S21-2 Production of lysosomal enzymes with M6P-type glycan by engineering of yeast glycosylation system

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Protein-based therapeutics, such as therapeutic antibodies, enzymes for replacement therapy for lysosomal diseases, and cytokines, has received attention in recent years. These therapeutic proteins and peptides are almost exclusively produced in mammalian expression systems, in particular Chinese hamster ovary (CHO) cell lines, however, it is expensive to produce adequate amounts of protein for therapeutic purposes from mammalian cells.

We have reported production of the recombinant human alpha-galactosidase A (GLA) and human beta-hexosaminidase A (HexA) in a yeast strain, *Ogataea minuta,* manipulated to synthesize glycoprotein that lacks the yeast-specific outer chain of *N*-glycan[1,2)]. After *in vitro* alpha-mannosidase treatment to expose M6P residues, the purified recombinant enzymes were effectively incorporated into the fibroblast cells of the patients. We also cloned and over-expressed *O. minuta MNN4* gene family, which may encode a positive regulator of mannosylphosphate transferase, in yeast. The recombinant HexA derived from *OmMNN4-1* over-expressing yeast strain was incorporated into Tay-Sachs fibroblast more effectively and degraded accumulated. An intracerebroventricular administration of the HexA improved motor function as well as prolongated the lifespan. We are attempting to produce the other lysosomal enzymes and related protein, and also purifying the recombinant human GLA enzyme by a large-scale cultivation of *OmMNN4-1* over-expressing *O. minuta* strain. The relationship of their glycan structures and incorporation into the cells will be discussed.