O-002 - IN VITRO REDUCTION OF LYSOSOMAL GANGLIOSIDE ACCUMULATION BY RECOMBINANT HUMAN BETA-HEXOSAMINIDASE PRODUCED IN PICHIA PASTORIS

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INTRODUCTION: Enzyme Replacement Therapy (ERT) is based on the capacity of heterologous lysosomal enzymes to be taken up and targeted to the lysosome, where they can degrade the accumulated substrate. β-hexosaminidases are hydrolases involved in lysosomal degradation of GM2 gangliosides. Recombinant human beta-hexosaminidases (rhHex-A and rhHex-B) produced in Pichia pastoris have been proved to be taken up by different culture cells via cation independent manner. ***OBJECTIVE:*** To evaluate the therapeutic effect of recombinant beta-hexosaminidases in lipid storage reduction using Tay Sachs (TSD) patient fibroblasts and TSD neural stem cells model (NSCs). ***MATERIALS AND METHODS:*** Recombinant hexosaminidases were produced in Pichia pastoris GS115 without any further modification. TSD patient fibroblasts were obtained from Coriell Institute. TSD-iPSCs were differentiated to TSD-NSCs at NCTAS-NIH using PSC neural induction medium kit. Nile Red and Lysotracker staining assays were used to evaluate lipids storage and lysosomal mass, respectively. Twenty-four hours before treatment, TSD fibroblasts and NSCs were seeded in 96-well black clear bottom plates. Purified rhHex-A and rhHex-B were added at 50 and 100 nM final concentration. Twenty-four, 48 and 72 h after treatment, the cells were treated with 50 nM LysoTracker Red DND-99 dye or 1 µM Nile Red in complete DMEM media at 37 °C for 1 h and 10 min respectively. The plates were fixed and stained simultaneously. The images were acquired using the INCell Analyzer 2200 imaging system. ***RESULTS:*** NCS derived from TSD iPSCs were a useful cellular model to evaluate the effect of recombinant hexosaminidases in the degradation of lysosomal stored lipids. Results showed that TSD fibroblasts and NSC treated with rhHex-A had a significant reduction of stored gangliosides respect to wild type cells. Normal levels of lysosomal lipids storage were observed after treatment of TSD-NCS and fibroblasts with rhHex-A. On the other hand, rhHex-B did not show consistent results. ***CONCLUSIONS:*** These results showed that rhHex-A produced in P. pastoris could be used for further preclinical and clinical assays towards the development of an ERT for TSD. In addition, these results confirm the potential of this host in the production of recombinant protein for other lysosomal storage diseases.