HRVATSKA AKADEMIJA ZNANOSTI I UMJETNOSTI
Zavod za biomedicinske znanosti u Rijeci
AKADEMIJA MEDICINSKIH ZNANOSTI HRVATSKE - Podružnica Rijeka
MEDICINSKI FAKULTET SVEUČILIŠTA U RIJECI
HRVATSKI LIJEČNIČKI ZBOR - Podružnica Rijeka

18. znanstvena tribina

NOVIJA POSTIGNUĆA RIJEČKE MEDICINE III:

PREDSTAVLJANJE ZNANSTVENOG DOPRINOSA NOVOIZABRANIH REDOVITIH ČLANOVA AMZH

20. rujna 2018.
u 11,00 sati

Medicinski fakultet Rijeka – Predavaonica br. 1,
Braće Branchetta 20, Rijeka

Registracija sudionika: 10,30 – 11,00

Ulaz je slobodan, a sudionici koji žele potvrdnicu HLK o sudjelovanju trebaju se registrirati. Sudjelovanje na simpoziju vrednovat će se prema Pravilniku Hrvatske liječničke komore.

Informacije

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P R O G R A M
OTVORENJE (11,00 – 11,15 h)

Uvodno slovo

Akademik Daniel Rukavina, predsjednik Podružnice Rijeka AMZH i voditelj HAZU Zavoda za biomedicinske znanosti u Rijeci

Prof. dr. sc. Davor Štimac, ravnatelj Kliničkog bolničkog centra u Rijeci, Rijeka; prvi dopredsjednik AMZH

Pozdravi uzvanika

Prof. dr. sc. Jasna Lipozenčić, predsjednica AMZH, Zagreb

Prof. dr. sc. Tomislav Rukavina, dekan Medicinskog fakulteta Sveučilišta u Rijeci, Rijeka

11,15 – 12,00 h

I. GOST TRIBINE

Predsjedaju: Jasna Lipozenčić i Tomislav Rukavina

Prof. dr. sc. Davor Štimac, osvrt na znanstveni opus Kristine Sepčić

Prof. dr. sc. Kristina Sepčić, Biotehnološki fakultet, Sveučilište u Ljubljani, Ljubljana, Slovenija
Primjena bjelančevina iz obitelji aegerolysina u biomedicini i biotehnologiji

12,00 – 14,15 h

II. STANIČNA FIZIOLOGIJA ENDOSOMALNOG SUSTAVA

Predsjedaju: Daniel Rukavina i Josip Španjol

Akademik Daniel Rukavina, osvrt na znanstveni opus Pere Lučina, redovitog člana u Kolegiju temeljnih medicinskih znanosti

Prof. dr. sc. Pero Lučin, Medicinski fakultet Sveučilišta u Rijeci, Rijeka
Virusna tvornica – nastanak i 3D rekonstrukcija odjeljka za sklapanje viriona tijekom citomegalovirusne infekcije

Prof. dr. sc. Gordana Blagojević Zagorac, Medicinski fakultet Sveučilišta u Rijeci, Rijeka
Rapidno recikliranje – neotkriveni putevi endosomalnog prometovanja prema staničnoj membrani

Prof. dr. sc. Hana Mahmutefendić Lučin, Medicinski fakultet Sveučilišta u Rijeci, Rijeka
Veliko preuređivanje – reorganizacija endosomalnog i sekretornog sustava tijekom rane faze infekcije citomegalovirusom

Doc. dr. sc. Kristina Grabušić, Odjel za biotehnologiju Sveučilišta u Rijeci, Rijeka
Pošiljka iz stanice: egzosomi i druge izvanstanične vezikule

14,15 – 14,30 h

III. OPĆA RASPRAVA

Predsjeda: Davor Štimac

Znanstveni i organizacijski odbor
Daniel Rukavina, predsjednik
Davor Štimac, Josip Španjol i Srđan Novak

ABSTRACTS

Biomedical and biotechnological applications of aegerolysin proteins

Kristina Sepčić
Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Aegerolysin protein family (Pfam 06355, InterPro IPR009413) comprises low molecular (15-20 kDa), acidic, beta-structured proteins, found in several eukaryotic and bacterial taxa. The family currently contains over 350 homologues from 285 diffeerent organisms. Despite the wide distribution of aegerolysins across the kingdoms of life, and although they appear to be among major proteins secreted by the organisms that produce them, their functions and biological roles remain poorly understood.
The common feature of the aegerolysins is their ability to bind different lipids and lipid derivatives, as well as biological and artificial lipid membranes. For example, some aegerolysins can target sphingomyelin/ cholesterol membrane nanodomains. Furthermore, aegerolysins from the fungal genus *Pleurotus* preferentially bind to ceramide phosphoethanolamine (CPE), which is the major membrane sphingolipid of invertebrates (particularly insects and molluscs). Moreover, the genomes of some aegerolysin-producing fungi have nucleotide sequences that encode proteins with membrane-attack complex/ perforin (MACPF) domain. In the presence of a protein with a MACPF domain, fungal aegerolysins can function as bi-component lytic complexes for target cell membranes.
In order to study the biomedical or biotechnological potential of aegerolysins and ae-

gerolysin-based binary lytic complexes, we isolated and characterized several recombinant aegerolysins (either unmodified or fused with fluorescent proteins) derived from fungi or bacteria, and evaluated their interactions with artficial and biological lipid systems, and with some target organisms.
We show that selected fluorescent fusion derivatives of fungal aegerolysins could be used as useful tools to track raft-like membrane nanodomains composed of sphingomyelin and cholesterol. Moreover, the selectivity of some aegerolysin-based cytolytic complexes for increased membrane sphingomyelin/ cholesterol contents can be exploited for selective killing of urothelial carcinoma cells. Finally, due to their specific interaction with CPE, some cytolytic complexes based on *Pleurotus*-derived aegerolysins could represent a novel promising class of biopesticides for controlling plant pests

Virus factory – development and 3D reconstruction of the virion assembly compartment during cytomegalovirus infection

Pero Lučin[1,2], Hana Mahmutefendić Lučin[1,2], Ljerka Karleuša[1], Gordana Blagojević Zagorac[1], Kristina Grabušić[3], Valentino Pavišić[1], Natalia Jug[1], Marina Marcelić[1], and Silvija Lukanović Jurić[1]

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Viruses rearrange intracellular compartments to develop the site for assembly and release of progeny virion, which is a key step in the biology of all viral infections and target for many researchers which explore the ways to prevent or to treat viral infections.
Herpesviruses are large DNA viruses which immediately after infection establish an extensive program of rearrangements of cellular functions. The viral program starts with the introduction of viral proteins embedded into virions and by expression of viral genes after integration of viral DNA into the nucleus of the infected cell. The expression of viral genes undergoes the cascade program through at least three phases: the immediate early (IE) and the early (E) which occur before viral DNA replication, and the late (L), after initiation of viral DNA replication. The late phase ends up with the assembly of virion progeny nucleocleocapsids, their embedment into the tegument matrix and envelopment with membranous cellular structures. The membrane envelopment occurs through two phases, the first in which newly formed nucleocapsids bud from the nucleus acquiring the primary envelope at the nuclear membrane (*primary envelopment*) and release into the cytosol, and the second (*secondary envelopment*) in which cytoplasmic capsids embed into the complex of cellular and viral proteins that from the matrix of the tegument followed by envelopment with cellular membranes containing viral glycoproteins. Cellular structures that participate in the secondary envelopment are usually called „virion assembly compartment," or „virus factory."
Virus factory is developed by rearrangements of cellular membranous organelles in a process that is initiated immediately upon infection (IE phase) and terminated at the end of the late phase. Although the morphological aspects of this rearrangement are relatively well known, most of the changes in the cellular physiology and regulation of these processes are less understood. Thus, we used murine cytomegalovirus infection to reveal the cellular program of membranous organelle rearrangements and formation of virus factory during herpesvirus infection.

The development of virus factory starts with intensive rearrangements of the endosomal system and the Golgi complex very early in the infection associated with a redirection of membranous trafficking and formation of a membranous organelle that concentrate viral glycoproteins and gather tegument components. The membranous organelle rearrangement during viral infection is associated with altered expression of small GTPases from the Rab and Arf family that shape identity and function of membranes and disruption of Arf/Rab regulatory cascades. Consequently, Arf/Rab regulatory network disruption is associated with alteration of recruitment of their effector proteins that regulate membrane dynamics and changes in phosphoinositide composition.
In this lecture, we will present the temporal and spatial stages in the development of virus factory and alteration of cellular physiology during all phases of infection, and 3D reconstruction of the final stage of the virus factory. The 3D reconstruction is based on phenotypic changes of membranous domains within the virus factory analyzed by membrane association of regulatory proteins and phosphoinositides using confocal microscopy. Understanding these processes during infection by murine cytomegalovirus enables understanding of virion assembly during herpesvirus infection and is essential for the development of antiviral drugs and therapeutic strategies.

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Rapid recycling – unrevealed pathways of endosomal traveling towards the cell membrane

Gordana Blagojević Zagorac[1], Hana Mahmutefendić Lučin[1,3], Senka Mačešić[2], Ljerka Karleuša[1], Valentino Pavišić[1], Natalia Jug[1], Marina Marcelić[1], Silvija Lukanović Jurić[1], and Pero Lučin[1,3]

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Endocytosis is an essential cellular process by which extracellular fluid (fluid phase endocytosis) and membrane proteins and their ligands (receptor-mediated endocytosis) enter the cell and enable maintenance of cell homeostasis. Following endocytosis, endocytosed molecules are directed either to late endosomal route for degradation or are returned to the plasma membrane by a process called endosomal recycling. Endosomal recycling is highly regulated and highly dynamic process that involves activation of multiple Rab and Arf proteins and takes place at different endosomal levels. The endosomal system is composed of different structures that continuously undergo fusion and fission reactions, exchange and sort cargo that flows from small peripheral pre-early endosomes (pre-EEs) to large central endosomes. Traditionally, recycling route is divided into two steps; fast recycling route that takes place at the level of early endosomes (EE) and the slow route that occurs from endosomal recycling compartment (ERC). However, some authors, by using fluorescent lipophilic dyes shown that recycling occurs very early after endocytosis and this recycling route is often called rapid recycling because it is activated 2-3 minutes after endocytosis. Rapid recycling route is mainly underestimated because

most of the data about endosomal recycling were generated from studying transferrin/transferrin receptor (Tf/TfR) intracellular trafficking due to the availability of a good tool for Tf labeling. However, an indicator of Tf recycling is the quantification of the ligand release from the cell, and the release of Tf only occurs when the Tf-TfR complex reaches sufficiently acidic compartment which converts holo- into apo-Tf. Therefore, the rapid recycling was invisible to conventional assays, and only development of the new techniques like TIRFM and live microscopy enabled visualization of rapid recycling vesicles. In our laboratory, we analyzed intracellular trafficking of seven different cargo molecules (transferrin receptor, fully conformed and non-conformed MHC-I, CD44, cholera-toxin B subunit, ICAM1, and G-protein coupled receptor Rae-1) which use different intracellular routes. For that purpose, we created a multicompartment mathematical model, and developed software by which we can follow the intracellular trafficking of endocytosed molecules. We have shown that all investigated molecules use rapid recycling route following endocytosis. From this data, we can conclude that rapid recycling takes place constitutively and it is essential for the maintenance of cell homeostasis and should not be underestimated when analyzing cellular processes.

This work was supported in part by the Croatian Science Foundation (grant IP-2014-9-9564) and by the University of Rijeka (grants 13.06.1.1.4, 13.06.2.1.55, and 13.06.2.1.56).

The extensive remodeling – reorganization of the endosomal and secretory system during the early phase of cytomegalovirus infection

Hana Mahmutefendić Lučin[1,2], Ljerka Karleuša[1], Gordana Blagojević Zagorac[1], Kristina Grabušić[3], Valentino Pavišić[1], Natalia Jug[1], Marina Marcelić[1], Silvija Lukanović Jurić[1], and Pero Lučin[1,2],

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Cytomegalovirus (CMV) infection causes the remodeling of the cellular endosomal system that terminates with the formation of the viral assembly compartment (viral factory). However, the core mechanisms and the consequences that consider the cell function are poorly understood.

We have shown that infection with murine cytomegalovirus (MCMV) induces early rearrangement (6 hrs p.i.) of intracellular membranes and formation of the juxtanuclear EPERC (early-phase endosomal retention compartment). The process includes the restructuring of early endosomes (EE), endosomal recycling compartment (ERC), and *trans*-Golgi network (TGN). EPERC is also the place where molecules that are typically transported through early/sorting endosomes are retained (i.e., transferrin receptor [TfR], MHC class-I [MHC-I] molecules, mannose-6-phosphate receptor [M6PR], epidermal growth factor receptor [EGFR]). Therefore, considering the involvement of EE and ERC in the generation of this remodeled compartment, we performed a functional analysis to determine the kinetics of recycling from the endosomal system to the plasma membrane. By using the flow cytometry and confocal microscopy, we have shown that the recycling of TfR, MHC-I, and Rae-1 is significantly retarded. The same conclusion was obtained with the NBD-sphingomyelin exit, a marker that labels the lipid domains of membranes,

indicating that MCMV infection causes the general block in endosomal recycling. Furthermore, we used the in-house-developed software to perform kinetic modeling of the recycling circuit and concluded that TfR recycling is inhibited at the stage of EEs and the ERC, but not from peripheral recycling endosomes (pre-EEs). It is important to notice that that endosomal rearrangement is not only crucial for the generation of the viral factory but also for modulation of important cellular processes. For example, by inhibition of endosomal recycling of MHC-I and Rae-1 molecules and their fast removal from the cell surface, the virus disables antigen presentation and recognition of infected cell by cytotoxic T lymphocytes and NK cells, respectively.
To reveal a mechanism by which MCMV generates the development of the EPERC, we analyzed the effects of early infection on the expression of small GTPases from Rab and Arf family that shape the endosomal recycling circuit. Western blot analysis demonstrated downregulation of Rab22a, Rab4 and Rab 11 already at 4-8 hrs p.i. Furthermore, by using confocal microscopy, we have found that this remodeled compartment is Arf6-, Rab5-, Rab22a-, and Rab11-positive, but Rab35-, Rab8- and Rab10-negative. Interestingly, the results from the literature indicate that the Rab8 and Rab10 molecules are expressed in the cascade fashion, following the Rab35 activation. The inhibition of recycling in infected cells could be partially explained by the fact that over-activation of Arf6 leads to the block in recycling processes. The same effect of recycling inhibition follows the depletion of Rab35, the molecule that works antagonistically with Arf6. EPI64, the Rab35-GAP, was upregulated whereas ACAP1/2, the Rab35 effector, and Arf6-GAP, was absent from EPERC, suggesting that MCMV disrupts the Arf6/Rab35 cascade in order to develop the EPERC.
Finally, we can conclude that MCMV in the early phase of infection remodels the EE, the ERC, the TGN system, and cause the intracellular retention of recycling molecules. The endosomal remodeling is achieved by alteration of expression of Arf and Rab regulatory proteins, including also their effectors, and by dysregulation of Arf/Rab regulatory cascades. The whole process is played to prepare the formation of the viral assembly compartment in the late phase of the infection.

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Shipments from cells - exosomes and other extracellular vesicles in translational medicine

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Cells can pack proteins and nucleic acids into nanoparticles enveloped by a lipid bilayer and secrete them into extracellular space. These membrane nanoparticles are 10 to 1000 nm in size, contained in body fluids, have a spherical shape and derive from the plasma membrane or endosomal compartment. They are mostly known as exosomes,

microvesicles or extracellular vesicles (EVs) in general. The final EV categorization and nomenclature is still under debate in the research community, since methods for EV isolation and characterization, which broadly define EV type and properties, have not yet been standardized. However, the last 15 years of EV research have shown: i) all cells analyzed by now are capable of secreting EVs; ii) EV composition is dynamic and reflects type and condition of originating cell; iii) EV are present in all body fluids.

Roles of EVs have been described in various physiologic and pathologic processes, like an immune response, viral infections, neurodegenerative diseases and tumor formation and metastasis. These processes are mediated by two types of EV-cell interactions: (i) binding to cell surface and subsequent triggering of intracellular signals, (ii) delivery of EV content into the cell. Thus, molecular EV content, which includes proteins, lipids, and nucleic acids, can indicate undergoing processes in tissues and organs. Thanks to high prognostic and diagnostic potential, EVs are one of the most intensively studied fields in biomedicine during the last decade.

Our research is focused on studying clinical EVs as neuroregeneration markers after traumatic brain injury. Severe traumatic brain injury is the leading cause of mortality and morbidity among young people. It is triggered by physical force which causes primary injury, leads to loss of consciousness and coma. Brain injury spreads to surrounding neural and non-neural tissue as a secondary injury which is mediated by the massive release of neurotransmitters, free radicals, and inflammatory molecules. Complete neurologic recovery is possible, but there are no prognostic markers of neuroregeneration to contribute to therapeutical decisions.

We have shown that severe brain injury causes secretion of enlarged EVs in clinical intracranial CSF during the first seven days after injury (Kuharic et al., 2018). Next, to changed EV morphology we also detected changes in EV- and neuroregeneration-associated proteins in correlation to the patient outcome: Rab7a, Arf6, and Flotillin-1. These findings are consistent with the recent view of EVs as newly discovered mediators of intercellular communication in the brain which is necessary for reconstruction of existing and forming of new synapses during neuroregeneration. Moreover, cerebral ventricles, the primary site of cerebrospinal fluid (CSF) production, have been shown to react to changes in the brain due to stroke or aging by producing CSF of different neuroregenerative capacity. Our ongoing studies are focused on describing the complete EV proteome from intracranial CSF at day 1, 3 and seven after injury. Such protein characterization should describe dynamics in biologic processes during the acute phase of severe traumatic brain injury and help to detect novel neuroregeneration markers.

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