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Improving the Bioprocess Phenotype of Chinese Hamster Ovary Cells Using CRISPR/Cas9 and Sponge Decoy Mediated MiRNA Knockdowns

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Abstract : Chinese Hamster Ovary (CHO) cells are the prominent cell line used in biopharmaceutical production. To improve yields and find beneficial bioprocess phenotypes genetic engineering plays an essential role in recent research. The miR-23 cluster, specifically miR-24 and miR-27, was first identified as differentially expressed during hypothermic conditions suggesting a role in proliferation and productivity in CHO cells. In this study, we used sponge decoy technology to stably deplete the miRNA expression of the cluster. Furthermore, we implemented the CRISPR/Cas9 system to knockdown miRNA expression. Sponge constructs were designed for an imperfect binding of the miRNA target, protecting from RISC mediated cleavage. GuideRNAs for the CRISPR/Cas9 system were designed to target the seed region of the miRNA. The expression of mature miRNA and precursor were confirmed using RT-qPCR. For both approaches stable expressing mixed populations were generated and characterised in batch cultures. It was shown, that CRISPR/Cas9 can be implemented in CHO cells with achieving high knockdown efficacy of every single member of the cluster. Targeting of one miRNA member showed that its genomic paralog is successfully targeted as well. The stable depletion of miR-24 using CRISPR/Cas9 showed increased growth and specific productivity in a CHO-K1 mAb expressing cell line. This phenotype was further characterized using quantitative label-free LC-MS/MS showing 186 proteins differently expressed with 19 involved in proliferation and 26 involved in protein folding/translation. Targeting miR-27 in the same cell line showed increased viability in late stages of the culture compared to the control. To evaluate the phenotype in an industry relevant cell line; the miR-23 cluster, miR-24 and miR-27 were stably depleted in a Fc fusion CHO-S cell line which showed increased batch titers up to 1.5-fold. In this work, we highlighted that the stable depletion of the miR-23 cluster and its members can improve the bioprocess phenotype concerning growth and productivity in two different cell lines. Furthermore, we showed that using CRISPR/Cas9 is comparable to the traditional sponge decoy technology.

Keywords : Chinese Hamster ovary cells, CRISPR/Cas9, microRNAs, sponge decoy technology
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