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Impact of apoptosis gene targeting on recombinant protein glycosylation

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Background

Chinese Hamster Ovary (CHO) cell is one of the major cell lines used for complex recombinant protein production. During CHO cell culture, loss in viability attributed to apoptosis often results in lower recombinant protein yield and affects protein quality. Through the individual targeting of four apoptosis genes identified via expression profiling studies, four apoptosis resistant CHO GT (Gene Targeted) cell lines were constructed. These cell lines enabled prolonged culture viability, higher maximum viable cell densities and significant increase in recombinant human interferon gamma (IFN-γ) yields. Furthermore, it was observed that the IFN-γ from CHO GT cells has a higher level of sialic acid content.

Results

To further characterize the impact of apoptosis gene targeting on recombinant protein glycosylation, a detailed analysis of the micro-heterogeneity of IFN-γ was performed. In addition, a quantitative real-time PCR (qRT-PCR) involving 24 N-glycosylation-related genes from CHO cells was established to profile the changes in expression of these genes across the exponential, stationary and death phase of the four CHO GT cell lines and compared to the parental cell line.

IFN-γ glycan analysis revealed that the CHO GT cell lines have higher proportions of tri- and tetra-antennary glycan branching as well as higher proportions of fully sialylated glycoforms. Interestingly, these changes in IFN-γ correlated with changes in N-glycosylation gene expression. Compared to the parental cells, higher expression of N-acetylglucosaminyltransferases IV and V, which are responsible for glycan branching, was observed in CHO GT cell lines. The enzymes involved in sialylation, β-galactoside α2,3-sialyltransferase and CMP-sialic acid transporter, were also upregulated in the CHO GT cells.

Conclusion

This study showed that apoptosis targeting could affect protein glycosylation but further experiments would be needed to establish the link between apoptosis and glycosylation. Nevertheless, it was demonstrated that the quantitative real time PCR method could potentially be used to identify possible 'bottlenecks' or 'compromised' pathways in N-glycosylation and subsequently allow for the development of strategies to improve glycosylation quality.