Evaluation of a potential of Chaperone Therapy for Mucopolysaccharidosis type IIID

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Abstract

Mucopolysaccharidosis type IIID (MPS IIID; Sanfilippo D) is a devastating pediatric neurodegenerative disorder with no cure or effective treatment available. The fundamental cause of MPS IIID is an inherited mutation in glucosamine (N-acetyl)-6-Sulfatase (GNS) required to catabolize heparan sulfate (HS). We will report our progress on evaluation of a potential chemical chaperone (JSF-3358) with purified rhGNS, with patient fibroblast cells and iPSC-derived neural stem cells. Our preliminary data indicated that JSF-3358 can activate rhGNS activity (40%) and slightly enhance cell survival of MPSIIID fibroblasts versus control fibroblasts. We are currently evaluating the ability of JSF-3358 to restore GNS activity in three cell types: MPSIIID fibroblasts, iPS cells, and neural stem cells. In addition, we are evaluating its potential to enhance GNS stability during production of GNS and cellular uptake.

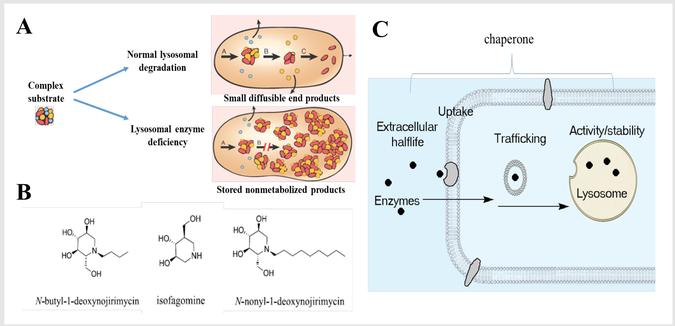

Figure 1. Mechanism of lysosomal storage diseases and pharmacological chaperones are potential therapy for lysosomal storage diseases. A. Lysosomal enzymes deficiency causing stored nonmetabolized products in lysosome. B. The structures of three known pharmacological chaperones[1]. C. Pharmacological action of chaperones on lysosomal storage diseases take place at different levels[2].

Results

1. JSF-3358 increases GNS activity and improves growth of MPS IIID patients derived fibroblasts

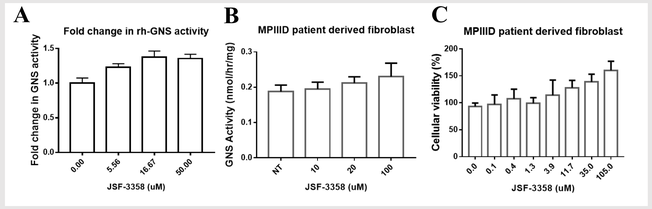

Figure 2. Effects of JSF-3358 on rhGNS and MPS IIID patient derived fibroblasts. (A) JSF-3358 increases GNS activity in a dose-dependent way. After 48 hours of treatment, JSF-3358 increase the GNS activity（B) and cell viability (C) of MPSIII D patient derived fibroblasts.

2. Reprogram MPS IIID patients derived fibroblasts into iPS cells.

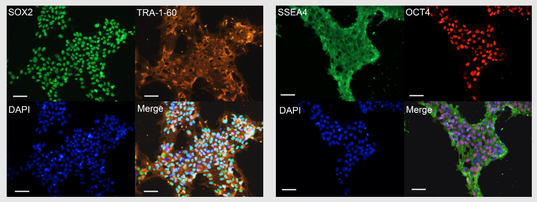

Figure 3. Immunostaining images of iPS cells. Cells are positive with iPS cells biomarkers, SOX2 and TRA1-60 (left), SSEA4 and OCT4 (right)

3. iPS cells are differentiated to human Neural Progenitor Cells (NPC)

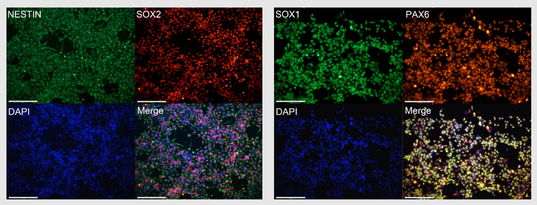

Figure 4. Immunostaining images of NPC. Cells are positive with NPC biomarkers, SOX2 and Nestin (left), SOX1 and PAX6 (right).

4. NPC differentiated from MPS IIID patients grow slowly and are deficiency in GNS activity.

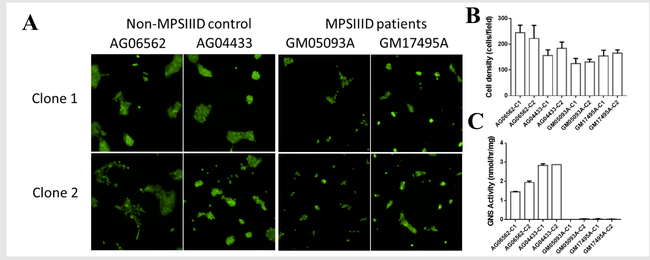

Figure 5. Comparison between NPC derived from healthy individuals and MPS IIID patients. (A) Live NPCs were stained with Calcein-AM and imaged 72 hours at plating. (B) Average cells number is counted from 6 field, triplicates. (C) GNS activity of NPC lysate.

5. Treated with JSF-3358 improve the growth of MPS IIID patients derived NPC, increase the activity and stability of GNS in NPC lysate.

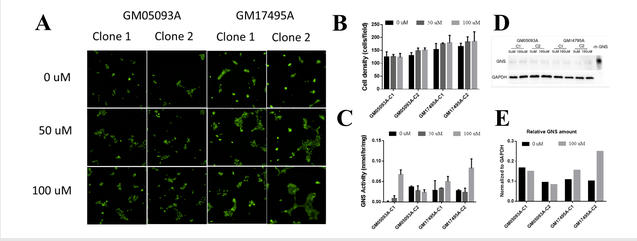

Figure 6. NPC were treated with JSF-3358. Images of NPC are taken after 48 hours of treatment (A) and average cells per well are counted (B). GNS activity of NPC lysate were determined (C), GNS protein level were measured by Western blot (D) and quantified relative to GAPDH (E).

Summary and Future Work

- JSF-3358 as a potential Chaperone therapy for MPS IIID.
- In vitro and in vivo PK and PD need to be further analyzed.

Refference

[1] Nakagome I, Kato A, Yamaotsu N, et al. Design of a New α-1-C-Alkyl-DAB Derivative Acting as a Pharmacological Chaperone for β-Glucocerebrosidase Using Ligand Docking and Molecular Dynamics Simulation[J]. Molecules, 2018, 23(10): 2683.

[2] Parenti G, Moracci M, Fecarotta S, et al. Pharmacological chaperone therapy for lysosomal storage diseases[J]. Future medicinal chemistry, 2014, 6(9): 1031-1045.

Acknowledgement

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