TARGETED EPIGENETIC GLYCO-ENGINEERING IN CHO CELLS

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Extensive knowledge has been gathered by applying and generating –omics techniques and data towards a holistic understanding of the Chinese Hamster Ovary (CHO) cell's regulatory network. However, these data are far from universally explanatory. The epigenome, i.e. the genetic signature that controls modulation of gene expression, has not yet been fully explored. To enable direct control of epigenetic regulation of individual genes, we constructed CRISPR-based epigenetic editing tools that induce site-specific DNA methylation or demethylation rather than double strand breaks at specific endogenous promoters.

The current design targets the promoter of the silenced α (2,6)-sialyltransferase (ST6GAL1) gene, which is actively transcribed in human and there part of the protein glycosylation machinery. It is present in the CHO genome, but silenced. We aimed to induce its expression in CHO by targeted demethylation of the ST6GAL1 promoter. Flow cytometric analysis (based on glycosylation specific lectin staining) showed upregulation of ST6GAL1 in up to 67% of cells in transfected cell pools. ST6GAL1 expression was also confirmed by RT-qPCR and MS glycan analysis. Stable upregulation of ST6GAL1 was monitored over a period of more than 80 days, showing the applicability in industrial cell development pipelines for long-term changes in cell behavior. The effect could be readily reversed by subsequent targeted re-methylation of the ST6GAL1 promoter by our epigenetic editing tool set.

In conclusion, this epigenetic tool does not only allow to build a new layer of cell control that complements existing techniques (e.g. genome engineering), but also enables a more sensitive investigation of gene function by induction and repression of genes without altering the DNA sequence. Finally, other than gene knockout or overexpression studies, the modulation is readily reversible, thus opening up a multitude of possibilities for fast, unbiased and stable testing of gene function and multiplex engineering approaches.