1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, *Medicinska fakulteta*
University of Ljubljana, Faculty of Medicine

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

Radovan Komel

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

1.05 Biokemija in molekularna biologija / *1.05 Biochemistry and Molecular Biology*

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

radovan.komel@mf.uni-lj.si

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

SLO

V predhodni raziskavi smo postavili metodologijo in tehnologijo nano-protiteles (nanoteles) kot orodja za iskanje novih biomarkerjev, specifičnih za glioblastomske matične celice (GMC). S pristopom reverzne proteomike z uporabo kamelidnih nanoteles smo identificirali vrsto potencialnih biooznačevalcev možganskega tumorja glioblastoma (GBM). Med proteini, ki so bili značilno nadizraženi v GBM, izstopa protein TRIM 28 (objavljeno v Jovčevska et al., *PLoS One*, 2014), vendar je njegovo vrednost kot potencialnega označevalca GMC še potrebno funkcijsko preiskati *in vitro* ter *in vivo*. Prvi poskusi na tej ravni, ki so bili pred kratkim izvedeni s pridobljenimi anti-TRIM nanotelesi na glioblastomskih celičnih linijah, so pokazali, da anti-TRIM nanotelesa dobro vstopajo v celice *in vitro* in da za več kot polovico znižajo preživetje celic GBM v primerjavi s kontrolo (normalna celična linija HACAT). Še več, preliminarni poskusi kažejo, da je njihov zaviralni vpliv razmeroma specifičen, saj enakega zaviralnega učinka niso pokazala na celični liniji TR-146 (human squamous cell carcinoma). Podobno je izrazit zaviralni učinek na GBM celice pokazalo tudi nanotelo Nb206, ki smo ga identificirali kot vezalca določenega mitohondrijskega proteina (v procesu objave), zelo potenciran učinek pa je imela kombinacija obeh nanoteles. Zanimivo je opažanje, da Nb206 v kontrolnih celičnih linijah označi pričakovano mitohondrijsko lokacijo, v celicah GBM pa poleg mitohondrijske označuje tudi periferno obmembransko citosolno lokacijo, ki se med delitvijo celic prerazporedi v področje centrosomov.

Z namenom nadaljevanja funkcijske analize obeh potencialnih biooznačevalcev in načrtovanja primerne terapevtske strategije želimo raziskati različne vrste lipidnih veziklov, posebno membranskih veziklov, ki jih razvijemo iz matičnih celic glioblastoma (GMC), za vnos skonstruiranega ekspresijskega vektorja, ki vsebuje cDNA izbranega nanotelesa za izraženje v GMC. Naloga mladega raziskovalca bo konstrukcija ekspresijskega vektorja, razvoj GMC membranskih veziklov in vključitev vektorja, izvedba poskusov fuzije/dostave *in vitro* na GMC kulturah in celičnih linijah GBM in na koncu opazovanje znotrajcelične lokalizacije izraženih nanoteles in njihovega vpliva na celično fiziologijo. Obstaja tudi možnost, da bi vzporedno mladi raziskovalec sodeloval tudi pri poskusih vnosa zasnovanega sistema CRISPR/Cas v GMC za utišanje izražanja izbranih biooznačevalcev in opazovanju vpliva na celično fiziologijo.

Raziskovalno delo bo potekalo na Medicinskem centru za molekularno biologijo (MCMB)

Medicinske fakultete UL. Mladi raziskovalec se bo seveda tudi vpisal na doktorski študij Univerze v Ljubljani BIOMEDICINA, smer Biokemija in molekularna biologija.

ANG

In our previous research we set-up the methodology and technology of nanobodies (Nb) as tool for looking for new biomarkers specific to glioblastoma stem cells (GSCs). With reverse proteomic approach using camelid nanobodies we identified a panel of potential glioblastoma (GBM) biomarkers. Among differentially expressed proteins TRIM 28 was found outstanding (published in Jovčevska et al., *PLoS One*, 2014), however its specific value remains to be investigated by functional *in vitro* and *in vivo* assays. The first experiments at this level, which have been recently carried out by the obtained anti-TRIM Nanobody on glioblastoma cell lines, showed that anti-TRIM Nanobody entered the cells *in vitro* and reduced GBM cell survival more than a half compared to the control (normal cell line HaCaT). Moreover, preliminary experiments suggest that its dampening impact is relatively specific, since the same inhibitory effect was not found in the cell line TR-146 (human squamous cell carcinoma). Similarly, a pronounced inhibition of GBM cells was also observed by Nanobody Nb206, which was identified as a binder of a particular mitochondrial protein (*in the process of publication*), whereas inhibitory effect was even more pronounced by the combination of both Nanobodies. It is interesting observation that in control cell lines Nb206 marked the expected mitochondrial location, while in GBM cells, in addition to mitochondrial location it indicated a peripheral cytosolic membrane location which was during cell division reallocated towards centrosomal position.

In order to continue functional analysis of both potential biomarkers and to design appropriate therapeutic strategy, we would like to examine various types of lipid vesicles especially membrane vesicles developed from glioblastoma stem cells (GSCs) in order to deliver the ingeneered expression vector containing Nb cDNA to be expressed in the GSCs. Tha task of the junior researcher will be construction of the expression vector, development of GSC membrane vesicles and incorporation of the vector, performance of fusion/delivery experiments *in vitro* on GSC cultures and GBM cell lines, and finally observation of intracellular localisation of the expressed Nbs and impact on the cell physiology. There is also possibility that in parallel junior researcher could be involved in the experiments of delivery of the designed CRISPR/Cas system into the GSCs in order to silence the expression of the selected biomarkers and observe the impact on cell physiology.

Research will be performed at the Medical Centre for Molecular Biology (MCMB) of the Faculty of Medicine of the University of Ljubljana. The young researcher will also be enrolled in the doctoral study BIOMEDICINE at the University of Ljubljana, division Biochemistry and Molecular Biology.