Univerza *v Ljubljani*
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Translational Medicine: Neuroglia

Translacijska medicina: Nevroglija

PROGRAMME AND BOOK OF ABSTRACTS
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Translational Medicine: Neuroglia

Neuroglia, also called the "other brain", consist of microglia, oligodendroglia and astroglia. The number of neuroglial cells in some areas of the brain outnumber neurons, as is especially the case with astrocytes. Defects in neuroglia leads to disease. For the development of new drugs, it is therefore important to understand the function of neuroglia. The aim of the workshop is to shed light on the role of astroglia in regulating metabolism, especially aerobic glycolysis and the role of organelles contacts, an emerging field of biology, in the function of glia. Moreover, we will address the role of astrocytes in infection with neurotropic viruses such as SARS-CoV-2, the invasion of glioblastoma cancer cells and their interaction with astrocytes, and how astrocytes contribute to pro-inflammatory conditions in the central nervous system. We will consider neurodegeneration of noradrenergic neurons, releasing noradrenaline, acting as an endogenous anti-inflammatory agent in the central nervous system. We will also be interested in how we could compensate for the lack of noradrenaline in aging and cognitive decline. Importantly, as the time of presenting the Nobel Prize for Physiology or Medicine in 2023 is approaching we will have a special lecture on this topic (Day of Physiology 2023). The workshop will be held in English and also online.

Translacijska medicina: Nevroglija

Nevroglija, imenovana tudi "drugi možgani", je sestavljena iz mikroglije, oligodendroglije in astroglije. Število celic nevroglije v nekaterih delih možganov presega število nevronov, kar zlasti velja za astrocite. Okvare nevroglije vodijo v bolezen. Za razvoj novih zdravil je zato pomembno razumeti delovanje nevroglije. Namen delavnice je osvetliti vlogo astroglije pri uravnavanju presnove, predvsem aerobne glikolize, kjer nastaja laktat, ter vlogo tega procesa in stikov med celičnimi organeli, novo področje v biologiji, v delovanju glije. Poleg tega bomo obravnavali vlogo astrocitov pri okužbi z nevrotropnimi virusi, kot je SARS-CoV-2, invazijo rakavih celic glioblastoma in njihovo interakcijo z astrociti ter kako astrociti prispevajo k vnetnim stanjem v centralnem živčnem sistemu. Osredotočili se bomo v nevrodegeneracijo noradrenergičnih nevronov, ki sproščajo noradrenalin, ki deluje tudi kot endogeno protivnetno sredstvo v centralnem živčnem sistemu. Zanimalo nas bo tudi, kako bi lahko nadomestili pomanjkanje noradrenalina pri staranju in kognitivnem upadu. Ker se bliža čas podelitve Nobelove nagrade za fiziologijo ali medicino za leto 2023, bomo imeli na to temo posebno predavanje (Dan fiziologije 2023). Delavnica bo potekala v angleškem jeziku in tudi preko spleta.

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Od odkritja lastnosti RNA do cepiv za SARS-CoV-2: Nobelova nagrada iz fiziologije in medicine 2023

Alojz Ihan

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Nobelova nagrada za odločilna odkritja, ki so omogočila nastanek mRNK cepiv, je bila zaradi pomena cepljenja proti covid-19 med pandemijo samo vprašanje časa. Prelomno odkritje, ki je omogočilo izdelavo mRNK cepiv, sega v leto 2005, ko sta v reviji "Immunity" dva vodilna avtorja (Katalin Karikó in Drew Weissman) s sodelavci objavila, zakaj evkariontska informacijska RNK (mRNK) v človeških celicah ne sproža vnetja. Za razliko od virusne ali prokariontske mRNK, ki takoj, ko vdre v človeške celice, vzdraži celične vnetne senzorje – tolične receptorje 3, 7 in 8. Opisano dražanje vnetnih senzorjev je dolga leta pred člankom v reviji "Immunity" preprečevalo uporabo sintetično proizvedene mRNK za krmiljenje naših celic – to so bile namreč dolgoletne sanje madžarske biokemičarke Katalin Karikó, ki se je že leta 1978 začela ukvarjati s to mislijo, sprva na Univerzi Szeged, ko so jo tam odpustili, pa je leta 1985 emigrirala v ZDA, kjer je na univerzi Pennsylvania lahko nadaljevala svoje eksperimente o uporabi sintetske mRNK za krmiljenje delovanja človeških celic.

Katalin Karikó in Drew Weissman sta leta 2005 v reviji "Immunity" objavila, da je vključitev psevdouridina v *in vitro* prepisano mRNK odpravila aktivacijo vnetnih odzivov, ko so bile te mRNK dodane dendritičnim celicam. Razvojni potencial opisanega odkritja in univerzitetnega patenta, kako izdelati sintetično mRNK in jo uporabiti za krmiljenje človeških celic, je kmalu prepoznal eminentni ameriški molekularni biolog Derrick Rossi, in leta 2010 odkupil patent in na njegovi osnovi ustanovil podjetje Moderna. Cilj podjetja je bil razvoj krmiljenja oz. "preprogramiranja" celic za tumorske terapije in razvoj "čistih cepiv". Pri slednjih mRNK zgolj z informacijo preprogramira človeške celice v kratkotrajno izdelovanje občutljivih delov mikroorganizmov, ki jih nato prepoznajo imunske celice in se vnaprej pripravijo na morebitno "pravo " okužbo.

Potem je prišla pandemija. Z nastankom pandemije covid-19 je Katalin Karikó postala del zgodovine, saj sta na njenem delu bliskovito zrasli dve podjetji, Moderna in BioNTech, ki sta omogočili izdelavo cepiv na povsem nov način. Zaradi te tehnologije ima svet za bodočnost zagotovljeno novo platform za pripravo cepiv, ki bo pri pojavu novih mikroorganizmov omogočala v nekaj mesecih "sprogramirati" in tudi tehnološko izdelati velike količine učinkovitih cepiv. S tega vidika pandemija covid-19 ni prinesla samo velike preizkušnje, ampak tudi veliko upanja in rešitev za obvladovanje epidemij in bolezni v bodoče.

From the discovery of RNA characteristics to SARS-CoV-2 vaccines: Nobel Prize in Physiology or Medicine 2023

Alojz Ihan

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia

The Nobel Prize for the decisive discoveries that led to the creation of mRNA vaccines was only a matter of time away since the successful introduction of the covid-19 vaccine. The breakthrough discovery that made mRNA vaccines possible dates back to 2005, when two lead authors (Katalin Karikó and Drew Weissman) published a ground-breaking discovery in the journal Immunity on why eukaryotic messenger RNA (mRNA) does not trigger inflammation in human cells. This is unlike viral or prokaryotic mRNA, which, as soon as it enters human cells, irritates the cell's inflammatory sensors - TLR 3, 7 and 8. This irritation of inflammatory sensors had prevented the use of synthetically produced mRNA to control eukaryotic cells for years before the Immunity paper appeared. However, mRNA controlling of human cells was a long-held dream of Hungarian biochemist Katalin Karikó, Karikó, who started working on this idea as early as 1978, initially at the University of Szeged, emigrated to the USA in 1985 when she was fired and was able to continue her experiments at the University of Pennsylvania on the use of synthetic mRNA to control human cell function.

In 2005, Katalin Karikó and Drew Weissman published in the journal Immunity that the incorporation of pseudouridine into *in vitro* transcribed mRNA abolished the activation of inflammatory responses when these mRNAs were added to dendritic cells. The potential of this discovery and the university patent on how to make synthetic mRNA and use it to control human cells was soon recognised by the eminent molecular biologist Derrick Rossi, who bought the patent in 2010 and founded Moderna on the basis of it. The company's goal was to develop cell control or "reprogramming" for tumour therapies and to develop "clean vaccines". In the latter, mRNA simply reprograms human cells with information to briefly produce antigenic parts of micro-organisms, which are then recognised

by immune cells and prepared in advance for a possible "real" infection.

Then came the pandemic. With the emergence of the covid-19 pandemic, Katalin Karikó became part of history, as two companies, Moderna and BioNTech, grew rapidly on her discovery, making it possible to produce vaccines in a completely new way. Thanks to this technology, the world is assured of a new vaccine platform for the future, which, when new micro-aggressions emerge, will make it possible to "programme" and technologically produce huge quantities of effective vaccines. In this respect, the covid-19 pandemic has not only been a terrible disaster, but also a great hope and a solution for the control of future epidemics and diseases.

Carbon nanotubes in translational astrogliology

Vladimir Parpura

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Carbon nanotubes (CNTs) have shown much promise in neurobiology and biomedicine. Their structural stability and ease of chemical modification make them compatible for biological applications. Parpura discusses the effects that chemically functionalized CNTs, applied as colloidal solute or used as strata, have on the morphological and functional (glutamate uptake and vesicular recycling) properties of astrocytes, with an insight into the potential use of CNTs in neural prostheses, as well as in pre-clinical treatment of astrocytoma/glioblastoma and traumatic brain injury model.

Neuronal and glial calcium signalling and glucose metabolism in aged *Drosophila* brain

Urška Černe[1], Anemari Horvat[1,2], Nika Kozoderc[1], Anne-Kristin Dahse[3], Robert Zorec[1,2], Nicole Scholz[3], Nina Vardjan[1,2]

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In ageing and neurodegeneration brain glucose utilization is reduced, likely contributing to the progression of cognitive decline. This may occur due to a malfunction of the noradrenergic system which controls the brain metabolism, as well as learning and memory formation. Release of noradrenaline from noradrenergic neurons triggers intracellular increases in Ca^{2+} and cAMP signals in brain cells through the activation of α_1- and/or β-adrenergic receptors, respectively. In astrocytes, at least in in vitro systems, noradrenergic signals increase glucose uptake, glycogen degradation and facilitate aerobic glycolysis with the end-product lactate. The latter has been considered to be shuttled from astrocytes to neurons to serve as a fuel during increased brain activity, enhancing cognition. Whether noradrenergic activation controlling neuronal and glial metabolism is impaired in aged brains is unclear and was assessed in this study.

By using binary expression system Gal4/UAS we have generated Drosophila melanogaster expressing fluorescent sensors for Ca^{2+}, cAMP, free glucose and lactate selectively in neurons or glial cells. We measured the changes in intracellular second messengers and metabolites by ex vivo whole brain confocal microscopy upon the exposure of brains to octopamine (an invertebrate analogue of noradrenaline). We performed the studies in the brains of young and aged flies. Aged flies exhibited reduced locomotion behaviour, increased neurodegenerative lesions and upregulation of octopamine/tyramine receptors in the brain. Octopamine triggered Ca^{2+} signals in neurons and glial cells in young

brains, but not in aged brains, suggesting age-related impairment in intracellular Ca^{2+}-signalling. Neurons (but not glial cells) responded to octopamine with an increase in intracellular cAMP and lactate, indicating that aerobic glycolysis occurs primarily in neurons and not in glial cells. Neuronal cAMP signals and aerobic glycolysis were not impaired in aged brains. Despite the absence of octopamine-induced aerobic glycolysis in glial cells, these cells responded to octopamine with cytosolic glucose increase, most likely due to extracellular free glucose uptake, which was reduced in aged brains. Both neurons and glial cells were able to uptake extracellular glucose and lactate to a similar extent. In neurons the glucose uptake was, however, reduced in aged brains.

These results indicate that neurons, but not glia-like cells, are the primary site of regulated aerobic glycolysis in *Drosophila*. In aged brains octopaminergic Ca^{2+} signalling, regulation of glial glucose uptake and the glucose delivery to neurons were impaired, which might contribute to the age-related cognitive-like deficits.

Density of GPR27 in astrocytes and neurons and cytosolic L-lactate levels stimulated by GPR27 agonists

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Astrocytes and neurons are metabolically coupled. According to the astrocyte-to-neuron lactate shuttle (ANLS) hypothesis, astrocytes produce L-lactate (LL), an end product of aerobic glycolysis (AG). Upon increased neuronal activity, LL transported from astrocytes is converted to pyruvate and used for energy production in oxidative phosphorylation. It has been shown that AG in astrocytes can be potently activated through certain GPCR agonists such as NA and LL. Recently, GPR27, an orphan GPCR with hitherto unknown functions, has also been proposed to modulate ERK and Akt signaling, both involved in promoting glycolysis. However, it has not yet been established whether GPR27 activation also affects LL metabolism in astrocytes and LCn. Unlike neurons, astrocytes are able to store glycogen and are therefore capable of rapid activation of glycolysis and LL production. Therefore, the metabolic handling of LL is expected to be different in the individual cell types.

First, we investigated the effect of stimulation with LL [10 mM] and NA [100 µM] on the dynamics of LLi changes in astrocytes and LCn and compared whether it differs in both cell types. Next, we investigated the presence and density of GPR27 on cortical astrocytes and LCn and the effect of its activation on LLi dynamics using ligands predicted to be specific for GPR27.

Isolated rat LCn and cortical astrocytes were transduced with fluorescence resonance energy transfer (FRET)-based L-lactate sensor (Laconic). We measured changes in the ratio of FRET signals, reflecting changes in LLi in real time after stimulation. The presence and density of GPR27 was determined by immunocytochemistry and using confocal microscopy.

The present data show that LLi dynamics in astrocytes and LCn differ significantly upon NA stimulation, as well as in the presence of elevated extracellular LL, suggesting a different handling of LLi in the two cell types. GPR27 is found to be present on the surface of both cell types, astrocytes and LCn, but appears to be at higher density in LCn. Also, in both astrocytes and LCn, an increase in LLi is induced by GPR27 stimulation, which may represent an important physiological role of this super-conserved receptor expressed in the brain.

Exploring New Mechanisms of Dopamine-induced L-lactate Production

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The role of dopamine in astrocytes is not fully understood. Our study aims to explore the potential crosstalk between astrocytes and dopamine, focusing on astroglial metabolism. Using FRET biosensors, we found that dopamine increased intracellular cAMP and L-lactate at the single-cell level. We will examine the dopamine-induced dynamics of intracellular glucose and Ca^{2+} in astrocytes to further clarify the regulatory role of dopamine in astroglial metabolism. In addition, our research plans to be conducted in both in vitro and in vivo experiments under the pathophysiology of neurological disorders, particularly Major Depressive Disorder. Ultimately, our goal is to develop the foundation for new drugs targeting the mechanism of action of dopamine on astrocytes.

Malignant Glioma – from Bench to Bedside

Vedrana Montana

International Translational Neuroscience Research Institute, Zhejiang Chinese Medical University, Hangzhou, P.R. China

Malignant gliomas or glioblastoma multiforme, GBM, primary brain tumors derived from glia or glial precursor cells, are characterized by invasive growth, which represents a significant obstacle to successful treatment with standard therapy of surgery followed by radiotherapy and temozolomide chemotherapy. High incidence of recurrent tumor growth and short median patient survival rate of 14 months are result of almost impossible complete surgical removal and, therefore, tumor dispersal throughout the brain. We have identified that bradykinin, BK, through binding to bradykinin 2 receptor, B2R induces calcium excitability followed by vesicular glutamate release which promotes migration and invasion of human GBM patient-derived xenolines/xenografts (PDX), the best pre-clinical model of GBM. The effects are reduced by blocking the receptor with icatibant, an FDA approved B2R blocker. Furthermore, we found that B2R is overexpressed in a subset of patient's tissue samples, while our analysis of RNAseq data available from The Cancer Genome Atlas (TCGA) indicates that high mRNA expression of B2R in glioma correlates with significantly shorter patient life span. Pre-clinical data indicate that the median GBM tumor volume in the icatibant-treated mice was 58% of that in the sham-treated mice, while survival of icatibant-treated mice was 25% longer than of the sham-treated mice. Taken together, B2R emerges as a therapeutic target and icatibant as the first adjuvant therapy that tempers the invasion of malignant glioma.

Immunohybridomas to treat glioblastoma? From understanding lysosomal fusion in reactive astrocytes to treating prostate cancer by immunohybridomas

Helena H. Chowdhury[1,2], Simon Hawlina[3,4], Marko Kreft[1,2,5] and Robert Zorec[1,2]

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In 2009, new EU legislation regulating advanced therapy medicinal products (ATMPs), consisting of gene therapy, tissue engineering and cell-based medicines, was introduced. Although less than 20 ATMPs were authorized since that time, the awarding of the Nobel Prize for Physiology or Medicine in 2018 reviwed interests in developing new cancer immunotherapies involving the manipulation of patient's own immune cells, including lymphocytes and dendritic cells. Lymphocytes are mainly thought to directly affect tumor cells. In contrast, dendritic cells are involved indirectly, by mediating antigen presentation to other leukocytes, thus orchestrating the immune response. It is the latter cells that have been used in the clinical trial treating patients with castration resistant prostate cancer (CRPC) with an immunohybridoma cell construct (termed aHyC), produced by electrofusion of autologous tumor and dendritic cells. The results of the clinical trial revealed that cancer-specifc survival and the time to next in-line therapy (TTNT) were both signifcantly prolonged versus controls. When patients were observed for longer periods since the time of diagnosis of CRPC, 20% of patients had not yet progressed to the next in-line therapy even though the time under observation was~80 months (Chowdhury et al., 2021 Clin Transl Med; Hawlina et al 2022, Biology Direct). It was concluded that autologous dendritic cell-based immunotherapy is a new possibility to treat not only CRPC but also other solid tumors. Currently a clinical trial to treat triple-

negative breast cancer is being started and the treatment of glioblastoma multiforme, a highly heterologous form of cancer is being considered for cell-based immunotherapy by aHyC.

Nuclear pathways affected by cytoplasmic RNA viruses, mechanism and implications

Alessandro Marcello

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Several RNA viruses with relevant human health impact have a cytoplasmic replication cycle. However, there are several indications that they are also able to modulate nuclear pathways to promote a conductive cellular environment. Here I will introduce the topic with different examples and dwelve in more detail on our recent observation that SARS-CoV-2 induces DNA damage, inflammation and cellular senescence (Gioia et al., Nature Cell Biology, 2023). Such mechanism may also be relevant to the long-term consequences of COVID.

SARS-CoV-2 infects human astrocytes but not human neurons

Borut Furlani[1], Maja Potokar[1,2], Miša Korva[3], Tomaž M. Zorec[2,3], Matjaž Stenovec[1,2], Tatjana Avšič-Županc[3], Robert Zorec[1,2], Jernej Jorgačevski[1,2]

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing global COVID-19 pandemic, exhibiting a number of neurological signs and symptoms. It is likely that SARS-CoV-2 infects neural cells directly, especially astrocytes, which exhibit aerobic glycolysis, a metabolic process, supporting morphologic plasticity, cell division and virus replication. Here we studied whether SARS-CoV-2 infects human neurons and astrocytes. We infected cells with two strains (B.1.1.1.7 and B.1.258.17) of SARS-CoV-2, with their identity determined by Illumina deep sequencing and mapping-consensus based complete genome analysis. The results revealed that human astrocytes but not neurons are the cell type that supports proliferation and further infection of neural cells. By using confocal microscopy and structured illumination microscopy (SIM), we have assessed further astrocyte respond to SARS-Cov-2 particles by monitoring the expression and distribution of ACE-2 and two viral proteins (RNA-dependent RNA polymerase - RDRP and envelope protein - E) immune-cytochemically in SARS-CoV-2-infected astrocytes. While ketamine-treatment did not significantly affect the gradual decrease in the viability of SARS-CoV-2-infected astrocytes, the percentage of astrocytes, infected with B.1.258.17, was lower in ketamine-treated cells. Infection of astrocytes with either strain of SARS-CoV-2 upregulated the expression of ACE-2 and triggered its redistribution to the cell periphery, whereas ketamine was shown to partially attenuate this effect. Furthermore, the density of RDRP- and E-proteins was

lower in ketamine-treated astrocytes, compared with the non-treated controls. In summary, our study on human astrocytes highlights the potential benefits of ketamine-treatment in COVID-19 hospitalized patients.

The role of mitochondria-lysosome contacts in health and disease

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Both mitochondria and lysosomes are multifunctional organelles that have been implicated in numerous human diseases. Mitochondria are necessary for cellular respiration and ATP synthesis, and they are also important storage sites for ions, lipids, and other metabolites. Similarly, lysosomes act as storage sites for ions such as calcium, iron, nucleosides and play a critical role in degrading cellular contents such as proteins and lipids.

We have previously described the functional interplay of mitochondrial and lysosomal dysfunction in neurodegeneration (Burbulla et al, Science), whereas our recent data demonstrated direct contacts between mitochondria and lysosomes in various cell types (Wong et al., Nature). Importantly, the formation of mitochondria-lysosome contacts is independent from autophagosome biogenesis or mitophagy. We found that mitochondria-lysosome contacts are regulated by multiple proteins on the mitochondrial and lysosomal membranes. In its active GTP-bound and lysosome-localized state, Rab7 mediates the tethering between mitochondria and lysosomes. Rab7 GTP hydrolysis is stimulated by Rab GAPs (GTPase-activating proteins) such as TBC1D15, resulting in an inactive, cytosolic GDP-bound form of Rab7. Interestingly, TBC1D15 is recruited to the outer mitochondrial membrane by the mitochondrial transmembrane fission protein FIS1, and TBC1D15- mediated Rab7 GTP hydrolysis was found to promote untethering of mitochondria-lysosome contacts.

Our recent studies have suggested that mitochondria-lysosome contact sites regulate organelle dynamics and metabolite transfer. Mitochondrial dynamics also depend on contacts with lysosomes, which affect the rate of mitochondrial

fission in a manner dependent on FIS1, TBC1D15, and Rab7 (Wong et al J. of Cell Biology). Beyond organelle dynamics, we found that mitochondria-lysosome contacts serve as sites of direct transfer of Ca^{2+} (Peng et al., PNAS) and amino acids (Peng et al Science Advances) from lysosomes to mitochondria.

Taken together, studies on the structure and function of mitochondria-lysosome contact sites reveal a clear role in cellular homeostasis, including in neurons. This raises the question of the importance of mitochondria-lysosome contacts to the development and progression of neurological disease. Indeed, we found that several proteins associated with contact sites have been implicated in the pathology of Parkinson's disease (Kim et al, Nature Communications, Peng et al Science Advances), Charcot-Marie-Tooth disease (Wong et a; Dev Cell), and other neurological disorders. We are currently pursuing further studies of the role of mitochondria-lysosome contacts in normal and diseased human neurons and glia. Thus, further insights into organelle contact site dynamics and regulation will shed important light on physiological and pathological cellular functions.


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