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CRISPR/CAS9 Targeting MicroRNA-24 in Chinese Hamster Ovary Cells Increases Growth and Boosts Productivity

Kevin Kellner[1]*, Nga T Lao[1], Paula Meleady[1] and **Niall Barron[2]**
[1]National Institute for Cellular Biotechnology, Ireland
[2]National Institute for Bioprocessing Research and Training, Ireland

Chinese Hamster Ovary (CHO) cells are the prominent cell line used in biopharmaceutical production. Although optimisation efforts have led to a vast increase in productivity, CHO cells yield less than other expression systems like yeast or bacteria. To improve yields and find beneficial bioprocess phenotypes, genetic engineering plays an essential role in recent research. The miR-23 cluster with its genomic paralogues (miR-23a and miR-23b) was first identified as differentially expressed during temperature shift, suggesting its role in proliferation and productivity. The common approach to deplete miRNAs is the use of a sponge decoy which, requires the introduction of reporter genes. As an alternative this work aims to knockdown miRNA expression using the recently developed CRISPR/Cas9 system which does not require a reporter transcript. This system consists of two main components: the single guide RNA (sgRNA) and an endonuclease (Cas9) which induces double strand breaks (DSBs). These DSBs can result in insertion or deletion (indels) of base pairs which can disrupt miRNA function and processing.

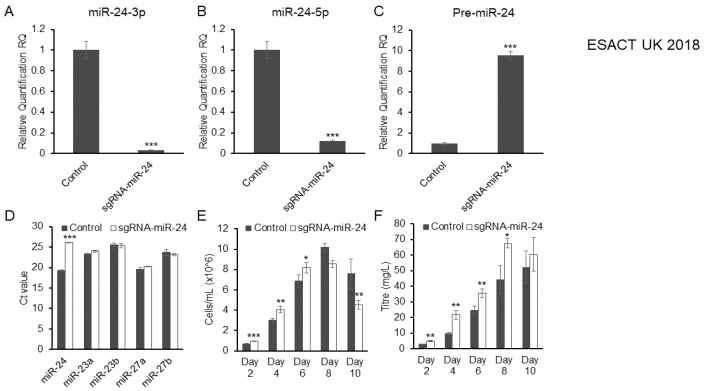

Figure 1 CRISPR mediated knockdown of miR-24. A and B) Knockdown of miR-24-3p as well as passenger miRNA miR-24-5p. C) Targeting miR-24 leads to an accumulation of pre-miR-24. D) Targeting miR-24 does not affect other members of the cluster. E and F) Depletion of miR-24 results in accelerated growth as well as boosted IgG titers (n=3, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

A CHO-K1 cell line stably expressing an IgG was used for knockdown experiments. SgRNAs were designed to target the seed region of miR-24-3p and stable mixed populations were generated.

It was shownthat miRNA expression for miR-24-3p as well as miR-24-5p was significantly reduced in mixed populations. Furthermore, an increase in pre-miR-24 was exhibited suggesting impaired processing by Drosha or Dicer. A knockdown up to 95% was achieved for miR-24 as well as the passenger miRNA (Figure 1A, B and C). The other members of the cluster which are located proximal to miR-24 were not affected by the knockdown (Figure 1D). Depletion of miR-24 showed increased proliferation in batch culture as well as boosted productivity (Figure 1E and F). However, faster growth led to increased nutrient demand of miR-24 depleted cultures and a reduction in culture time was exhibited. Quantitative label-free LC/MS was used to identify 81 more abundant protein targets. Pathway analysis revealed proteins potentially involved in enhanced ribosomal RNA biogenesis, recycling and assembly of ribosomal subunits. Furthermore, proteins involved in catalysing the loading of cognate aminoacyl-tRNAs and release of deacetylated tRNAs were higher expressed. These targets were highlighted as potential cell line engineering targets to improve productivity of CHO cells.

Out of all 81 upregulated proteins, 50 were predicted targets of either miR-24-3p or miR-24-5p. In this work, we have shown that CRISPR/Cas9 can be successfully applied as a tool to knockdown miRNA expression in CHO cells. The data was generated using mixed pools and it remains to be established if both alleles can be successfully targeted e.g. using next-generation sequencing ofindividual clones.