TREHALOSE ENHANCES MIR-124 INHIBITOR EFFICIENCY IN NEURAL STEM CELL CULTURE

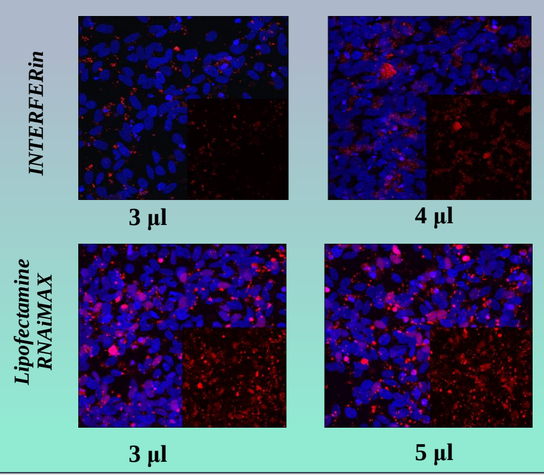
Ivan Arzhanov, Yuriy Petrenko, Nataliya Romanyuk

Institute of Experimental Medicine ASCR v.v.i., Prague, Czech Republic

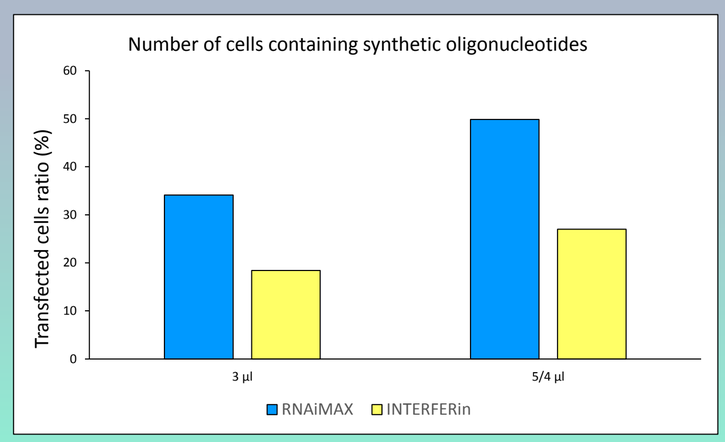
Background

MicroRNAs are small non-coding RNAs which regulate gene expression. MiRNA-124 is one of the most abundant miRNAs in the adult brain. It is highly expressed in neural cells during their differentiation and able to inhibit proliferation and upregulate differentiation. The expression of miR-124 is also significantly decreases in the nervous tissue after injury, which can lead to severe outcome. However, using miR-124 inhibitor may contribute to faster recovery after injury. The effect of the inhibitor of miR-124 was demonstrated on human induced pluripotent stem cell-derived neural precursors (IMR-90). To enchance the absorption of the miR-124 inhibitor into cells, we increased the osmolarity of the medium by adding trehalose at concentrations of 50 mM and 100 mM. Our results show that the presence of trehalose in the medium increases the absorption of the miR-124 inhibitor by neural stem cells. However, though the important roles of miR-124 in regulating differentiation and development are known not completely, the mechanism which is underlying in this process is remained to be addressed.

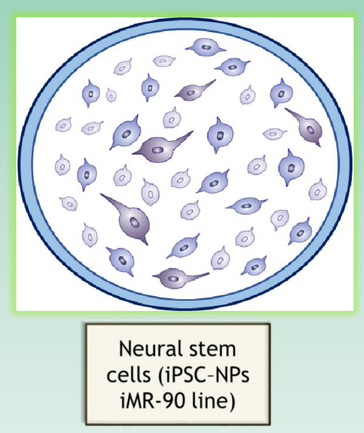
Determination of the most effective transfection reagent



To transfected miRNAs into the cells, we decided to use two lipofectamine reagent – RNAiMAX and INTERFERin. Instead of miRNAs, we have used oligonucleotides. To estimate which one could be better, we transfected the same concentration of oligonucleotides with a different volume of lipofectamine. In 48 hours after transfection, we fixed cells and determined the number of positively transfected cells with confocal microscopy. **RNAiMax lipofectamine provides higher permeabilization of synthetic oligonucleotides into neural stem cells**.

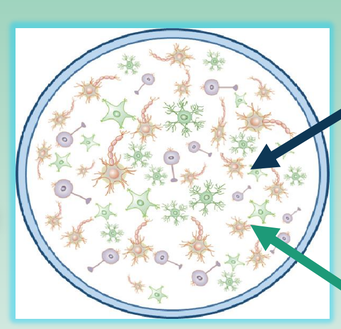


Experimental design

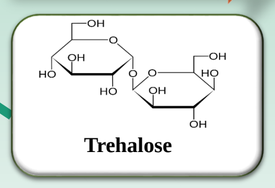


Neural stem cells (iPSC-NPs iMR-90 line)

2 weeks of differentiation



miR-124 inhibition



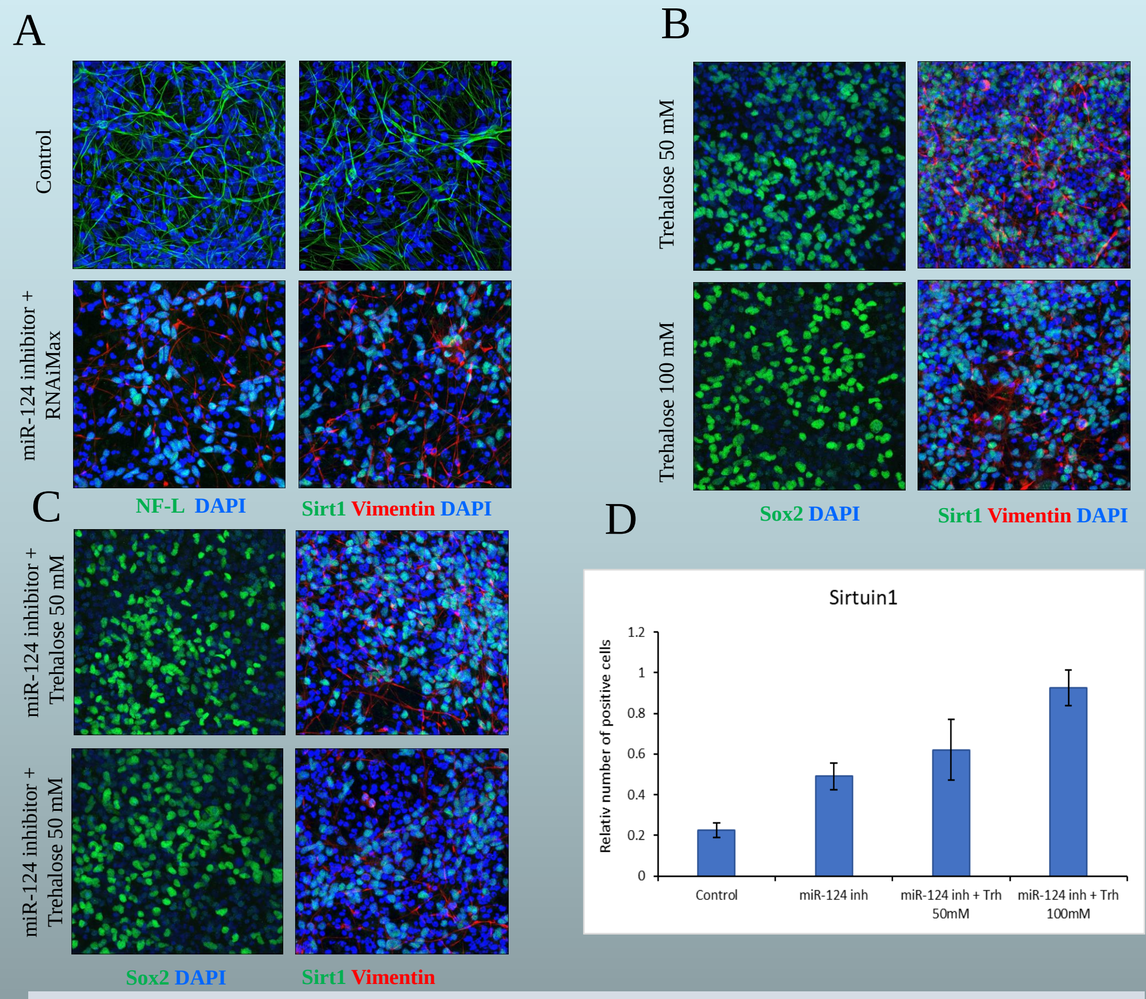
Trehalose

48 hours incubation

Collection samples for Western blotting

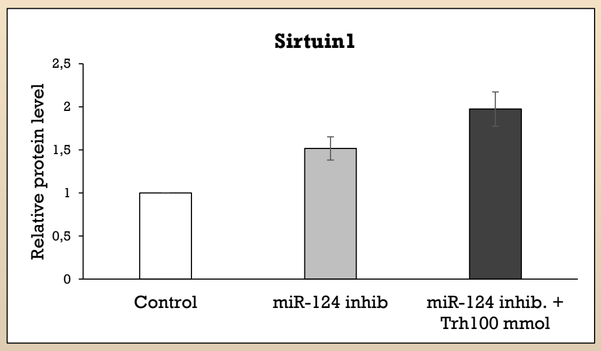
Fixation and immunocytochemical staining of samples

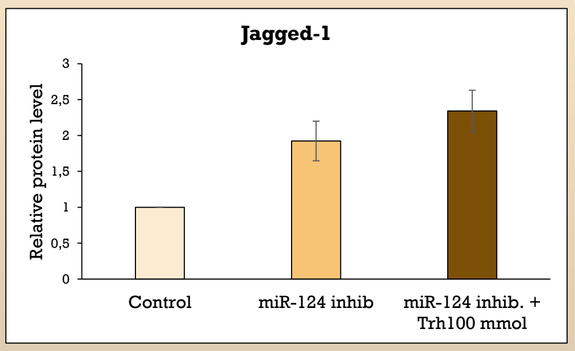
Immunocytochemical staining of control and treated NSCs



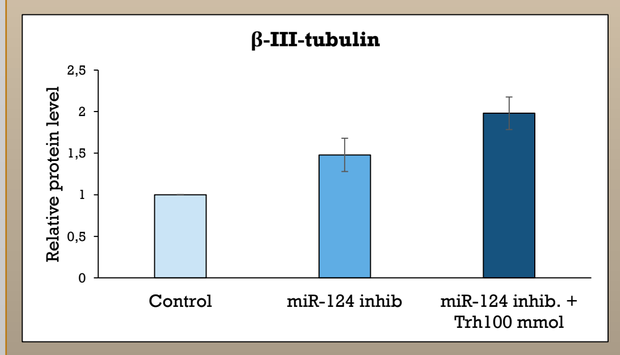
Immunocytochemical staining demonstrates, that treatment of NSCs with MiR-124 inhibitor alters the level of several target proteins in neural stem cells. The most significant changes were demonstrated for Neurofilament light chain (NF-L) and Vimentin. The effect was even stronger in the presence of Lipofectamine RNAiMax (**A**). The addition of trehalose to the culture medium enhances the miR-124 inhibitor effect in a concentration-dependent manner. We suggest that trehalose may affect cell membrane permeability for the miR-124 inhibitor due to an increase in the osmolarity of culture medium. The levels of Sox 2 and NF-L were significantly altered (**B** and **C**), however, the strongest effect was shown for Sirtiun1(**D**). The mechanism of trehalose action need to be clarified.

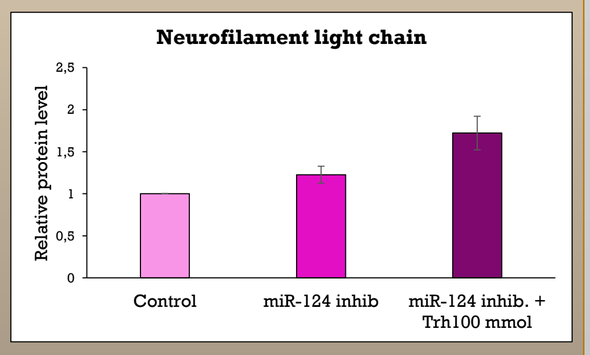
The treatment of neural stem cells with trehalose can enhance miR-124 inhibitor effect





We have confirmed that the inhibition of miR-124 leads to an increase in the expression of its main targets, which are involved in cell development. However, this effect was enhanced in the presence of trehalose and was on 23.2% for Sirt1 and 17.8% for Jagged1 higher compared to cells treated with miR-124 inhibitor alone.





Pretreatment with miR-124 inhibitor leads to an increase of expression of neural markers Neurofilament light chain and β-III-tubulin. The level of β-III-tubulin was higher on 25,3% and NF-L on 29% in samples which include trehalose compared to cells that were treated only with miR-124 inhibitor.

Conclusion:

1. RNAiMax lipofectamine transfection reagent provides efficient permeabilization for synthetic oligonucleotides into neural stem cells. Despite its high concentration any harmful effects were not observed.

*2. MiR-124 inhibitor enhances the level of several target proteins such as **Sirtuin1, Jagged-1**, **Neurofilament light chain** and **β-III-tubulin** in neural stem cells. Its effect is stronger in the presence of RNAiMax.*

3. The addition of trehalose to the culture medium enhances the miR-124 inhibitor effect in a concentration-dependent manner.

4. Further studies are necessary to understand the role of miRNAs in CNS injury.

Support by LQ-1604 (BIOCEV-FAR Project) and GACR 18-21942S