SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17103
STSM title: Delivery of in vitro Antisense RNA Therapeutics to Mucopolysaccharidosis III
STSM start and end date: 02/03/2020 to 13/03/2020
Grantee name: Nurselin Ateş

PURPOSE OF THE STSM:

This STSM involved application of antisense oligonucleotides delivery as a substrate reduction therapy for patient cell lines of Mucopolysaccharidoses type III which is a type of lysosomal storage disorder. Substrate reduction therapy is a common approach to treat lysosomal storage disorders and in this project siRNA-mediated substrate reduction therapy was performed. The purpose of STSM was to expand our knowledge about delivery techniques of antisense RNAs to patient fibroblasts (*in vitro* studies) in order to use this technology as substrate reduction therapy. As in the Seyrantepe research group, we are working on a project with antisense therapies applied to in vitro cell cultures and animal models within the scope of CA17103 CostAction project. Thus it was very beneficial to learn some experimental and theoretical protocols and methods to work with delivery of antisense RNA to Tay-Sachs mouse model and patient cell lines in our laboratory.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Mucopolysaccharidoses are one of the major group of lysosomal storage diseases caused by impaired degredation of glycosaminoglycans (GAGs). Currently, the MPS group comprises 11 different conditions caused by individual deficiencies of enzymes that catalyze GAGs stepwise degradation. Some types of the disease can be treated by recombinant enzyme therapy, even if there is no fully effective treatment. ERT therapy cannot correct neurological symptoms because of inability to cross blood brain barrier. MPS III is charactherized by severe neurological degeneration and in this work siRNA-mediated substrate reduction therapy was used to overcome these challenges. Xylotransferase1 (XYLT1) is an enzyme involving in very early stages of the GAGs biosynthesis and was targeted to knockdown in this project. Firstly; previously grown MPSIII fibroblast cells were seeded on 6-well plates for subsequent transfection. In order to do that cells were harvested by trypsinization and counted by using neubauer chamber. The concentration of the cells was calculated and the cells were seeded so that there are 150000 cells on each well of 6-well plate and grow in presence the DMEM + Glutamax medium supplemented with 10% FBS, 5% antibiotics and 5% fungizone, at 95% humidity and 5% CO_2 in an incubator at 37°C. For transfection; siRNA pool (composed of 4 different siRNAs targeting the same gene: XYLT1) was used to assess the potential of RNAi to act as a mechanism to promote substrate reduction in MPS cell lines. After checking the confluence of cells on the next day; a working solution at 500nM was prepared (40 μL siRNA + 760 μL RNAse free water) and solutions for siRNA transfection 1. siRNA (680 μL siRNA stock solution + 170 μL Opti-MEM®); 2. Transfecting agent (85 μL LP2000 + 765 μL Opti-MEM®) were prepared. Then the two solutions were mixed and incubated. For transfection purposes Opti-MEM is used (because of its optimal properties and reduced serum) without antibiotics. Antibiotics have been shown to accumulate to toxic levels in permeabilized cells. Some cells and transfection reagents require serum-free conditions for optimal siRNA delivery. 200 ul of the solution (LP2000/siRNA) were added to each well and the plates

were placed in the incubator at 37ºC, 5% CO2 for the next 24h, 48h, 72h and 7days. The cell pellets were collected by harvesting with trypsin on post-transfection time points for further experiments.
Separately from siRNA transfection, I had opportunity to observe bacterial transformation of pRL-TK plasmid encoding for luciferase gene on HEP3B competent cell line by using heat-shock process. The plasmid were amplified in transformed cells and isolated by using both mini and maxi-prep plasmid isolation kit (Endotoxin-free plasmid DNA purification,Macherey-Nagel). The plasmids were run on 1% agarose gel to check for the quality and purity. Amplified and isolated plasmids were stored for in vivo transfection.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Our purpose with this STSM were to expand our knowledge about basic principles and techniques related to in vitro transfection. In this short (12 days) period of time we didn't obtained the expression results of siRNA-transfected cells. For plasmid amplification and isolation, the agarose gel results showed no contaminations of bacterial chromosome in the plasmid and the concentration of the plasmid were around 3500 ng/ul which was a satisfactory outcome for further in vivo injection.

FUTURE COLLABORATIONS (if applicable)

I have attached a photo with Host instructer and people in the lab