knock-out cells are stable with regard to this phenotype and marker genes can be excised. Also, RNAi expression is more easily controlled since a saturating level is usually reached and the expression level is less critical as for protein overexpression.

SUMMARY

In the present disclosure it is shown that knocking-down selected target genes increased production of secreted proteins, particularly reducing ATF6B activity, or reducing CERS2 and TBC1D20 activity, exerts a positive effect on secreted product concentration, protein productivity and viable cell density. These results suggest that the targeting of ATF6B, or the combination of CERS2 and TBC1D20, are effective strategies for improving titers of therapeutic proteins, including antibodies.

By virtue of downregulation of ATF6B, CERS2 and TBC1D20 protein activity, it is now possible to engineer mammalian cells to improve their cell productivity and/or cell viability, in particular, to increase the amount of protein secreted by the cell culture. The mammalian cells, methods and uses provided herein allow for a more efficient and cost-effective production of secreted proteins, especially for antibody production. This may speed up drug development, since the generation of sufficient amounts of material for pre-clinical studies is critical with regard to overall development timelines. The objects of the examples disclosed herein can further be used for the generation of one or several specific secreted proteins for either diagnostic purposes, research purposes (target identification, lead identification, and lead optimization) or manufacturing therapeutic proteins either on the market or in clinical development.

In one aspect, the disclosure relates to a mammalian cell having enhanced secretion of a recombinant therapeutic protein comprising (a) reduced expression of the host cell proteins TBC1 domain family member 20 (TBC1D20) and ceramide synthase 2 (CERS2); or (b) reduced expression of the host cell protein activating transcription factor 6 beta (ATF6B); wherein the mammalian cell further comprises one or more expression cassette(s) encoding a recombinant secreted therapeutic protein.

Another aspect of the disclosure relates to a mammalian cell having enhanced secretion of a recombinant therapeutic protein comprising reduced expression of the host cell proteins TBC1D20 and CERS2. Optionally the mammalian cell may further comprise one or more expression cassette(s) encoding a recombinant secreted therapeutic protein.

In the mammalian cell of the disclosure (a) the gene encoding the host cell protein comprises a genetic modification that inhibits expression of said host cell protein, or (b) the mammalian cell comprises a RNA oligonucleotide that inhibits expression of the gene encoding said host cell protein by RNA-interference, wherein the protein expression of TBC1D20 and CERS2 or the protein expression of ATF6B in the mammalian cell is reduced compared to the same mammalian cell not containing said genetic modification(s) or RNA oligonucleotide(s).

In a further aspect, the disclosure relates to a method of producing a mammalian cell with enhanced secretion of a recombinant therapeutic protein comprising (a) reducing expression of the host cell

proteins TBC1D20 and CERS2, or of the host cell protein ATF6B in the mammalian cell by introducing (i) a genetic modification into a gene encoding the host cell protein that inhibits expression of said host cell protein, or (ii) a RNA oligonucleotide into the mammalian cell that inhibits expression of the gene encoding said host cell protein by RNA-interference, and (b) introducing one or more gene(s) encoding a recombinant secreted therapeutic protein. The method of the disclosure may further comprise the following steps: (c) selecting cells with enhanced secretion of the recombinant therapeutic protein; and (d) optionally culturing the cells obtained in step (c) under conditions which allow expression of one or more gene(s) encoding a recombinant secreted therapeutic protein. According to the method of the disclosure the protein expression of TBC1D20 and CERS2 or the protein expression of ATF6B in the mammalian cell is reduced compared to the same mammalian cell not containing said genetic modification(s) or RNA oligonucleotide(s).

The RNA-interference in the cell of the disclosure or in the method of the disclosure may be mediated by small hairpin RNA (shRNA) or short interfering RNA (siRNA). Preferably the mammalian cell is transfected with one or more expression vector(s) comprising a nucleotide sequence encoding said siRNA(s) or shRNA(s), more preferably the mammalian cell is stably transfected with one or more expression vector(s) encoding said siRNA(s) or shRNA(s). The siRNA used in the cell or the method of the disclosure may be (a) siTbc1D20#1 (SEQ ID NO: 7) or siCerS2#1 (SEQ ID NO: 8), or a combination thereof if TBC1D20 and/or CERS2 are targeted; or (b) one or more of siAtf6b#1 (SEQ ID NO: 9), siAtf6b#2 (SEQ ID NO: 10), and siAtf6b#3 (SEQ ID NO: 11) if ATF6b is targeted. The shRNA may comprise (a) shTbc1D20#1 (SEQ ID NO: 12), or one or more of shCerS2#1 (SEQ ID NO: 13) and shCerS2#2 (SEQ ID NO: 14), or a combination thereof, if TBC1D20 and/or CERS2 are targeted; or (b) one or more of shAtf6b#1 (SEQ ID NO: 15) and shAtf6b#2 (SEQ ID NO: 37), if ATF6b is targeted. The exemplary siRNAs and shRNAs are particularly suitable for hamster cells, such as CHO cells.

The genetic modification in the gene(s) encoding the host cell protein(s) TBC1D20 and CERS2, or ATF6B may be independently (a) a gene deletion; or (b) a mutation in the gene that inhibits expression of the host cell protein, preferably in the coding region of the gene and/or the promoter or regulatory region of the gene. The host cell protein TBC1D20 preferably has a sequence identity of at least 80% to the amino acid sequence of SEQ ID NO: 4, the host cell protein CERS2 preferably has a sequence identity of at least 80% to the amino acid sequence of SEQ ID NO: 5; and the host cell protein ATF6B preferably has sequence identity of at least 80% to the amino acid sequence of SEQ ID NO: 6.

According to the disclosure the recombinant secreted therapeutic protein may be an antibody, preferably a monoclonal antibody, a bi-specific antibody or a fragment thereof. Alternatively the recombinant secreted therapeutic protein may also be a Fc-fusion protein. The cell according to the disclosure or used in the method of the disclosure is preferably a rodent or a human cell, preferably a rodent cell, such as a CHO cell.

In a further aspect the disclosure also relates to a method for the production of a recombinant secreted therapeutic protein in a mammalian cell comprising (a) providing the mammalian cell of the

disclosure, wherein the cell is transfected with a recombinant secreted therapeutic protein or providing the mammalian cell produced by the method of the disclosure, (b) culturing the mammalian cell of step (a) in a cell culture medium at conditions allowing production of the recombinant secreted therapeutic protein, (c) harvesting the recombinant secreted therapeutic protein, and optionally (d) purifying the recombinant secreted therapeutic protein.

In yet another aspect, the disclosure relates to the use of the mammalian cell of the disclosure or to the mammalian cell produced by the method of the disclosure for increasing the yield of a recombinant secreted therapeutic protein.

DESCRIPTION OF THE FIGURES

FIGURE 1 shows IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TARGET GENES OF MICRORNAS BY NEXT GENERATION SEQUENCING

Panel (A) is a table showing that CHO-DG44 cells stably secreting an IgG1 antibody (mAb1) (CHO-mAb1 cells) were transfected with each of the two microRNAs miR-1287 and miR-1978 having strong effects on antibody production and specific productivity. 12 hours after transfection transcriptome profiling by next generation sequencing (NGS) was used to identify direct target genes of the two miRNAs. Genes that were significantly downregulated with a $|\log2|$ fold change > 1 (gene expression in untransfected control cells (mock) was more than 2-fold) were defined as hits. The miR-1287 target gene ATF6B and the miR-1978 target genes CerS2 and Tbc1D20 were chosen for further analysis. Shown is the normalized fold change in expression (mock vs miRNA transfected) of the respective genes and its $|\log2|$.Effects of selected target genes on antibody production and specific productivity were first assessed by siRNA-mediated gene knockdown, followed by shRNA-mediated knockdown to investigate long-term depletion of the target genes. Panel (B) is a table listing the names and sequences of the siRNAs and shRNAs used in this study.

FIGURE 2 shows VALIDATION OF TARGET GENES VIA QPCR

(A) and (B), CHO-DG44 cells stably secreting the IgG1 antibody mAb1 were transfected with microRNA miR-c#1, miR-1287 or miR-1978. One day after transfection, RNA was extracted to quantify levels of mRNA of ATF6B, CerS2 and Tbc1D20 by qPCR. (C) and (D), Expression levels of these target genes were quantified for CHO-DG44 cells stably secreting the antibody mAb1 and further stably overexpressing either miR-1287 or miR-1978. Relative expression was calculated by normalizing to the reference gene beta actin. Panel (A) is a bar graph showing that transient transfection with miR-1287 resulted in a reduced expression level of ATF6B compared to untransfected cells (mock) (n=3, error bars = SEM of triplicates). Panel (B) is a bar graph showing that transient transfection with the miRNA miR-1978 resulted in decreased levels of CERS2 mRNA and TBC1D20 mRNA compared to untransfected