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

1. Raziskovalna organizacija (*Research organisation)*:

Medicinska fakulteta, Univerza v Ljubljani, Inštitut za patološko fiziologijo / Faculty of Medicine, University of Ljubljana, Institute of pathophysiology

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

Nina Vardjan, nina.vardjan@mf.uni-lj.si

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

3. Medicina (3.03. Nevrobiologija) / 3. Medicine (3.03. Neurobiology)

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

Navedite tudi morebitne druge zahteve, vezane na usposabljanje mladega raziskovalca (npr. znanje angleškega jezika, izkušnje z laboratorijskim delom, potrebne licence za usposabljanje…).

Raziskovalno delo mladega raziskovalca bo potekalo na Inštitutu za patološko fiziologijo Medicinske fakultete v Ljubljani. Začetek raziskovalnega dela je predviden v jeseni 2020.

Vsebina raziskovalnega dela

Uravnavanje presnove astrocitov prek z G-proteini sklopljenih receptorjev v normalnih in bolezenskih stanjih

Astrociti so številčne in heterogene celice glije v možganih s številnimi 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 astrocitih razgradi do glukoze, ki se v procesu aerobne glikolize presnovi v laktat. Laktat se sprosti iz astrocitov in kot energijsko gorivo prenese v nevrone. Presnova v astrocitih je zelo uravnana, preko z G-proteini sklopljenih receptorjev (GPCR) na površini astrocitov. Napake v presnovni povezanosti med astrociti in nevroni lahko privedejo do bolezni.

Nedavno je bilo pokazano, da je presnova v celicah lahko uravnana tudi preko celične adhezije, vendar pa molekularni mehanizmi, ki bi v astrocitih uravnavali presnovo preko adhezijskih GPCR (aGPCR), ki so mehansko občutljivimi proteini, niso poznani. Po podatkih možganske baze RNA-seq laboratorija Bena Barresa (2015) astrociti izražajo več aGPCR-jev, za katere je znano, da se lahko povezujejo preko ligandov z zunajceličnim matriksom in sosednjimi celicami. Vpleteni so v različna patološka stanja v možganih. Te povezave bi lahko vplivale na presnovo v astrocitih. V projektu bomo proučevali interakcijo astrocitnih GPCR, s poudarkom na aGPCR, z ligandi in agonisti GPCR, in njen vpliv na znotrajcelične signalne poti in presnovo. Pri tem bomo uporabili gensko kodirane fluorescenčne nanosenzorje za sekundarne prenašale (Ca^{2+}, cAMP) in presnovke (glukoza, laktat), ki jih bomo v celice vnesli s transfekcijo. Spremembe v fluorescenci bomo spremljali s fluorescenčno mikroskopijo v realnem času po stimulaciji celic z ligandi in agonisti za GPCR. Za preučevanje presnovnih odzivov na aktivacijo GPCR v astrocitih v možganih *in vivo* v normalnih in bolezenskih stanjih bomo uporabili živalski model drozofile.

Rezultati projekta bodo zagotovili nova spoznanja o vlogi GPCR v astrocitih pri uravnavanju presnove v možganih v normalnih in bolezenskih stanjih.

Metode dela

Mladi raziskovalec bo izvajal meritve znotrajceličnih sekundarnih prenašalcev in metabolitov v astrocitih po aktivaciji receptorjev GPCR. Pri tem bo uporabljal fluorescenčne označevalce in pa genetsko kodirajoče nanosenzorje in visokoločljivo fluorescenčno mikroskopijo v realnem času (konfokalna in dvofotonska mikroskopija, SIM (»structured illumination microscopy«). Meritve bo izvajal v posameznih astrocitih v i) kulturi (*in vitro*), ii) tkivnih možganskih rezinah (*in situ*) oz. iii) *in vivo* v možganih živali (*Drosophila melanogaster*).

Kandidate, ki bodo do predvidenega roka septembra 2020 zaključili magistrski študij na 2. stopnji naravoslovnih smeri, kot so biologija, biokemija, medicina, biotehnologija, mikrobiologija, farmacija, kemija in imajo željo po raziskovanju vabimo, da pošljejo življenjepis in motivacijsko pismo na naslov: nina.vardjan@mf.uni-lj.si. Prednost pri izbiri bodo imeli kandidati z visoko povprečno oceno študija in izkušnjami z delom v celični biologiji in biokemiji/molekularni biologiji.

eng:
The research work of a young researcher will be carried out at the Institute of Pathophysiology of the Faculty of Medicine in Ljubljana. The beginning of the research work is planned for autumn 2020.

Content of the research work

Regulation of astrocyte metabolism via G-protein coupled receptors in normal and disease states

Astrocytes are an abundant and heterogeneous subtype of glial cells in brain involved in many homeostatic functions, including in the regulation of brain metabolism. They store blood-derived glucose in the form of glycogen as the brain fuel reserve, which is during intense neuronal activity degraded to glucose and metabolized in aerobic glycolysis to lactate. The latter is released from astrocytes and distributed as an energy fuel to neurons. Astroglial metabolism is highly regulated though G-protein coupled receptors (GPCRs) on the surface of astrocytes and any malfunction in astrocyte-neuron metabolic coupling can lead to disease.

While the relation between the cell adhesion and the metabolism has been previously suggested in other cell types, the molecular mechanisms of such a cross-talk in astrocytes particularly in relation to mechanical force-sensing proteins, i.e. adhesion GPCRs (aGPCR). is not known. According to the Ben Barres brain RNA-seq database (2015) astrocytes express several aGPCRs, which are known to generate cell adhesions with extracellular matrix and neighbouring cells, what might affect astroglial metabolism. They are involved in various brain pathologies. In this project asroglial GPCR-mediated interaction, with the focus on aGPCRs, with ligands and agonist, and its effect on astroglial intracellular signaling pathways and metabolism will be studied. For this we will use genetically encoded fluorescent nanosenors for secondary messengers (Ca^{2+}, cAMP) and metabolites (glucose, lactate), which will be introduced into cells by transfection, and monitored by real-time fluorescence microscopy and stimulate cell with ligands and agonists for GPCRs. To study metabolic responses to GPCR activation in astrocyte-like cells in brain *in vivo* in normal and disease states *Drosophila* GPCR model will be used.

The results of the project will provide new insights into the role of astroglial GPCR in the regulation of brain metabolism in health and disease.

Methods

A young researcher will perform measurements of intracellular second messengers and metabolites in astrocytes upon activation of GPCR receptors, using fluorescence markers, genetically encoded nanosensors and real-time high-resolution fluorescence microscopy (confocal, two-photon microscopy, structured illumination microscopy (SIM)). Measurements will be performed in individual astrocytes in i) culture (*in vitro*), ii) tissue brain slices (*in situ*) or iii) *in vivo* in the brain of the animals (*Drosophila melanogaster*).

Candidates who will complete the Master's studies of natural sciences by the scheduled deadline of September 2020, such as Biology, Biochemistry, Medicine, Biotechnology, Microbiology, Pharmacy, Chemistry, and who wish to become researchers are invited to send a CV and a motivation letter to the address: nina.vardjan@mf.uni-lj.si. Priority will be given to the candidates with a high average grade of study and with the working experience in the field of cell biology, biochemistry/molecular biology.