Glycoengineering of Chinese Hamster Ovary Cells for Modulating Glycoprotein N-linked Sialylation

Cheng-Yu Chung[1], Bojiao Yin[1], Jake Yang[2], Qiong Wang[1], Hui Zhang[2] and Michael J. Betenbaugh[1]

1. Department of Chemical and Biomolecular Engineering, Johns Hopkins University

2. Department of Pathology, Johns Hopkins University

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Erythropoietin (EPO), a highly glycosylated protein was widely used in anemia of kidney failure. It has been confirmed that enhancing sialic content on EPO can prolong its circulatory half-life, improving EPO therapeutic efficacy. On the other hand, limiting EPO sialylation can be beneficial in medical and diagnostic application. EPO has been evaluated as a potential radiolabeled diagnostic candidate. Thus, the development of genetic engineering strategies to finetune the sialic acid content on EPO as well other protein therapeutics is needed.

To enhance sialylation, we focused on engineering Chinese Hamster Ovary cell (CHO) to overexpress Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1). Combinantorial overexpression of ST6GAL1 with the glycan brancing enzyme, UDP-*N*-acetylglucosamine: α-1,3-D-mannoside β1,4-*N*-acetylglucosaminyltransferase (GnTIV/Mgat4) and UDP-*N*-acetylglucosamine: α-1,6-D-mannoside β1,6-*N*-acetylglucosaminyltransferase (GnTV/Mgat5), can further increase the sialylation level of EPO up to 45 %.

As to limiting the sialylation, we aimed to investigate which enzymes in the α2,3-sialyltransferases family are responsible for N-linked sialylation. ST3GAL3, ST3GAL4 and ST3GAL6 were chose as knockdown targets and siRNA transfection was performed to study the level of reduction in sialylation. Our result indicated three sialyltransferases all partook in protein sialylation. Triple genes siRNA knockdown exhibited the most drastic reduction in sialylation in total protein and EPO. What's more, ST3GAL4 may play the critical role in protein especially N-linked sialylation.