was: CD19, CD38, CD138, CD45, CD81, CD28, CD27, CD117, CD56, Kappa and Lambda. Bone marrow samples from patients diagnosed with MM between February 2018 - iulie 2019 were processed with Ficoll-Histopaque®-1077 (Sigma Aldrich) for separation in density gradient of the mononuclear cells. CD138 + cells were screened with magnetic bead anti-CD138 antibody (MicroBeads - MiltenyiBiotec). Percentage of PC was assessed by flow cytometry using anti-CD38, CD56, CD19, CD45 antibodies and an 87% enrichment of the sample was found.
The FC immunophenotyping indicates different expressions of the analyzed patients, as follows: for the light chains, 56% Lambda,44% Kappa and also 12% CD81-, 17% CD28+, 34% CD27+, 25% CD56-, 39% CD117+. The CD138 + cell fraction's MLPA assay highlights that 68% of the analyzed cases showed odd chromosomes (chr.) trisomy, trisomy of 12 chr. (2.85%), trisomy 14 chr. (8.57%); deletions of chr. 13 (51%), chr. 17 (11.4%), chr. 1 (22%), chr. 16 (25.71%), chr. 12 (5.71%), chr.14 (8.57%). For the 4.87% of the investigated patients, no deletions and duplications were found. Due to the fact that classical karyotyping analyzes do not provide relevant results for MM diagnosis, presence of duplication and deletion of the chromosomes can be successfully evaluated by MLPA on samples enriched by screening of CD138+ cells. The MLPA and immunophenotyping techniques have the ability to provide key information for the correct diagnosis of MM patients.

[1] Regional Institute of Oncology, Iasi, Romania

[2] Aexandru Ioan Cuza University of Iasi, Romania

*Corresponding author: Iuliu Ivanov, e-mail: iuliuic@gmail.com and Lucian Gorgan, e-mail: lucian.gorgan@uaic.ro

PDI PROTEINS CONTRIBUTE TO THE FORMATION OF EXTRACELLULAR MATRIX

IOANA MILITARU[1], GABRIELA CHIRIȚOIU[1], CRISTIAN V. A. MUNTEANU[1], ȘTEFANA M. PETRESCU[1], FLORENTINA PENA[1*]

Abstract: The extracellular matrix represents a connective material, which includes polysaccharides and large proteins that are secreted by cells in the near environment. ECM role is not only mechanic - to serve as a support for tissue architecture but also has a dynamic role in normal tissue physiology (such as embryogenesis, tissue repair, and immune response) or in the pathologic response (tumor invasion and metastasis). Some of ECM proteins are characterized by complex disulfide bonds and their formation may be catalyzed by proteins belonging to protein disulfide isomerase (PDI) family. Using mass-spectrometry (MS)-based interaction proteomics, we identified ECM proteins coprecipitating with PDI proteins. Among these, collagen IV and laminin were also found in the same complex with PDIs by western blot analysis. To study the role of PDI proteins in the maturation and secretion of ECM proteins we created HT1080 derived cell lines that either overexpress or are depleted (using CRISPR-Cas9 knock-out technique) of PDI proteins. We used these cells to evaluate ECM deposition and found that P5 - a member of PDI family modulates the maturation and/or secretion of some types of collagen and laminin. Because we want to see how oxygen influences ECM deposition, we combined different techniques (western blotting and immunofluorescence-based methods) to study all of these modifications under normal conditions and low oxygen levels. In conclusion we found the not only PDI but also P5 has a role in ECM formation and further studies are required to elucidate its exact function in the endoplasmic reticulum.

[1] Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independenței 296, 060031, Bucharest, Romania

*Corresponding author: florentinapena@yahoo.com

THE ROLE OF ERAD PATHWAY ON INSULIN SECRETION IN PANCREATIC β-CELL

PETRUTA R. ALEXANDRU[1], GABRIELA N. CHIRITOIU[1], DANIELA LIXANDRU[2], CONSTANTIN IONESCU-TARGOVISTE[3], STEFANA M. PETRESCU[1*]

Abstract: Pancreatic β-cells secrete insulin in response to high blood glucose levels. Type I diabetes occurs when the body fails to produce insulin and the people must take artificial insulin. Type 2 diabetes is the most common type of diabetes and in this case the cells in the body do not respond to insulin and insulin resistance takes place gradually. The present study examined the role of Endoplasmic-reticulum-associated protein degradation (ERAD) on insulin secretion in pancreatic beta cells and diabetic rat model. We demonstrate that overexpression of some ER-stress induced proteins that targets misfolded glycoproteins for ER-associated degradation in INS-1 cells increased preproinsulin mRNA, facilitates proinsulin transport to the secretory vesicles, conversion and secretion of mature insulin. Such effects were also observed in human pancreatic islets and streptozotocin diabetic rats. Our findings identify a role of ERAD pathway as potential target for the treatment of diabetes.

[1] Department of Molecular Cell Biology, Institute of Biochemistry, Romanian Academy, Splaiul Independentei 296, 060031 Bucharest, Romania

[2] Department of Biochemistry, 'Carol Davila' University of Medicine and Pharmacy, 050474 Bucharest, Romania

[3] N. C. Paulescu Institute, Bucharest, Romania

*Corresponding author: *stefana.petrescu@biochim.ro*

LEVODOPA THERAPY ASSOCIATED CHANGES IN PLASMA MICRORNA PROFILES IN PARKINSON'S DISEASE

MIHAELA SIMU[1], CRISTINA BEJINAR[2], CRISTINA DIJMARESCU[1], ANDA VIZITIU[2], CECILIA ROSCA[1], RALUCA TUDOR[1], ANDREI ANGHEL[2], CATALIN MARIAN[2], MIHAI UDRESCU[3], AND IOAN-OVIDIU SIRBU[2*]

Keywords: microRNA, Parkinson, Levodopa

Abstract: Parkinson's disease (PD) is one of the most common and investigated neurodegenerative disorders, the molecular pathology of which is still not fully understood. Excepting the minority of monogenic forms of PD, there are no reliable diagnostic, prognostic and therapy evaluation biomarkers available. Furthermore, basically all PD-related mRNA molecules have been shown to be modulated by microRNAs, substantiating the role of microRNAs in PD pathogenic processes like mitochondrial damage, programmed cell death, and autophagy.

The studies attempting to characterize the profile of circulant microRNAs associated to PD have shown a wide variety of data, quite often contradictory, most probably due to the heterogeneity of the disease and the differences in the technical platforms have been used to analyze the different fractions of the blood.

Here we describe a two-step qRT-PCR biomarker analysis of plasma microRNAs associated with PD, based on PCR array (discovery step) and TaqMan assays (validation step) and propose a set of 5 plasma microRNAs as being specifically associated with PD. We show data validating three of these microRNAs not only as putative diagnostic biomarkers, but also as therapy-monitoring markers in a cohort of patients diagnosed with sporadic PD. Furthermore, we combine miRWalk machine learning prediction algorithms with clustering and topological community detection (CTCD) approach to describe the signaling pathways putatively targeted by the changes in the expression of the three microRNAs in LEVODOPA/CARBIDOPA treated PD patients.

[1] Department of Neurology, Victor Babes University of Medicine and Pharmacy Timisoara, Romania

[2] Department of Biochemistry, Victor Babes University of Medicine and Pharmacy Timisoara, Romania

3 Department of Computer and Information Technology, Polytechnic University of Timisoara, Romania

*Corresponding author: Dr. Ioan-Ovidiu Sirbu, ovidiu.sirbu@umft.ro; Tel: 0756136272; Biochemsitry Department,University of Medicine and Pharmacy from Timisoara, 2, E. Murgu street

NEW DEVELOPMENTS IN MOLECULAR DIAGNOSTICS BASED ON RNASE H – DEPENDENT PCR

VICTOR IONESCU[1], DAN SOARE[1], IOANA LAMBRESCU[1], GISELA GAINA[1], ALINA MICU[1], VALERIU CISMASIU[1,*]

Keywords: RNase H-dependent PCR, acute myeloid leukemia, chimerism

Abstract: Several technologies for detection and quantitation of sequence variations have been developed for genomics research and molecular diagnostics, namely polymerase chain reaction (PCR), isothermal amplification, hybridization, and next-generation sequencing (NGS). NGS allows the massively multiplexed sequence analysis of DNA and RNA and, thus, it is optimal method for multiplexed analysis of many genes and their variants. However, NGS has a significant error rate due to signal ambiguity, enzyme infidelity, imperfect deprotection and others, making the method very inefficient in case of the low frequency targets. PCR is more accurate than microarrays or NGS, it has high molecular sensitivity, and ease of use. Most of the FDA approved assays for molecular diagnostics are based on PCR methods. The main weakness of PCR is the primer dimer formation that result in false positives or false negatives. The RNase H2 dependent PCR, the most recent development of this technology, has several advantages: eliminate the primer dimers, allows multiplexing for a high number of targets, improved precision for detection of low abundance targets. In this assay, the primers are modified at 3′ end and carry a removable PCR blocker. The primers are not expandable by the DNA polymerase unless the 3′ blocker is not removed by a hyperthermophilic RNase enzyme (*Pyrococcus abyssi* RNase H2). We have developed several assay for molecular diagnostics using the RNase H2 dependent PCR.

Acknowledgments: *Partially supported by Ministry of Research and Innovation grants PN19.29.01.03 and under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No.7PFE/16.10.2018*

[1]"Victor Babes" National Institute of Pathology, Bucharest, Romania

*Corresponding author: valeriu.cismasiu@ivb.ro; tel. 0213192732, 99-101 Spl. Independentei, District 5, Bucharest 050096,

NOVEL TI-APATITE COMPOSITE SCAFFOLD BASED ON 3D-PRINTING TECHNOLOGY INTENDED FOR SPINAL RESTORATION: AN IN VITRO STUDY

MARIA DANIELA VLAD[1,2,*], ENRIQUE FERNÁNDEZ AGUADO[3], SERGIO GÓMEZ GONZÁLEZ[3], IULIU CRISTIAN IVANOV[2], ION POEATĂ[4], JOSÉ LÓPEZ LÓPEZ[3]

Keywords: 3D printing; hybrid scaffolds; osteogenic gene expression; bone restoration.

Abstract: In this study, new hybrid scaffolds based on 3D-printed titanium alloy with spongy bone-like architecture filled with porous hydroxyapatite (i.e. which also mimic compositional and micro geometric features of bone inorganic matrix) were investigated in vitro in terms of cell osteogenic differentiation, matrix maturation and mineralization. MTT-assay proved the high proliferation profile of the osteoblastic cells coincubated with the studied scaffold. RT-PCR evidenced enhanced expression level of several key osteogenic markers, such as Runt-related transcription factor 2, collagen, alkaline phosphatase, bone sialoprotein, osteocalcin. Extracellular matrix staining showed highly collagen synthesis. Alizarin-red staining confirmed nodule bone formation, i.e. matrix mineralization. The results put forward the osteogenic behaviour of the studied scaffolds and consequently their potential for using in bone restoration after further in vivo study.

[1] Faculty of Medical Bioengineering, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Str. Kogalniceanu 9-13, 700454-Iasi, Romania;

[2] TRANSCEND Research Centre, Regional Institute of Oncology, Str. G-ral Henri Mathias Berthelot 2-4, 700483-Iași, Romania;

[3] Research Group of Interacting Surfaces in Bioengineering and Materials Science (InSup), Technical University of Catalonia (UPC), Avda. Diagonal 647, 08028-Barcelona, Spain;

[4] Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Sstr. Universității no. 16, 700115-Iasi

*Corresponding author: Dr. Maria Daniela Vlad, Faculty of Medical Bioengineering, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Str. Kogălniceanu 9-13, 700454-Iasi, Romania; Phone/fax: +40 232 213573.

TIME DEPENDENT EXPRESSION OF MMU-MIR-195 IN MOUSE HEART

AIMEE CHIS[1], ALEXANDRA MOATAR[1*], MIHAI UDRESCU[2], GINA ISVORANU[3], RODICA BADEA[4], VALERIU CISMASIU[3], STEFAN SZEDLACSEK[4], CRISTINA BEJINAR[1], IOAN-OVIDIU SIRBU[1*]

Keywords: microRNA, heart, gene network

Abstract: Member of the miR-15 precursor family, mmu-miR-195 is a conserved small non-coding RNA known for its role in cell cycle and cell death/apoptosis regulation. miR-195 is differentially expressed in various organs and tissues of the postnatal mouse. Recent analyses have associated miR-195 with different diseases such as cancer, chronic obstructive pulmonary disease, heart failure, Parkinson disease.
We evaluated the expression of the mature and precursor mmu-miR-195 in the heart of male and female, wild-type mice at different stages of pre-natal and post-natal life. Quantitative Real-Time PCR (qRT-PCR) analysis revealed coordinated, concordant changes of the mature form, which are not paralleled by the pri-miRNA changes. Furthermore, we used a combined machine learning algorithm for miR targets prediction, and a clustering and topological community detection (CTCD) approach followed by gene ontology analysis to describe the impact of miR-195 changes upon heart transcriptome.
We show that miR-195 regulates multiple novel signaling pathways associated with cardiac maturation and provide data validating - both at mRNA and protein level – our predictions.

[1] *Department of Biochemistry, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[2] *Computer and Information Technology Department, Polytechnic University, Timisoara, Romania*

[3] *Cell Biology Department, Victor Babes National Institute of Pathology, Bucharest, Romania*

[4] *Enzymology Department, Biochemistry Institute, Romanian Academy, Bucharest, Romania*

*Corresponding author: Dr. Ioan-Ovidiu Sirbu, ovidiu.sirbu@umft.ro; Tel: 0756136272; Biochemsitry Department,University of Medicine and Pharmacy from Timisoara, 2, E. Murgu street

MEASLES VIRUS ISOLATION ON CELLULAR SUBSTRATE

CARMEN MARIA CHERCIU[1], AURORA STĂNESCU[2], MARIA ELENA MIHAI[1], CATALINA PASCU[1], MIHAELA LAZAR[1*]

Keywords: measles, virus, hVero SLAM cells, isolation

Abstract: *Measles virus* (MV) is an enveloped negative-strand RNA virus of the *Morbillivirus* genus in the *Paramyxoviridae* family. The signaling lymphocytic activation molecules have been shown to act as cellular receptors for MV. The ability to successfully produce a viable virus stock from clinical specimens collected from cases of measles is important for molecular epidemiologic investigations of outbreaks, particularly those that involve additional gene targets or extended sequencing.
The purpose of this study was to identify measles virus on nasopharyngeal swabs from measles patients use signaling lymphocytic activation molecule, SLAM (CDw150).
The viruses on nasopharyngeal swabs (n=219) from patients with measles in Romania were inoculated on Vero cells stably expressing human SLAM (African green monkey-genetic modified organism).
Virus RNA was extracted from both nasopharyngeal swabs and virus isolates with commercial kits: NucleoSpin RNA virus Macherey Nagel or semi-automated Maxwell 16 Viral Total Nucleic Acid Purification Kit, Promega.
Real-time RT PCR was performed for detection of measles virus using SuperScript™III Platinum™One-Step qRT-PCR, Invitrogen.
Samples from 92 patients produced numerous plaques, structural changes caused on SLAM-expressing Vero cells by measles viral invasion. The cytopathic effect was observed by optical microscopy after 4 - 5 days from inoculation time when the measles virus caused lysis of the host cell.
The successfully isolation of measles virus permit to carry out gene sequencing and molecular characterization of MVs that can help strengthen the measles laboratory monitoring network and improve monitoring efficiency.

Acknowledgement:
We are very grateful to all our colleagues from the Romanian local public health departments for sending specimens of possible measles cases. Financial support: projects ”Instalaţii de Interes Naţional” and Nucleu: PN 19.14.02.06., The Research Institute of the University of Bucharest (ICUB).
We thank to the technical staff in the Viral Respiratory Infections Laboratory: Mirela Ene, Oana Vitencu, Luiza Ustea, Nicoleta Paraschiv and Emilia Dobre.

[1] Cantacuzino, National Military-Medical Institute for Research and Development, Bucharest, Romania

[2] National Centre for Communicable Diseases Surveillance and Control, National Institute of Public Health, Bucharest, Romania

*Corresponding author: mlazar@cantacuzino

MODULATING EXPRESSION OF TRPC1 CHANNEL INVOLVED IN STORE-OPERATED CALCIUM ENTRY INFLUENCES HBV ASSEMBLY *IN VITRO*

MIHAELA-OLIVIA DOBRICA[1], OTILIA BARBIERU[1], ALEXANDRA MANOLACHE[2], ALEXANDRU BABES[2], NORICA NICHITA[1*]

Keywords: Hepatitis B Virus, SOCE, TRPC channels

Abstract: Hepatitis B Virus (HBV) is responsible for a large number of infections in the world, with more than 320 million people being chronically infected. Around 20-30% of the chronic infections lead to severe liver complications, such as fibrosis, cirrhosis, hepatocellular carcinoma or liver disfunction that cause 500.000 deaths/year. There are several therapies available against HBV, but none of them cures the disease. In this context, intensive research focuses on the unknown details of the viral life cycle to unravel novel antiviral targets. Previous reports have indicated that Ca^{2+} homeostasis is important for HBV replication, as Ca^{2+} stimulates nucleocapsid assembly. Our study investigates whether TRPC (Transient Receptor Potential Canonical) channels that are involved in SOCE (Store-Operated Calcium Entry) play a role in HBV life cycle in hepatic cells. We show that TRPC channels (TRPC1, TRPC6) are well-expressed in human hepatoma cell lines, such as Huh7, HepG2, HepG2 2.2.15 and HepaRG. The effect of TRPC1 expression on the HBV life cycle was analysed by using an in vitro system for HBV infection. In calcium microfluorimetry experiments we observed a reduced SOCE influx in hepatic cells in which TRPC1 expression was reduced by small interfering RNA. Subsequently, analysis of HBV infectivity in these cells showed a significantly lower viral secretion; however, an increased HBsAg level was detected in extracellular media. These results suggest that Ca^{2+} influx mediated by TRPC1 promotes HBV nucleocapsids assembly and further virus release. Hepatoma cells with either stable TRPC1 overexpression or downregulation will be generated in order to investigate in more detail the observed effect. In addition, mechanisms involved in TRPC1-mediated Ca^{2+} signaling in HBV infected or HBV replicating cells are currently being investigated. Our preliminary data suggest that TRPC1 channel or other interactors required for SOCE represent possible candidates to target HBV infection.

[1] Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy

[2] Department of Anatomy, Physiology and Biophysics, Faculty of Biology, University of Bucharest

*Corresponding author: nichita@biochim.ro, Institute of Biochemistry, 296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031, Tel: (+4).021.223.90.69, FAX: (+4).021.223.90.68

COMPLEX NETWORK GENE NETWORK ANALYSIS OF GENOME WIDE METHYLATION DATA OF ORAL CQARCINOMA FORMALIN FIXED TISSUE

ALEXANDRA MOATAR[1], AIMEE CHIS[1], VIKTORIAN MIOK[1], CORINA SAMOILA[1], EDWARD SECLAMAN[1], ANDREI ANGHEL[1], IOAN-OVIDIU SIRBU[1,*], CATALIN MARIAN[1]

Abstract: Oral scuamocellular cancer (OSCC) is the sixth most common malignancy and the fourth cause of death due to cancer in men worldwide, due to its' (usually) late diagnostic. Recent work has involved multiple epigenetic mechanisms in the pathogenesis of OSCC, the most common of which are specific DNA methylation profiles and changes in microRNA expression, both at tissue and biological fluids level. The aim of our study was to identify the methylation profile of OSCC tissues with a potential impact on microRNA expression.
We have used the Infinium array-basedMethylation Assay to quantitatively assessthe genome wide methylation status at single-CpG-site level using DNA purified from formalin fixed OSCC pooled samples from the University of Medicine archive. After quality control, filtering out poor performing probes and normalization of data (using the preprocessQuantile method, to minimize the unwanted variation within and between samples), we performed PCA analysis and generated MultiDimensional Scaling (MDS) plots to visualize and explore the differences between OSCC and normal tissue samples. Following the identification of the differentially methylatedmicroRNA loci, we performed qRT-PCR analysis of microRNA expression to validate the predicted effect of DNA methylation upon miR expression. Complex network analysis of prediction data and OSCC GEO data sets reveal the impact of DNA methylation and microRNA changes upon OSCC transcriptome.
Our data depict a complex image of the intertwining between the two major epigenetic mechanisms and contribute to a better understanding of OSCC pathogeny.

[1] Department of Biochemistry, Victor Babes University of Medicine and Pharmacy Timisoara, Romania

*Corresponding author: Dr. Ioan-Ovidiu Sirbu, ovidiu.sirbu@umft.ro; Tel: 0756136272; Biochemsitry Department,University of Medicine and Pharmacy from Timisoara, 2, E. Murgu street

ISOLATION AND CHARACTERIZATION OF THE EXOSOMES RELEASED BY THE TRIPLE NEGATIVE BREAST CANCER CELL LINE MDA-MB-231

SORINA DINESCU[1,2]*, LEONA CHIȚOIU[3], TUDOR EMANUEL FERTIG[3], OTILIA ZĂRNESCU[1], MARIETA COSTACHE[1,2]

Keywords: breast cancer, exosomes, miRNAs, communication.

Abstract: Breast cancer remains the most prevalent type of cancer occurring in women worldwide. Breast cancer cells (BCCs) are therefore involved in complex studies in order to develop personalized therapies to fight cancer and metastasis. It has been shown that exosomes released from tumor cells are able to transfer cancer-specific molecules to other cells, to manipulate their environment, encouraging tumor growth. An in vitro study involving MDA-MB-231 cell line revealed that exosomes released by BCCs were capable of conferring the transformed characteristics of cancer cells to normal fibroblasts and epithelial cells. Exosomes are nanovesicles of approximately 30-150 nm, produced in the endosomal pathway, that play a vital role in cell communication. The purpose of this work was to isolate and to characterize the exosomes secreted by MDA-MB-231 cells, in order to identify the non-coding RNAs cargo.

Cells from MDA-MB-231 cell line were cultured in plates at a density of $2x10^4$ cells/cm^2 and maintained in complete culture media with exosome-depleted fetal bovine serum. After 72 hours, culture media was harvested and processed for exosome isolation using dedicated extraction kit. Isolated extracellular vesicles (EVs) were further characterized for size and exosomal markers by cryo-transmission electron microscopy (cryo-TEM) and western blotting. After exosomes validation, the fraction of small non-coding RNA (miRNAs) was isolated and breast cancer-specific miRNA expression profile was obtained by qPCR.

EVs isolated from MDA-MB-231 culture media were examined in cryo-TEM and most of them were validated as exosomes by having diameters of 40-150 nm. Additionally, vesicles were validated as exosomes after western blotting profile, as they expressed both CD63 and CD81. During miRNA screening, a series of miRNA species were found to be overexpressed in BCCs as compared to normal cells- let-7i-5p registered a 10-fold higher level, miR-206 and miR-132-3p were 4-times overexpressed, whereas miR-10a-5p and miR-7-5p displayed a 2-times higher expression level in MDA-MB-231 cells as relative to control cells.

Breast cancer cells are able to modulate the microenvironment surrounding them by secreting exosomes that carry signals. Among them, miRNAs such as let-7i-5p, miR-206, miR-132-3p, miR-10a-5p and miR-7-5p were found to be overexpressed in these cells.

Acknowledgements: This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2016-2057 (46PD/2018).

[1] Department of Biochemistry and Molecular Biology, University of Bucharest

[2] Research Institute of University of Bucharest

[3] Laboratory of Ultrastructural Pathology, Victor Babeș National Institute of Pathology

*Corresponding Author: sorina.dinescu@bio.unibuc.ro; Postal address: Splaiul Independentei 91-95, 050095, Bucharest, Romania

GENERATION AND CHARACTERIZATION OF HCV-E2 ANTIGEN IN *NICOTIANA BENTHAMIANA* LEAVES FOR VACCINE DEVELOPMENT

MIHAELA-OLIVIA DOBRICA[1], LISA PARUCH[2], DOMINIC ALONZI[3], NICOLE ZITZMANN[3], COSTIN-IOAN POPESCU[1], JEAN DUBUISSON[4], JIHONG LIU-CLARKE[2*], NORICA NICHITA[1*]

Keywords: Hepatitis C Virus, antigens, plant expression, N-glycosylation

Abstract: More than 70 million people are infected with Hepatitis C Virus (HCV) and about 400,000 people/year die due to associated liver complications. An efficient antiviral therapy has been recently developed, but the associated costs are high, making it less accessible to all patients. In these circumstances, prevention by vaccination remains the most effective way to control HCV infections. Currently, there is no commercial vaccine against HCV infection. Research in this field remains a real challenge, mainly due to the high genetic diversity of HCV. Our study aims to develop and characterize HCV antigens as vaccine candidates in a cost-effective manner. For this purpose, the E2 envelope protein, the major HCV vaccine candidate, was transiently expressed in premiere in *Nicotiana benthamiana* as a low-cost alternative production system and in mammalian cells, as control. Our results indicated comparable expression levels of the viral antigen in either expression system. As the heavy N-glycosylation of E2 is known to contribute to its immunogenic properties, we further investigated the N-glycan profiles in both expression systems. Sensitivity to Endo H treatment demonstrated the attachment of high-mannose N-glycans in both plants and mammalian cells. The E2 protein was purified by affinity binding to lectin-agarose beads followed by elution with methyl α-D-mannopyranoside. N-glycans were released from the protein by digestion with either PNG-ase F or Endo H glycosidases followed by 2-anthranilic acid labelling and analysis by normal phase HPLC and fluorescence detection. The comparative analysis shows similar glycosylation pattern for E2 expressed either in plants or mammalian cells. These results suggest that plants are promising biotechnological platforms for cost-efficient production of complex HCV antigens. Further studies will address the immunogenic properties in animals.

Acknowledgement: The research leading to these results has received funding from the EEA Grants 2014-2021, within the GreenVac and SmartVac Projects, contracts no. 5/2014 and no. 1/2019.

[1] Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy

[2] NIBIO - Norwegian Institute for Bioeconomy Research, Ås, Norway

[3] Institute of Glycobiology, Department of Biochemistry, University of Oxford, UK

[4] Center for Infection & Immunity of Lille (CIIL), Inserm U1019, CNRS UMR8204, Université de Lille, Institut Pasteur de Lille, Lille, France

[*]Corresponding author: nichita@biochim.ro, Institute of Biochemistry, 296 Splaiul Independentei, sector 6, Bucharest, Romania, 060031, Tel: (+4).021.223.90.69, FAX: (+4).021.223.90.68 and jihong.liu-clarke@nibio.no, Norwegian Institute for Bioeconomy Research, Høgskoleveien 8, 1433 Ås, Norway, Phone: +47 406 04 100

EMPLOYING ARTIFICIAL INTELLIGENCE TO DESIGN INTELLIGENT BIOMATERIALS

TU LE[1,*], ALEXE BOJOVSCHI[2]

Abstract: The design and synthesis of materials with useful, novel properties is one of the most active areas of contemporary science, generating a veritable explosion of scientific activity in areas such as biomaterials, cell and tissue engineering, organic photovoltaics and light-emitting materials, and nanomaterials for a myriad of medical and nonmedical applications. This new era of materials design and discovery covers many disciplines from chemistry and biology to physics and engineering. Conventionally, it takes at least 20 years to move a material from initial discovery to the marketplace. To accelerate the pace of novel materials discovery, computational methods such as artificial intelligent machine learning techniques can be used to construct predictive materials property models and allow rapid scanning of large chemical datasets to systematically identify attractive candidates for specific applications. This presentation will showcase recent studies on data-driven design of functional biomaterials for a broad spectrum of applications such as drug delivery, and antifouling materials.

RMIT University,GPO Box 2476,Melbourne VIC 3001Australia; The University of Melbourne, GPO Box 2476,Melbourne VIC 3001Australia

+61 3 9925 2216, tu.le@rmit.edu.au