O-004 - CELL-DELIVERED AND LYSOSOMAL-ACCUMULATION REDUCTION BY GLYCOENGINEERED N-ACETYLGALACTOSAMINE-6-SULFATE SULFATASE (GALNS) FOR MORQUIO A DISEASE TREATMENT

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INTRODUCTION: Mucopolysaccharidosis IV A (MPS IVA, Morquio A disease) is a lysosomal storage disease produced by mutations in GALNS enzyme. We had reported the production of recombinant GALNS in Pichia pastoris yeast as an alternative for Morquio A ERT. The modification in the N-glycosylation pathway in P. pastoris NRRL Y-11430 strain, allows to obtain a more humanized and homogeneous glycan profile. Glycoengineered recombinant GALNS produced in P. pastoris have been proved to be taken up by different culture cells. ***OBJECTIVE:*** To evaluate the effect of recombinant glycoengineered GALNS in lysosomal mass reduction using MPS IVA fibroblasts and iPSC-derived cardiomyocytes and chondrocytes. ***MATERIALS AND METHODS:*** Recombinant glycoengineered GALNS was produced in the yeast Pichia pastoris NRRL Y-11430. Fibroblasts from MPS IVA patients were obtained from Coriell Institute. MPS IVA iPSC were generated by using the non-integrating CytoTune-Sendai viral vector kit. Cardiomyocite differentiation was performed using STEMdiffTM Cardiomyocyte Differentiation Kit; while chondrocyte differentiation was performed following the protocol reported by Suchorska W et al. 2017. The MPS IVA fibroblasts and iPSC-derived cardiomyocytes and chondrocytes were treated with 50 and 100 nM of recombinant GALNS. Control cells were treated with PBS 1x. After treatment, the cells were treated with 50 nM LysoTracker Red DND-99 dye. The images were acquired using the IN Cell Analyzer 2200 imaging system. ***RESULTS:*** iPSC-derived cardiomyocytes and chondrocytes were successfully obtained and expressed the expected protein markers. MPS IVA fibroblasts and iPSC-derived cardiomyocytes and chondrocytes showed significant increase in lysosomal mass. Results showed a significant reduction ($p<0.05$) in the lysosomal accumulation in MPS IVA fibroblasts, which ranged between 25 and 40% of WT levels. Reduction between 11 to 16% of the lysosomal accumulation was observed after treatment of MPS IVA cardiomyocytes and chondrocytes. ***CONCLUSIONS:*** The results showed that developed cells models can be used in the evaluation of new therapies for MPS IVA. It was observed that recombinant GALNS produced in a glycoengineered yeast showed significant reduction of the lysosomal mass in all treated cells. These results pave the way in the development of a new ERT for MPS IVA patients.