1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, Medicinska fakulteta, Vrazov trg 2, 1000 Ljubljana, Slovenija

2. Ime in priimek mentorja (*Name and surname of a mentor*):

Nina Vardjan

3. Področje znanosti iz šifranta ARRS (*Primary research field*):

3.03. Medicinske vede. Nevrobiologija

4. Kontaktni e-naslov mentorja (*Contact of a mentor)*:

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5. Kratek opis programa usposabljanja (*Short description of the program)*:

SLO

Astrociti so celice glije v centralnem živčnem sistemu (CŽS), ki v možganski skorji številčno prekašajo nevrone. Dolgo časa so jim pripisovali zgolj vlogo vezivnega tkiva, vendar danes vemo, da so aktivno vključeni v uravnavanje številnih fizioloških kot tudi patoloških procesov v CŽS. Sodelujejo pri sinaptičnem prenosu in procesiranju informacij v možganih, tvorbi spomina in učenju (preko receptorjev na svoji površini se odzivajo na nevrotransmitorje in izločajo svoje lastne signalne molekule), uravnavajo pretok krvi po žilah, zunajcelično homeostazo ionov (npr. K^+), pH, sodelujejo pri odstranjevanju odpadnih snovi iz intersticijske tekočine, uravnavajo dihanje, sodelujejo pri vnetnem odgovoru, itd.

Astrociti pa ključno prispevajo tudi k energetskem metabolizmu CŽS. Astrociti so celice v CŽS, v katerih se skladišči glikogen. Ob povečani aktivnosti nevronov in aktivaciji GPR na površini astrocitov iz glikogena lahko nastajata glukoza in laktat, ki ju astrociti kot vir energije posredujejo nevronom. V astrocitih pa so lahko prisotne tudi lipidne kapljice (LD, angl. »lipid droplets«), celični organeli obdani s fosfolipidnim monoslojem, v katerih se skladiščijo v obliki trigliceridov proste maščobne kisline (FFA, angl. »free fatty acids«) in pa sterolni estri (holesterol). Predvideva se, da so LD mobilni, dinamični organeli, ki najverjetneje nastajajo iz membran endoplazemskega retikuluma (biogeneza LD) in služijo kot vir FFA in holesterola v stresnih situacijah. FFA, ki nastajajo pri razgradnji LD, se lahko porabijo kot alternativen vir energije (β-oksidacija FFA v mitohondrijih) ali pa se izločijo iz celice, kjer so kot metabolni substrat na voljo sosednjim celicam. FFA pa lahko delujejo tudi kot signalne molekule, saj številne celice na svoji površini izražajo z G-proteini sklopljene receptorje (GPR) za FFA (FFAR). Recentne raziskave so pokazale, da je kopičenje LD v celicah glije v CŽS pri vinski mušici in glodalcih povezano z razvojem nevrodegenerativnih bolezni, kar kaže na vlogo LD v celicah glije tudi pri patoloških procesih CŽS. Molekularni mehanizmi uravnavanja lipidnega metabolizma in biogeneze LD v astrocitih in pa signalne poti, ki pri tem sodelujejo, so še neraziskani. Prav tako ni raziskana vloga oziroma sklopitev astrocitnega lipidnega metabolizma z glukoznim metabolizmom in pomen le-tega za metabolizem v možganih.

Kandidat/-ka bo z uporabo konfokalne in dvofotonske mikroskopije ter specifičnih fluorescenčnih označevalcev LD (Nile Red, Bodipy 493/503) preveril/-a, kako aktivacija GPR na površini astrocitov (npr. adrenergičnih, laktatnih GPR, FFAR), ki se kaže kot porast v znotrajcelični koncentraciji Ca^{2+} in/ali cAMP (ciklični adenozin monofosfat), vpliva na biogenezo LD v astrocitih. Pri tem bo astrocite v kulturi in možganskih tkivni rezinah izpostavil/-a agonistom GPR in kvantitativno ovrednotil/-a spremembe v številu in velikosti LD. Aktivacija GPR bi lahko podobno kot v adipocitih sprožila lipolizo trigliceridov v LD in nastanek FFA, ki se nato bodisi razgradijo v procesu β-oksidacije ali pa izločijo iz celic. Preveril/-a bo ali FFA v zunajcelični (npr. oleinska kislina) preko FFAR na površini astrocitov vplivajo na znotrajcelične signalne poti (Ca^{2+}, cAMP) in glukozni metabolizem v astrocitih. Meritve bo izvajal/-a v realnem času na astrocitih v kulturi in tkivnih rezinah. V astrocite bo predhodno s

transfekcijo ali s sistemom »gene gun« vnesel/-a plazmidno DNA, ki bo imela zapis za nanosenzor (npr. cAMP, glukozni, laktatni, piruvatni nanosenzor), katerega detekcija bo temeljila na metodi FRET (angl. »fluorescence resonance energy transfer«). Znotrajcelične spremembe v Ca^{2+} pa bo spremljal/-a v astrocitih, ki bodo predhodno označeni s Ca^{2+}-indikatorjem Fluo4. S pomočjo imunocitokemije bo proučil/-a subcelično lokalizacijo LD v astrocitih. S poskusi v realnem času pa bo preveril/a ali so LD v astrocitih povezane s citoskeletom ter kako na mobilnost LD vpliva porast znotrajceličnih signalnih molekul kot sta cAMP in Ca^{2+}. Raziskave bodo pomembno prispevale k razumevanju fiziologije lipidnega metabolizma v astrocitih in širše v CŽS.

ANG

Astrocytes are glial cells in the central nervous system (CNS) that in the cortex area largely outnumber neurons. For a long time they were considered as a “connective tissue“, but it is known today that they are actively involved in the regulation of many physiological and pathological processes in the CNS. They contribute to synaptic transmission and brain information processing, memory formation and learning (respond to neurotransmitters via their surface receptors and secrete their own signalling molecules), regulate blood flow, extracellular ion homeostasis (K^+), pH, they participate in the removal of debris from the interstitial fluid, regulate breathing, CNS inflammatory response, etc.

Astrocytes also importantly contribute to the CNS energy metabolism. These cells store glycogen in CNS. During increased neuronal activity and upon activation of astrocytic surface receptors glycogen in astrocytes could be degraded to glucose and lactate, which are then released from astrocytes and used as an energy source by neighbouring neurons. Astrocytes also contain lipid droplets (LD), cellular organelles surrounded by a phospholipid monolayer, where free fatty acids (FFA) in the form of triglycerides and sterol esters (cholesterol) are stored. It is believed that LD are mobile, dynamic organelles formed from the membranes of the endoplasmic reticulum (biogenesis of LD) and serve as a source of FFA and cholesterol during stress. FFA released from LD can be utilized as an alternative energy source (mitochondrial β-oxidation of FFA) or, when released from cells, as a metabolic substrate in neighbouring cells. Moreover, extracellularly FFA may act as signalling molecules, as many cells on their surface express the G-protein coupled receptors (GPR) for FFA (FFAR). Recent studies have shown that the accumulation of LD in glial cells in the CNS of *Drosophila* flies and rodents is associated with the development of neurodegenerative diseases, suggesting a role of glial LD in the CNS pathologies. Molecular mechanisms underlying lipid metabolism and biogenesis of LD in astrocytes and the signalling pathways involved in these processes are still unexplored. Furthermore, the role and potential coupling of lipid metabolism with the glucose metabolism in astrocytes as well as its importance in the general brain metabolism is unknown.

The candidate will use confocal, two-photon microscopy and specific fluorescent LD markers (Nile Red, Bodipy 493/503) to study, how activation of plasma membrane GPR (e.g. adrenergic and lactate GPR, FFAR), which may trigger increases in the intracellular concentration of Ca^{2+} and/or cAMP (cyclic adenosine monophosphate), affects the biogenesis of LDs in astrocytes. She/he will expose astrocytes in culture and brain tissue slices to various GPR agonists and quantitatively evaluate changes in the number and size of LD. As was observed in adipocytes GPR activation could cause lipolysis of triglycerides in LD and FFA formation also in astrocytes. When formed, FFA could be either broken down in the process of β-oxidation and used as energy source or released from cells. The candidate will explore, whether extracellular FFA, such as oleic acid, via binding to plasma membrane FFAR triggers in astrocytes intracellular signalling pathways (Ca^{2+}, cAMP) and activates glucose metabolism. These measurements will be carried out using real-time confocal imaging on single astrocytes expressing FRET (fluorescence resonance energy transfer) - based nanosensors for cAMP, glucose, lactate, pyruvate, both in culture and tissue slices. pDNA vectors carrying FRET nanosensors will be introduced into astrocytes by transfection or with the gene gun delivery system. Changes in intracellular Ca^{2+} will be monitored in astrocytes pre-labeled with a Ca^{2+} indicator Fluo4. Moreover, immunocytochemistry will be used to examine the subcellular localization of LD in fixed astrocytes. To determine whether LD in astrocytes associate with cytoskeleton and how the mobility of LD is affected by the increase in signalling molecules (cAMP and Ca^{2+}), the real-time confocal imaging of fluorescently labeled LD will be performed. This study will importantly contribute to the understanding of physiology of lipid metabolism in astrocytes and CNS.