Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training)*

1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, Medicinska fakulteta, Inštitut za patološko fiziologijo, Laboratorij za nevroendokrinologijo-molekulsko celično fiziologijo

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

akad.prof.dr. Robert Zorec; robert.zorec@mf.uni-lj.si

3. Šifra in naziv raziskovalnega področja (*Research field*):

3.03. Medicinske vede. Nevrobiologija

4. Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training*):

slo: - Raziskovalno delo mlade(ga) raziskovalke(ca) bo potekalo v Laboratoriju za nevroendokrinologijo-molekulska celična fiziologija, na Inštitutu za patološko fiziologijo Medicinske fakultete v Ljubljani. Začetek raziskovalnega dela je predviden v jeseni 2023. Kandidate(ke), ki bodo do predvidenega roka septembra 2023 zaključili magistrski študij na 2. stopnji naravoslovnih smeri, kot so biologija, biokemija, medicina, biotehnologija, mikrobiologija, farmacija, kemija in imajo željo po raziskovanju vabimo, da se prijavijo prek spletne aplikacije ter pošljejo življenjepis in motivacijsko pismo na naslov: robert.zorec@mf.uni-lj.si. Prednost pri izbiri bodo imeli kandidati z visoko povprečno oceno študija in izkušnjami z delom v celični biologiji/fiziologiji in biokemiji/molekularni biologiji/biotehnologiji.

Vsebina raziskovalnega dela:

Astrociti so celice glije v možganih z mnogimi homeostatskimi funkcijami, med drugim uravnavajo presnovo. Privzemajo glukozo iz krvi in jo shranjujejo v obliki glikogena kot rezervno možgansko gorivo. Glikogen se ob povečani aktivnosti nevronov v procesu aerobne glikolize presnovi v laktat. Laktat se sprosti iz astrocitov in kot energent prenese v nevrone. Presnova v astrocitih je uravnana, prek z G-proteini sklopljenimi receptorji (GPCR) na površini astrocitov. Napake v presnovni povezanosti med astrociti in nevroni lahko privedejo do bolezni, kot je kognitivni upad zaradi nevrodegeneracije. Najpogostejša oblika demenc je povezana z Alzheimerjevo boleznijo (AB), ki pa je povezana s hipometaboličnim stanjem, kar se redno izmeri klinično. Eden od razlogov za hipometabolično stanje je degeneracija jedra *Locus coeruleus* (LC) v možganskem deblu, kjer je sicer zelo malo nevronov, ki prek difuznega pleteža aksonov inervirajo praktično vse dele možganov in hrbtanjače in na teh mestih izločajo noradrenalin. Ker nevroni, ki izločajo noradrenalin propadejo, je količina noradrenalina zmanjšana. S tem pa tudi upade stimulacija astrocitne produkcije laktata v astrocitih. Ena od strategij je, da se zmanjšane količine noradrenalina nadomesti s transplantacijo nevronov, kar je zelo zahtven in kompleksen postopek. Alternativa je stimulirati aerobno glikolizo z ligandi, ki delujejo stimulativno na aerobno glikolizo, podobno kot noradrenalin. Ker smo pred kratkim odkrili molekule, ki aktivirajo ta proces in tudi odkrili nov receptor (gprB), ki se aktivira s temi molekulami, bo predmet raziskave določiti sekundarne prenašalce (Ca^{2+}, cAMP), ki se aktivirajo prek novega receptorja gprB in tudi prek katerih G-proteinov je učinek povečane produkcije laktata povezan (Gs, Gi Gq). Zanimalo nas bo tudi, kako se produkcija laktata uravnavana z intermediati ciklusa citronske kisline, saj slednjo izločajo v možganih prav astrociti in ni znano, kako citrat deluje na uravnavanje proizvodnje laktata.

Rezultati projekta bodo zagotovili nov vpogled v celično uravnavanje produkcije laktata v astrocitih.

Metode dela

Mladi(a) raziskovalec(ka) bo izvajal(a) meritve znotrajceličnih sekundarnih prenašalcev in metabolitov v astrocitih po aktivaciji receptorjev GPCR. Pri tem bo uporabljal(a) fluorescenčne označevalce in pa genetsko kodirajoče nanosenzorje in visokoločljivo fluorescenčno mikroskopijo v realnem času. Meritve bo izvajal v posameznih astrocitih v i) kulturi (*in vitro*).

eng: - The research work of the selected young researcher will be carried out at the Lab on Neuroendocrinology-Mol. Cell Physiology, Institute of Pathophysiology of the Faculty of Medicine in Ljubljana. The beginning of the research work is planned for autumn 2021. Interested candidates, who hold a Master degree in Biology, Microbiology, Pharmacy, Medicine, Chemistry, Physics are invited to apply via the web and send their CV and motivation letter to: robert.zorec@mf.uni-lj.si . Priority will be given to the candidates with a high average grade of study and with working experience in the field of cell biology, biochemistry/molecular biology/Biotechnology.

Content of the research work

Astrocytes are a subtype of glial cells in the brain with many homeostatic functions, including the regulation of brain metabolism. They store blood-derived glucose in the form of glycogen as the brain fuel reserve, which is used during intense neuronal activity. It is degraded to glucose and metabolized in aerobic glycolysis to lactate. The latter is released from astrocytes and distributed as an energy fuel to neurons. Metabolism in astrocytes is regulated, via G-protein coupled receptors (GPCRs) on the surface of astrocytes. Defects in the metabolic connection between astrocytes and neurons can lead to diseases such as cognitive decline due to neurodegeneration. The most common form of dementia is associated with Alzheimer's disease (AD), which is associated with a hypometabolic condition that is regularly measured clinically. One reason for the cognitive decline is the demise of the brainstem Locus coeruleus neurons, which through a diffuse axon plexus innervate virtually all parts of the brain secrete norepinephrine at these sites. As norepinephrine-secreting neurons fail, the amount of norepinephrine is reduced. This also reduces the stimulation of astrocyte lactate production in astrocytes. One of the strategies to rectify this is to replace the reduced amounts of norepinephrine with neuronal transplantation, which is a very demanding and complex procedure. An alternative is to stimulate aerobic glycolysis with ligands that have a stimulating effect on aerobic glycolysis, similar to norepinephrine. As we have recently discovered molecules that activate this process and also found a new receptor (gprB) that is activated by these molecules, the subject of the study will be to identify secondary messengers (Ca^{2+}, cAMP) that are activated through a new gprB receptor and also through which type of G-protein is the increased lactate production mediated (i.e. Gs, Gi Gq). Moreover as astrocytes are the key cell type producing citrate, a produced in astroglial mitochondria and secreted form astrocytes, it may be that this Krebs cycle intermediate provides a feedback regulating the production of lactate.

The results of this project will provide a new insight into the cellular regulation of lactate production in astrocytes.

Methods

The young researcher will perform measurements of intracellular second messengers and metabolites in astrocytes upon activation of GPCR receptors, using fluorescence markers, using the FRET-based approach with genetically encoded nanosensors and real-time high-resolution fluorescence microscopy. Measurements will be performed in individual rodent and human astrocytes in culture.