NENS STIPEND FOR TRAINING STAY REPORT

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Institution visited: Medical University Innsbruck
Division for Neuroanatomy
Mentor Professor: Prof. Dr. Lars Klimaschewski

Project Title: **STAM2 analysis *in vitro* using RNA silencing technique**

Reporting period: 28/02/2011 – 08/04/2011

Objectives:

The general objective of the fellowship was to identify the function of STAM2 molecule *in vitro* using RNA silencing technique. Used technique is a powerful tool for downregulation or complete suppression of gene activity by an introduction of small interfering RNAi, which are a class of double-stranded RNA molecules.
STAM2 molecule is a part of ESCRT-0 protein machinery that initiate sorting of ubiquitinated receptors for final degradation in the lysosomes. Although several hundred scientific publications have been published concerning the subject of the ESCRT machinery, extensive efforts are still required for fully understanding of the cellular roles of ESCRT proteins and their interactors.

Initial aims:

- Learning the principles and basic procedures of cell culture
- Learning the RNA interference method
- Transfer of technology at the Croatian Institute for Brain Research where the technique will be implemented with the help of staff from the Medical University of Innsbruck

Technologies in which training was requested:

Cell cultures techniques, cell transfection, siRNA technique, RNA extraction, cDNA synthesis, real-time PCR, protein extraction, western blot, immunocitochemistry, confocal microscopy.

Methods:

Experiments were performed on human cell lines U373 (Human glioblastoma-astrocytoma, epithelial-like cell line) and SH-SY5Y (Human derived neuroblastoma cell line).
Our first experiment was to verify *Stam1* and *Stam2* expression in neuronal tumour cell lines U373 and SH-SY5Y. RNA was extracted from the cells and subsequently transcribed to cDNA for performing the real-time PCR technique. PCR results confirmed strong expression of both genes in the tested cell lines.
Next experiment was immunocytochemical analysis of STAM proteins in the cell lines using STAM1 and STAM2 antibody. These experiments showed that STAM2 antibody was mainly localized to punctuate structures in the soma and nerve fibers cytoplasm, and we assumed that punctuate structures in the cytoplasm were the early endosomes.
RNA silencing method and cell trensfection experiments were performed in the next step. I learned about culturing cells specifically used for the transfection procedure. I acquired skills for working in cell culture and the precautions to be taken in the laboratory to prevent contamination at various stages of the procedures. The siRNA oligonucleotides were purchased from Dharmacon. U373 and SH-SYY cells were transfected with three different concentration of STAM2 siRNA (20, 50, 100 nM). After 2 days, RNA lysate was taken and real time PCR was performed. PCR result showed strong *Stam2* downregulation with 100 nM concentration of STAM2 siRNA. Unexpectedly there *Stam1* gene was downregulated as well.
Afterwards transfection experiments for the imaging analysis were performed with STAM2 siRNA on U373 cell line and SY5Y cell line. Each cell line was divided in four groups. One group was transfected with siRNA STAM2 and co-transfected with FGFR1 (Fibroblast growth factor receptor 1) plasmid. The second cell group was transfected with siRNA STAM2 and co-transfected with FGFR1, and in addition treated with FGF (Fibroblast growth factor). The other two cell groups were control groups and were not transfected with siRNA. One of them was transfected with FGFR1 plasmid, and the other one was transfected with FGFR1 plasmid and treated with FGF. There were two time points per experiment: 2 days and 4 days. Each cell group was then stained with: Transferrin (recycled endosome marker), Lysotracker (multivesicular body and lysosome marker), EEA1 (Early Endosome Antigen 1; early endosome marker), STAM1 and STAM2 antibody. After antibody incubation, sections were coverslipped and partially analysed by confocal microscope. At present, the imaging analysis is not fully evaluated. Future work will include further analysis on confocal microscope and will be performed at my home institution, School of Medicine University of Zagreb.

The benefits of NENS fellowship:

This work, which has been funded by NENS fellowship, led to important collaboration with scientist from Medical University of Innsbruck and I hope that these results will be a basis of a new bilateral project between our two institutions (submitted few days ago).

My visit to the Division for Neuroanatomy gave me the opportunity to meet a dedicated and very enthusiastic group of people who were more than willing to demonstrate, teach, and discuss all aspects of the silencing analysis. I would like to thank NENS Programme for giving me this opportunity. As you could imagine, my scientific environment has limited resources, therefore this fellowship was the only way I could learn such novel technique.

My motivation was enhanced by expected benefits to me and my research community. Learning the innovative approach methods will allow me the transfer of technologies to my home institution and the establishment of novel approaches for my future research.

How were the NENS funds spent?

1.) TRAVEL COSTS 417,30 €

a) Flight ticket Zagreb – Munich 200,00 € (1467,00 HRK)
b) Bus ticket Munich – Innsbruck 85,00 €
c) Travel insurance 32,00 € (234,60 HRK)
d) Laboratory baggage 40,00 €
e) Bus ticket Innsbruck – Schuzenstrasse street 60,30 €
(43,30 € - one monthly ticket; 13,20 € - one weekly ticket; 3,80 € - two daily tickets)

2.) ACCOMODATION COSTS 549,50

a) Accomodation at Akademikerhilfe, student house 517,50 €
(300,00 € + 137,50 € + 70,00 € + 10,00 €)
b) Bank transfer
(26,00 € (111,90 HRK + 80,00 HRK)+ 6,00 € 32,00 €

TOTAL: 966,80 €