A NOVEL SYSTEM FOR GLYCOSYLATION ENGINEERING BY NATURAL AND ARTIFICIAL MICRORNAS

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N-linked glycosylation is a common post-translational modification of many biopharmaceuticals capable of modifying their biological effect in patients and therefore is considered as a critical quality attribute potentially impairing the safety and efficacy. However, expression of desired and consistent glycosylation patterns remains a constant challenge and constitutes the need for tools to engineer glycosylation. Small non-coding microRNAs (miRNAs) have been successfully used for engineering various phenotypes of production cell lines in the past, but their potential of being used as a tool for glyco-engineering remained concealed. Due to their complex mechanism of regulation, where hundreds of target genes are regulated by a single miRNA causing a variety of phenotypic effects, the application of miRNAs for a specific regulation remains challenging. Our study aimed to (i) identify and establish miRNAs as novel tools for glycosylation-engineering in mammalian expression cell lines and (ii) use artificial miRNAs design to modulate the degree of protein glycosylation to fine-tune phenotypic effects allowing an adjustment towards the exact needs of the patients.

For the experimental approach, a functional transient high-content screen of a miRNA mimic library consisting of ~1900 different miRNA sequences was performed in an industrial monoclonal antibody (mAb) expressing Chinese hamster ovary (CHO) cell line using state of the art intact-mass spectrometry analysis of resulting glycosylation patterns. Identified glycosylation modulatory miRNAs were processed in a subsequent validation pipeline for characterization of their impact on cellular behavior relevant for bioprocessing and their mode of action on molecular level by target gene regulation. Selected miRNAs with identified enzyme targets in the fucosylation pathway were artificially in silico adapted based on miRNA binding rules, transfected in CHO production cells and gene regulation, cellular parameters and mAB fucosylation analyzed.

The screening of the miRNA mimic library resulted in the identification of 84 miRNA sequences capable of modifying various moieties including α-1,6 linked core-fucosylation and galactosylation on the glycan structure of the produced mAb. Validation experiments identified 9 novel miRNA sequences regulating different enzymes of the cellular fucosylation pathway inducing reduced amount of core-fucosylated mAb glycoforms. In addition, miRNAs increasing or decreasing the abundance of galactose were identified and target genes characterized. A multiplex modulation approach demonstrated gradual and additive regulation capacity and thereby the potential of miRNAs to be used as a fine-tuning tool of expressed glycosylation patterns. Artificial desgin of selected miRNAs was successful to (i) increase the strength of a weak fucosyltransferase 8 (FUT8) regulator miRNA with intermediate sequence variants covering the whole spectrum of regulation, (ii) eliminate negative side effects as reduced cell viability of a novel FUT8 regulatory miRNA, and (iii) create sequence variants of a novel fucokinase regulator miRNA inducing a metabolic shift to bypass regulation. Finally, stable overexpression of selected native and artificial miRNAs in an industrial CHO production cell line confirmed reliable and modulatory glyco-regulation capacity underlining the applicability for industrial mAb production.

In conclusion, our study unveiled miRNAs as novel tools for gradual modulation of N-linked glycosylation and opened new avenues to artificially design miRNAs for fine-tuning of phenotypic parameters to meet industrial needs. These novel tools will further expand the toolbox for rational design of production cells to ensure the production of safe biopharmaceutical products with high quality standards.