CRISPR/CAS9 MEDIATED KNOCKOUT OF MICRORNAS FOR PECISE CELL ENGINEERING

Nadja Raab, University of Applied Sciences Biberach
raab@hochschule-bc.de
Kerstin Alt, Genetikum Neu Ulm
Sven Mathias, University of Applied Sciences Biberach
René Handrick, University of Applied Sciences Biberach
Vaibhav Jadhav, BOKU Vienna
Kerstin Otte, University of Applied Sciences Biberach

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Recent advances in the development of molecular tools available for cell line engineering has opened avenues for precise modulation of production cell lines to optimize cellular process relevant parameters. Among those, the CRISPR/Cas9 system represents a rapid and straightforward cell line engineering tool which allows for precise gene editing of host genomes to modify relevant signaling pathways. In addition, microRNAs (miRNAs) have proven to serve as versatile molecular tools to improve production cells regarding protein production and growth characteristics. These small non-coding RNA molecules are regulators of gene expression and regulate process relevant cellular pathways as growth, apoptosis, protein expression or stress. With an individual microRNA being able to regulate up to several hundred target genes, the modulation of miRNA expression may mimic simultaneous modification of several hundred target genes. Therefore, miRNAs may offer great potential to serve as targets for CRISPR/Cas9-mediated genome editing, as an individual microRNA can regulate up to several hundred target genes and therefore mimics multiplexing of hundreds of target genes in one single step. While the overexpression of miRNAs to improve the performance of biopharmaceutical production hosts has received major interest in the past years, effects of precise knockout of unfavorable miRNAs in Chinese hamster ovary (CHO) production cells have not been reported yet.

Therefore, the current study aimed at applying a novel strategy to increase product yields by enhancing viability and culture longevity of CHO cells using CRISPR/Cas9-mediated deletion of a miRNA causing adverse effects. In a previous high content screen with more than 1000 miRNAs we were able to identify miR-744 as being involved in the regulation of apoptosis. After the apoptosis-inducing capacity of miR-744 in monoclonal antibody-producing CHO-mAb1 cells was revalidated, the genomic precursor sequence of miR-744 was deleted by two sgRNA-Cas9-mediated DNA double-strand breaks flanking the miR-744 gene in the CHO genome. A plasmid encoding both required sgRNAs in a tandem array, Cas9 and GFP was used to allow for simultaneous delivery of all required components and thereby reducing the screening effort for potential *knockout* (KO) cell lines. After fluorescent-activated cell sorting (FACS) seven putative, clonal miR-744-KO cell lines could be recovered. From these, three were identified and confirmed as miR-744-KOs by sequencing of their miR-744 locus respectively. In a subsequent growth experiment over a period of nine days considerable differences in the investigated parameters viable cell count, viability, apoptosis, necrosis and antibody titers of the miR-744-KO cell lines in comparison to untreated cells and non-targeting sgRNA transfected cells could be observed. Further experiments are conducted to analyze the observed effects.

In summary, the present study elucidates the role of microRNAs, which bear adverse effects on production cells, as targets for the novel CRISPR/Cas9 genome editing technique in the context of cell line engineering. This approach contributes to the idea of a rational design of CHO production hosts with controlled modulation of signaling pathways or metabolic characteristics to enable the generation of optimized production cells.