SUMMARY

[008] The present disclosure demonstrates that three variants of a mammalian glutamine synthetase, harboring a single point mutation, result in the generation of highly productive and stable host cell pools expressing a therapeutic protein, particularly a therapeutic antibody, showing a more stringent selection behavior due to the attenuated activity compared to wildtype glutamine synthetase and beneficial cell culture performance in CHO cells. All three mutations confer an increased selection stringency and unexpectedly resulted in stable host cell pools with higher productivity and/or stability when compared to wildtype GS, making these variants superior in the cell line development. The mutated amino acid positions have not been described yet and are not directly associated with the catalytic binding pocket of the enzyme, but rather seem to influence the stability of the protein by driving the structural interaction. These protein variants are particularly advantageous as they carry mutations outside of the substrate binding site, e.g. at protein interfaces, which are important for the assembly of a multimeric GS protein complex – giving additional means to modulate the attenuation in a more moderate fashion.

[009] More specifically, in one aspect, the present disclosure relates to a modified mammalian glutamine synthetase comprising a mutation at amino acid position 10 and/or 298 in a mammalian glutamine synthetase having an amino acid sequence of SEQ ID NO: 1, 2, 3 or 4 or an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 1, 2, 3 or 4, wherein the mutation is selected from the group consisting of R298K, N10S, N10T and N10Q. Particularly, the mutation does not interfere with substrate-binding.

[010] In certain examples, the modified mammalian glutamine synthetase comprising a mutation selected from the group consisting of R298K, N10S, N10T and N10Q (a) has diminished enzymatic activity compared to the same mammalian glutamine synthetase not comprising the mutation; and/or (b) mediates increased selection stringency and/or genetic stability upon transfection with a polynucleotide encoding the modified mammalian glutamine synthetase and a transgene compared to the same mammalian glutamine synthetase not comprising the mutation.

[011] In another aspect, the disclosure relates to an expression vector comprising a polynucleotide encoding the modified mammalian glutamine synthetase according to the disclosure. In certain example, the expression vector further comprises at least one polynucleotide encoding a protein of interest and/or a non-coding RNA, preferably wherein the protein of interest is a therapeutic protein selected from the group consisting of a cytokine, a hormone, a fusion protein, an antibody, an antibody-derived molecule and an antibody mimetic.

[012] In yet another aspect, the disclosure relates to a nucleic acid sequence comprising a polynucleotide encoding the modified mammalian glutamine synthetase according to the disclosure operably linked to a eukaryotic promoter, optionally further comprising at least one polynucleotide encoding a protein of interest and/or a non-coding RNA.

[013] In yet another aspect, the disclosure relates to a eukaryotic host cell comprising a polynucleotide sequence encoding the modified mammalian glutamine synthetase according to the disclosure, the expression vector according to the disclosure or the nucleic acid sequence according to the disclosure.

The eukaryotic host cell is preferably a mammalian host cell, more preferably a rodent cell, even more preferably a CHO cell. In addition or alternatively the eukaryotic host cell is a GS knockout mutant.

[014] In yet another aspect, the disclosure further relates to a method for preparing a cell stably expressing a protein of interest and/or a non-coding RNA, comprising (a) introducing the expression vector according to the disclosure or the nucleic acid according to the disclosure, optionally further comprising at least one polynucleotide encoding a protein of interest and/or a non-coding RNA, into a eukaryotic host cell, preferably into a mammalian host cell, more preferably a CHO cell; and (b) culturing the eukaryotic host cell in a medium comprising no glutamine under conditions to select for the modified mammalian glutamine synthetase, wherein the optional at least one polynucleotide encoding a protein of interest and/or a non-coding RNA is co-integrated with the polynucleotide encoding the modified mammalian glutamine synthetase into the host cell genome. The method may further comprise a step of a step of isolating a single clone for clonal expansion to prepare a monoclonal cell line.

[015] In yet another aspect, the disclosure provides a method of producing a protein of interest, comprising (a) introducing the expression vector comprising a polynucleotide encoding a modified mammalian glutamine synthetase according to the disclosure or the nucleic acid according to the disclosure, comprising a mutation selected from the group consisting of R298K, N10S, N10T and N10Q and further comprising at least one polynucleotide encoding a protein of interest into a eukaryotic host cell, preferably into a mammalian host cell, more preferably into a CHO cell; (b) culturing the eukaryotic host cell in a medium comprising no glutamine under conditions to select for the modified mammalian glutamine synthetase, wherein the at least one polynucleotide encoding a protein of interest is co-integrated with the polynucleotide encoding the modified mammalian glutamine synthetase into the host cell genome; (c) optionally isolating single clones for clonal expansion to prepare a monoclonal cell line; (d) culturing the eukaryotic host cell under conditions to produce the protein of interest; and (e) harvesting and optionally purifying the protein of interest. The expression vector may be introduced by transfection or transduction.

[016] In yet another aspect, the disclosure provides a method of producing a protein of interest, comprising (a) providing the eukaryotic host cell according to the disclosure comprising a polynucleotide encoding a mammalian glutamine synthetase comprising a mutation at amino acid position 10 and/or 298 in a mammalian glutamine synthetase having the amino acid sequence of SEQ ID NO: 1, 2, 3 or 4 or an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 1, 2, 3 or 4, wherein the mutation is selected from the group consisting of R298K, N10S, N10T and N10Q and at least one polynucleotide encoding a protein of interest; (b) culturing the eukaryotic host cell under conditions to produce the protein of interest; and (c) harvesting and optionally purifying the protein of interest. The eukaryotic host cell of the methods of the disclosure is (a) a mammalian host cell, preferably a rodent cell, more preferably a CHO cell; and/or (b) a GS knockout mutant.

[017] In yet another aspect, the disclosure also provides a kit comprising the expression vector according to the disclosure and a cell culture medium not comprising glutamine.

[018] In yet another aspect, the disclosure relates to a use of the modified mammalian glutamine synthetase according to the disclosure as a selection marker for monoclonal eukaryotic cell line

generation, preferably a mammalian cell line generation, more rodent cell line generation, even more preferably a CHO cell line generation.

[019] In yet another aspect, the disclosure relates to a use of the vector according to the disclosure for producing a protein of interest in a eukaryotic host cell, preferably mammalian host cell, more preferably a rodent cell, even more preferably a CHO cell.

DESCRIPTION OF THE FIGURES

[020] FIGURE 1: CHO-K1-GS cell pools expressing mAb1 and wildtype CHO GS as selection marker. CHO-K1-GS cell pools stably transfected with a mammalian expression vector to express mAb1 and CHO wildtype GS as a metabolic selection maker (n=5) were cultured in medium not containing L-glutamine. Titer [mg/L] of mAb1 was determined at the indicated days post transfection.

[021] FIGURE 2: Viability of CHO-K1-GS cell pools expressing mAb1 and CHO wildtype GS or R298 variants. CHO-K1-GS cell pools stably transfected with an expression cassette to express mAb1 and CHO wildtype GS (wtGS) or one of 6 variants of CHO GS with various mutations at position R298 were cultured in medium not containing L-glutamine. Viability [%] was determined at the indicated days in selection.

[022] FIGURE 3: Productivity and mean productivity of CHO-K1-GS cells expressing mAb1 and CHO wildtype GS or CHO GS R298K mutant. CHO-K1-GS cell pools stably transfected with an expression cassette to express mAb1 and CHO wildtype GS or CHO GS R298K mutant cultured in medium not containing L-glutamine. Titer [µg/mL] of mAb1 was determined at the indicated days in culture. A: Productivity of CHO GS R298K mutant and wildtype CHO GS over the course of 20 days. B: Mean productivity of CHO WT GS and CHO GS R298K mutant (** $p < 0.01$).

[023] FIGURE 4: Viability of CHO-K1-GS cells expressing mAb1 and CHO wildtype GS or CHO GS N10S, N10Q or N10W mutants. CHO-K1-GS cell pools stably transfected with an expression cassette to express mAb1 and CHO wildtype GS or single point mutants CHO GS N10S, N10Q or N10W were cultured in medium not containing L-glutamine. Viability [%] was determined at the indicated days post transfection.

[024] FIGURE 5: Productivity of CHO-K1-GS cells expressing mAb1 and CHO wildtype GS compared to N10S, N10Q or N10W CHO GS variants as selection marker. CHO-K1-GS cell pools stably transfected with an expression cassette to express mAb1 and CHO wildtype GS or CHO GS N10S, N10Q or N10W mutants cultured in medium not containing L-glutamine. Titer [mg/L] of mAb1 was determined at the indicated days once cells reached viabilities >70%.

[025] FIGURE 6: Viability of CHO-K1-GS cells expressing mAb1 and CHO wildtype GS or additional CHO GS N10 variants. CHO-K1-GS cell pools stably transfected with an expression cassette to express mAb1 and CHO wildtype GS or single point mutants CHO GS N10S, N10Q, N10W, N10F, N10Y or N10G cultured in medium not containing L-glutamine. CHO-K1-GS cells carrying N10 mutations were found to be phenotypically unstable, except for cells carrying CHO GS N10S or N10Q. Viability [%] was determined at the indicated days post transfection.