REPORT

Name: Jelena Katić

Home institution: Faculty of Biology, University of Belgrade, Belgrade, Serbia

Visiting institution: Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Turin, Italy

Duration of stay: 31 March 2012- 29 April 2012

Problematic addressed: **„In vitro model systems to study the role of tenascins in astrocyte migration and development“**

Techniques learnt:

1. Isolation and culturing primary cortical astrocytes from postnatal mice- C57 black 6 strain, wild type and TNR KO
2. Isolation and culturing neurosphera from SVZ, postnatal mice, C57 black 6 strain
3. Scratch wound assay and stimulation of migration with exogenous tenascin R fragments
4. Immunocytochemistry (GFAP and DAPI)

Tenascin R fragmens were sent from University of Hamburg. Tenascin protein consisting of several different functional domains, each responsible for different role on cell migration, adhesion and interaction with other ECM and cell surface proteins. In order to follow different effect of tenascin R on astrocyte migration and morphology we used four different fragments (domains) separately: TNR/EGF-L, TNR/FN 1-2, TNR/FN 3-5 and TNR/FG fragments.

Postnatal P10 mice, wt and TNR KO, were decapitated and heads were moved in cold dissection buffer (PBS with 0.6% glucose). Skin and skull were removed with forceps and scissors in order to release brain. Cerebrum were separated from cerebellum and brain stream, divided into lobes and released from meninges. Lobes were cut into small pieces, transferred in 15ml tube with L-15 medium and mechanically dissociated using Pasteur pipette and centrifuge. Cell pellet was discarded in astrocyte culture medium (DMEM/F-12 Ham with 10% FBS, 2M glucose, 2mM L-glutamine, 1mM Na-pyruvate and penicillin/streptomycin). Cells were plated (3x100000 cells/ml) in poly-L-ornithine coated 90mm culture dishes and placed in incubator. Culture medium was changed every 48h. Cells formed confluent monolayer 10-12 after dissection and became ready to pass onto poly-L-ornithine coated plates and cover slips using 0.25% trypsine and 0.02% EDTA. Secondary astrocyte culture was grown to confluence and after that became ready for scratch wound assay which is appropriate model to study cell migration.

Scratch wound assay was establish on confluent astrocyte monolayer plated on cover slips using sterile 100µl tips. We flashed cells in PBS and change the culture medium immediately after wounding to prevent the medium to be conditioned with cell debris. Each tenascin fragment was added in a medium in the same concentration (10µg/ml). Namely, on every cover slip we added different TNR fragment and one cover slip was negative control. Then we followed wound healing by measuring a wound diameter on every cover slip, both wt and TNR, in different time points (1h, 6h, 24h after wound). In order to examine the role of TNR fragments on astrocyte migration we compared wound diameter in every cover slip with different TNR fragment.

Conclusions: Results showed that TNR/FN 1-2 and TNR/FN 3-5 fragments have positive effect of on astrocyte migration both in TNR KO and wt culture (diameter of the wound was smaller with TNR/FN 1-2 and TNR/FN 3-5 addition than in control). On the other side, negative effect on astrocyte migration with TNR/FG and TNR/EGF-L addition was shown only in TNR KO culture (diameter of the wound in TNR KO culture was bigger with TNR/FG and TNR/EGF-L addition than in control). In wt culture TNR/FG and TNR/EGF-L fragments show signs of pro-migratory effect on astrocytes (wound diameter in wt culture was smaller with TNR/FG and TNR/EGF-L addition than in control).
In my future experiments I will try to repeat these results with TNR fragments, first. The idea is also to establish the same experiments with TNC KO animals, which we have in our home laboratory, and TNC fragments homologue with used TNR fragments.

In order to follow astrocyte morphology and migration after wound we did imuno-staining with GFAP antibody. Astrocytes from TNR KO mice were seeded onto cover slips and after scratch wound fixed in 4% PFA and stained using primary antibody rabbit@GFAP (Dako- 1:1000 solution) and secondary antibody goat@rabbit (alexa 488- 1:1000 solution).
After successful maintaining immunocytochemistry I am planning to compare wound healing (migration and morphology) in TNR KO, TNC KO and control astrocytes (GFAP stained) in different time points.

First steps of SVZ isolation from postnatal mice P10 was the same like mention. We cut lateral wall of ventricle carefully and put in L-15 medium. First, we used enzymatic digestion with 0.25% trypsine and then mechanical dissociation with centrifuge. Cell pellet was discarded in neurosphera culture medium (DMEM/F-12 Ham with B27 supplement, 1M HEPES, 30% glucose, 2mM L-glutamine, 1mM penicillin/streptomycin, EGF and BFGF growth factors). We changed only growth factors every 48h and after 8 days first primary neurospheras appeared.