Opis delovnega mesta mladega raziskovalca/ke (D*escription of the Young Researcher's position)*

1. Članica UL (*UL member)*:

Medicinska fakulteta

2. Ime, priimek in elektronski naslov mentorja/ice (*Mentor's name, surname and email*):

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3. Raziskovalno področje (*Research field*):

3.03 Nevrobiologija

4. Opis delovnega mesta mladega raziskovalca/ke (D*escription of the Young Researcher's position)*:
Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce.

*slo: Mehanizmi prožen*ja *avtofagije/mitofagije z zunajceličnim laktatom noradrenalinom in agonisti za GPR27 v astrocitih v kulturi* *Astrociti so najštevilčnejše celice glije, ki prispevajo k homeostazi v osrednjem živčevju in uravnavajo številne funkcije živčevja. Avtofagija je proces v katerem se razgradi celični material in ima pomembno vlogo pri uravnavanju energije v stresnih razmerah, recikliranju makromolekul, odstranjevanju napačno zvitih beljakovin, skupkov beljakovin in poškodovanih organelov, kot so mitohondrij (v tem primeru se proces imenuje mitofagija) (Glick et al., 2010). Med procesom avtofagije se sestavni deli citosola, vključno z beljakovinami, organeli in drugim materialom znotraj celic dostavijo v lizosome, kjer poteka razgradnja (Martinez-Vicente, 2017). Recentne raziskave kažejo, da so okvare v procesih avtofagije povezane z bolezenskimi stanji, med katerimi so tudi nevrodegenerativne bolezni, kot so Alzheimerjeva bolezen, Parkinsonova bolezen in Huntingtonova bolezen, kardiovaskularne bolezni, rak (Glick et al., 2010) in ishemija (Chen et al., 2014).* *V naši raziskavi bomo preučevali makroavtofagijo, posebej pa se bomo osredotočili na mitofagijo, tj. selektivno avtofagijo mitohondrijev. Pri procesu avtofagije se deli citoplazme namenjeni za razgradnjo dostavijo v lizosome prek avtofagosoma, ki se zlije z lizosomom v avtolizosom (Glick et al., 2010; Martinez-Vicente, 2017). Proces avtofagije se prične z nastankom membranske strukture fagofora, ki zori v mešiček, imenovan avtofagosom. Proces nastanka avtofagosomov in njihovih povezav s citoskeletom je še v veliki meri neraziskan. Kaže, da je različen izvor avtofagosomov vezan na tip celic, posledično pa to pomeni, da je lahko tudi njihova vpetost v mrežo citoskeleta ter premikanje ob citoskeletu celično specifična. Različne beljakovine povezane s citoskeletom bi lahko imele pomembno vlogo pri transportu avtofagnih predelkov v nevralnih celicah (Winter et al., 2015). Predlagana tema doktorskega dela je osredotočena na vlogo zunajceličnega laktata pri stimulaciji avtofagije. Nastanek laktata stimulira aktivacija adrenergičnih receptorjev, med drugim tudi receptorja GPR27. Ker se s starostjo in pri nevrodegenerativnih boleznih količina noradrenalina zniža, nas zanima ali lahko nadomestimo pomanjkanje noradrenalina s stimulacijo omenjenega receptorja. V ta namen bomo določili ali laktat, aktivacija noradrenalina in receptorjev GPR27 deluje podobno na mitofagijo. Za aktivacijo receptorjev GPCR je pomembna vpetost teh receptorjev s citoskeletom. Astrociti v osrednjem živčnem sistemu privzemajo glukozo iz krvi in jo presnavljajo v laktat, ki ga, ko je to potrebno, posredujejo nevronom. Vpliv zunajceličnega laktata na avtofagijo astrocitov, ki je tema predlagane raziskave, še ni bil raziskan. V raziskavi se bomo osredotočili na ta cilj, posebno pozornost pa*

bomo namenili mitofagiji.

Delo raziskovalne naloge bo dopolnjevalo raziskave programske skupine, ki se med drugim ukvarja s preučevanjem znotrajceličnega transporta organelov v celicah nevralnega izvora. Poskuse bomo izvajali na celicah glije (astrocitih) ali/in na nevronih osrednjega živčnega sistema. S fluorescentnim označevanjem znotrajceličnih mešičkov in organelov ter delov citoskeleta bomo s konfokalno mikroskopijo in z visokoločljivostnimi mikroskopijami preučili vpliv laktata na avtofagijo/mitofagijo in njihovo povezavo s citoskeletom.
Spremljanje dinamike laktata in sprememb v ravni avtofagije zahteva dobro poznavanje dela z optofiziološkimi in elektrofiziološkimi sistemi z veliko časovno in prostorsko ločljivostjo. Prednost bodo imeli kandidati z izkušnjami z laboratorijskim delom in izkazanim znanstvenim udejstvovanjem (predstavitev lastnega raziskovalnega dela v pisni in ustni obliki).

Reference:
Chen, W., Sun, Y., Liu, K. and Sun, X. (2014). Autophagy: a double-edged sword for neuronal survival after cerebral ischemia. Neural Regen Res 9, 1210–1216.
Glick, D., Barth, S., and Macleod, K.F. (2010). Autophagy: cellular and molecular mechanisms. J Pathol 221, 3-12.
Martinez-Vicente, M. (2017). Neuronal Mitophagy in Neurodegenerative Diseases. Front Mol Neurosci 10, 64.
Winter, L., Kuznetsov, A.V., Grimm, M., Zeöld, A., Fischer, I., and Wiche, G. (2015). Plectin isoform P1b and P1d deficiencies differentially affect mitochondrial morphology and function in skeletal muscle. Hum Mol Genet 24, 4530-4544.

eng: Mechanisms of autophagy/mitophagy induced by extracellular lactate, noradrenalin and GPR27 in cultured astrocytes

Astrocytes are the most abundant glial cells in the central nervous system that are closely associated with neuronal synapses. Autophagy is a conserved process by which cells capture cytoplasmic components for delivery to lysosomes and is of vital importance for recycling of molecules, cellular homeostasis, and degradation of damaged proteins and organelles, such as mitochondria (in this case the process is termed mitophagy) (Glick et al., 2010). Autophagy is a dynamic process associated with the formation of autophagosome that engulfs cellular components, including proteins and organelles, and eventually fuses with lysosomes to be degraded (Martinez-Vicente, 2017). Dysregulated autophagy can result in different neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, cardiovascular diseases, cancer (Glick et al., 2010), and ischemia (Chen et al., 2014).
In our study we will focus on macroautophagy, especially mitophagy, where the aged and damaged mitochondria are selectively removed. During the process of autophagy the targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membraned vesicle known as an autophagosome (Glick et al., 2010; Martinez-Vicente, 2017). Initiation of autophagy begins with the formation of a double-membrane sequestering compartment termed the phagophore, which matures into an autophagosome. This process, as well as the role of the cytoskeleton in it, remain largely unexplored. It appears that the different source of autophagosomes as well as the traffic along the cytoskeleton is cell specific. Some of the cytoskeleton-related proteins are likely vital for proper traffic of autophagic structures in cells of neuronal lineage (Winter et al., 2008 in 2015). The proposed topic is focused on the role of extracellular lactate in the stimulation of autophagy. The formation of lactate is stimulated by the activation of adrenergic receptors, including the GPR27 receptor. Since the amount of noradrenaline decreases with age and in neurodegenerative diseases, we are interested whether we can replace the lack of noradrenaline by stimulating the aforementioned receptor. For this purpose, we will determine whether lactate, noradrenaline and GPR27 receptor activation act similarly on mitophagy. The association of these receptors with the cytoskeleton is important for the activation of GPCR receptors. Astrocytes take up glucose from the blood capillaries via glucose transporters (GLUTs). In astrocytes, glucose is either stored as glycogen or metabolized to

pyruvate. Pyruvate, in turn, is converted into lactate, which, when needed, is delivered to the neurons. Possible effects of extracellular lactate on autophagy in astrocytes has not yet been investigated yet. Therefore, we will address this question in the proposed research, where special attention will be dedicated to mitophagy.

The proposed project will converge with the research focuses of our laboratory, which are, among others, intracellular transport of different organelles and the role of cytoskeleton in this process. Experiments will be performed on glial cells and neurons, preparations, which are well established in our laboratory. Using different fluorescent markers in combination with confocal and superresolution microscopies and electrophysiological approaches, we will focus our research on the autophagy induced by extracellular lactate and the contribution of the cytoskeleton-related proteins.

References:

Chen, W., Sun, Y., Liu, K. and Sun, X. (2014). Autophagy: a double-edged sword for neuronal survival after cerebral ischemia. Neural Regen Res 9, 1210–1216.

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