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

1. Raziskovalna organizacija (*Research organisation)*:

Medicinska fakulteta Univerza v Ljubljani (Faculty of Medicine, University of Ljubljana)

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

Mirjana Liović, mirjana.liovic@mf.uni-lj.si

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

4.06.02 Biotehnologija – Bioinženirstvo (Biotechnology –Bioengineering)

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 tujih jezikov, izkušnje z laboratorijskim delom, potrebne licence za usposabljanje…).

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Bulozna epidermoliza (EB) je skupina dednih bolezni krhkosti kože. Razvrstimo jo v 4 glavne klinične tipe, EB simpleks (EBS), junkcijska EB (JEB), distrofična EB (DEB) in Kindler sindrom (KINDS), ki zajemajo 30 različnih podtipov, katere povzročajo mutacije v 18. genih (1, 2). Pri številnih bolnikih (predvsem pri DEB) se v času življenja pojavi tudi več ploščato-celičnih lezij (spinocelularni karcinom), ki so glavni vzrok njihove predčasne smrti (3). EB prizadane okoli 500.000 ljudi na svetu in je še vedno neozdravljiva bolezen. Kljub napredku medicine in znanosti, zdravijo le simptome bolezni. V zadnjem desetletju so razvili številne eksperimentalne terapevtske pristope. Med te uvrščamo lentivirusne in retrovirusne konstrukte za dostavo zdrave kopije gena ali popravilo mutiranega gena v *in vitro* pogojih, celične terapije z uporabo alogenih mezenhimskih matičnih celic ali fibroblastov, ter transplantacijo kostnega mozga (4, 5, 6). V poskusnih raziskavah so pripravili EB iPSC linije z namenom popravila okvarjenega gena (7, 8). Pred kratkim so pacienta z JEB obliko zdravili celo s kombinacijo *ex vivo* popravila gena v pacientovih keratinocitih, klonalno selekcijo popravljenih celic in nato s transplantacijo epidermalnih plasti pripravljenih iz selekcioniranih celic (9). Tkivo in celice EB pacientov so zelo omejeni, zato presejalnega testiranja spojin, ki bi lahko imele pozitivne učinke na fenotip bolezni, še vedno niso opravili. Delno je to posledica neobstoječega *in vitro* 3D modela EB kože, ki bi omogočil zanesljivi model za potrebe presejalnega testiranja. Pomanjkljivost je tudi nepoznavanje dobro definiranih tarč, ki bi omogočili potencialne pristope za sistemske terapije v prihodnosti.

Usposabljanje mladega raziskovalca na tem področju bo zajemalo preučevanje skupine strukturnih proteinov in signalnih poti, ki sodelujejo tudi pri adheziji celic kožnega epitelija na ekstracelularni matriks. Pri tem bomo uporabili najsodobnejše tehnologije s področja celične biologije in regenerativne medicine, kot so to npr. "gene editing", iPSC linije, *in vitro* 3D kožni ekvivalenti, masna spektrometrija, in številni drugi biokemijski in biofizikalni raziskovalni pristopi (10, 11). Raziskave bodo v povezavi s sodelujočimi raziskovalnimi skupinami iz Belgije (Univerza v Leuven-u), Velike Britanije (Kings College London in University of Manchester), ter Hrvaške (Inštitut Rudjer Bošković) in Slovenije (Kemijski inštitut v Ljubljani), in se navezujejo na obstoječa EU projekta (4D-HEALING in Celsa Alliance), ki sta v teku.

Glede na to, da gre za raziskavo, ki temelji na celičnih in tkivnih kulturah, je zaželeno, da kandidati že imajo izkušnje s celičnimi kulturami in različnimi biokemijskimi pristopi (ni pa pogoj).

1. Fine JD et al., J Am Acad Dermatol. 70(6):1103-26, 2014; 2. Fine JD et al., JAMA Dermatol. 152(11):1231-1238, 2016; 3. Fine JD et al., J Am Acad Dermatol. 60(2):203-11, 2009; 4. Rashidghamat E and McGrath JA. 6(1):6-20, 2017; 5. Gorell E et al., Gene therapy for skin diseases. Cold Spring Harb Perspect Med. 4(4):a015149, 2014; 6. Abdul-Wahab A et al., Semin Cutan Med Surg. 33(2):83-90, 2014; 7. Tolar J et al., J Invest Dermatol. 131(4):848-56, 2011; 8. Itoh M et al., Proc Natl Acad Sci U S A. 108(21):8797-802, 2011; 9. Hirsch T, et al. Nature. 551(7680):327-332, 2017. 10. Khurana P et al., Stem Cell Res 45:101827, 2020; 11. Gouveia M et al., Int J Mol Sci 21(7):2596, 2020.

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EB is a heterogeneous group of inherited skin blistering diseases. The 4 main clinical types, EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome (KINDS), include 30 different subtypes that are caused by mutations in 18 genes [1, 2]. Almost all patients with recessive DEB (RDEB) develop squamous cell carcinoma lesions, which are the main cause of their premature death [3]. EB affects around 500.000 people worldwide and is incurable. Despite the progress of medicine and science, patients are treated only symptomatically. In the last decade several experimental approaches for treatment have been devised. These included lentiviral and retroviral constructs to supplement the missing gene or to repair the mutation in vitro, and cell therapy through mesenchymal stromal cells, allogeneic fibroblasts and bone marrow transplantation [4, 5, 6]. EB patient-derived iPSC lines have been also generated for gene repair studies [7, 8]. Recently a JEB patient was even treated by a combination of ex vivo gene repair of patient-derived keratinocytes, clonal selection of repaired cells and epidermal sheet transplantation [9]. EB patient-derived material (cells/tissue) is limited, and high-throughput testing of compounds that may help improve patients' phenotype has not yet been done. This is partly due to the lack of a reliable in vitro EB 3D skin model for high-throughput testing purposes, but also the abscence of well defined targets for potential systemic therapy.

This training program will focus on investigating the role of structural proteins and signalling pathways involved in cell-to-matrix adhesion. It includes daily use of cutting edge technology from the field of cell biology and regenerative medicine, such as gene editing, iPSC lines, in vitro 3D skin equivalents, mass spectrometry, as well as many other biochemical and biophysical techniques. This research program will be in collaboration with several collaborating groups from Belgium (University of Leuven), Great Britain (Kings College London and University of Manchester), as well as Croatia (Rudjer Bošković Institute) and Slovenija (National Institute of Chemistry, Ljubljana), and is linked to two ongoing EU projects (4D-HEALING in Celsa Alliance).

As the training program is heavily based on cell and tissue culture, pre-existing knowledge on this is an advantage (but not a must).

1. Fine JD et al., J Am Acad Dermatol. 70(6):1103-26, 2014; 2. Fine JD et al., JAMA Dermatol. 152(11):1231-1238, 2016; 3. Fine JD et al., J Am Acad Dermatol. 60(2):203-11, 2009; 4. Rashidghamat E and McGrath JA. 6(1):6-20, 2017; 5. Gorell E et al., Gene therapy for skin diseases. Cold Spring Harb Perspect Med. 4(4):a015149, 2014; 6. Abdul-Wahab A et al., Semin Cutan Med Surg. 33(2):83-90, 2014; 7. Tolar J et al., J Invest Dermatol. 131(4):848-56, 2011; 8. Itoh M et al., Proc Natl Acad Sci U S A. 108(21):8797-802, 2011; 9. Hirsch T, et al. Nature. 551(7680):327-332, 2017. 10. Khurana P et al., Stem Cell Res 45:101827, 2020; 11. Gouveia M et al., Int J Mol Sci 21(7):2596, 2020.