Engineering of M6P-type glycan in yeast to produce a potential enzyme for replacement therapy of lysosomal diseases

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For the treatment of lysosomal storage diseases (LSDs), several treatments, such as chemical chaperones, substrate deprivation, enzyme replacement, gene therapy, and bone-marrow transplantation, have been attempted. Of these methods, enzyme replacement therapy (ERT) is a strategy that has been clinically approved for some LSDs. In ERT, administrated recombinant enzymes are incorporated into the cell *via* mannose-6-phosphate (M6P) receptor localized at the cell surface. At present, the recombinant enzymes for ERT have been produced in mammalian cells because the glycan structure is similar to that of native enzyme. However, it is expensive to produce adequate amounts of protein for therapeutic purposes from mammalian cells.

We produced recombinant human α-galactosidase A and human β-hexosaminidase A in a yeast *Ogataea minuta* manipulated to synthesize glycoprotein that lacks the yeast-specific outer chain of *N*-glycan. After *in vitro* α-mannosidase treatment to 'uncover' the M6P residues, the purified recombinant enzymes were effectively incorporated into the fibroblast cells of the patients. These results support the possibility of using yeast as a host to produce recombinant enzymes for ERT. We also cloned and over-expressed *O. minuta MNN4* gene, which may encode a positive regulator of mannosylphosphate transferase, in the yeast. The recombinant β-hexosaminidase A derived from *OmMNN4* over-expressing yeast strain was incorporated into Tay-Sachs fibroblast more effectively and rapid degradation of accumulated GM2 was observed, indicating that the enzyme produced from the strain contained *N*-glycans with higher M6P content. The *O. minuta MNN4* over-expressing strain may be useful for production of other lysosomal enzymes for ERT.