CLAIMS

What is claimed is:

1. A method for producing a recombinant protein with a human-like glycosylation pattern, the method comprising expressing the recombinant protein in a non-human mammalian cell line deficient in cytidine monophosphate-N-acetylneuraminic acid hydroxylase (Cmah) and/or glycoprotein alpha-1,3-galactosyltransferase-1 (Ggta1).

2. The method of claim 1, wherein the cell line comprises an inactivated chromosomal sequence encoding Cmah and/or an inactivated chromosomal sequence encoding Ggta1, and the cell produces a reduced amount of Cmah and/or Ggta1.

3. The method of either claim 1 or claim 2, wherein the recombinant protein has a reduced content of N-glycolylneuraminic acid (Neu5Gc) residues and/or galactose-alpha-1,3-galactose (alpha-Gal) residues compared to the same recombinant protein produced by a comparable cell line not deficient in Cmah and/or Ggta1.

4. The method of claim 2, wherein all copies of the chromosomal sequence encoding Cmah and/or Ggta1 are inactivated, and the cell line produces no Cmah and/or Ggta1.

5. The method of claim 4, wherein the recombinant protein lacks N-glycolylneuraminic acid/or (Neu5Gc) residues and galactose-alpha-1,3-galactose (alpha-Gal) residues.

6. The method of any one of claims 1-5, wherein the recombinant protein has at least one property that is improved relative to the same recombinant protein produced by a comparable cell line not deficient in Cmah and/or Ggta1.

7. The method of claim 6, wherein the property that is improved is chosen from reduced immunogenicity, increased bioavailability, increased efficacy, increased stability, increased solubility, improved half-life, improved clearance, and improved pharmacokinetics.

8. The method of any one of claims 1-7, wherein the recombinant protein is chosen from an antibody, an antibody fragment, a growth factor, a cytokine, a hormone, and a clotting factor.

9. The method of any one of claims 1-8, wherein the cell line is a Chinese hamster ovary (CHO) cell line.

10. The method of claim 1, wherein the cell line is a CHO cell line, all copies of the chromosomal sequence encoding Cmah are inactivated, the cell line produces no Cmah, and the recombinant protein lacks Neu5Gc residues.

11. The method of claim 1, wherein the cell line is a CHO cell line, all copies of the chromosomal sequence encoding Ggta1 are inactivated, the cell line produces no Ggta1, and the recombinant protein lacks alpha-Gal residues.

12. The method of claim 1, wherein the cell line is a CHO cell line, all copies of the chromosomal sequence encoding Cmah and all copies of chromosomal sequence encoding Ggta1 are inactivated, the cell line produces no Cmah or Ggta1, and the recombinant protein lacks Neu5Gc and alpha-Gal residues.

13. A non-human mammalian cell line deficient in cytidine monophosphate-N-acetylneuraminic acid hydroxylase (Cmah).

14. The non-human mammalian cell line of claim 13, wherein the cell line comprises an inactivated chromosomal sequence encoding Cmah.

15. The non-human mammalian cell line of claim 14, wherein the inactivated chromosomal sequence encoding Cmah comprises a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.

16. The non-human mammalian cell line of either claim 14 or claim 15, wherein the chromosomal sequence is inactivated with a targeting endonuclease.

17. The non-human mammalian cell line of claim 16, wherein the targeting endonuclease is a zinc finger nuclease.

18. The non-human mammalian cell line of any one of claims 14-17, wherein the inactivated chromosomal sequence encoding Cmah comprises no exogenously introduced sequence.

19. The non-human mammalian cell line of any one of claims 14-18, wherein the inactivated chromosomal sequence encoding Cmah is monoallelic and the cell line produces a reduced amount of Cmah.

20. The non-human mammalian cell line of any one of claims 14-18, wherein the inactivated chromosomal sequence encoding Cmah is biallelic and the cell line produces no Cmah.

21. The non-human mammalian cell line of any one of claims 13-20, wherein the cell line produces at least one protein having a reduced content of or no N-glycolylneuraminic acid (Neu5Gc) residues.

22. The non-human mammalian cell line of any one of claims 13-21, wherein the cell line is further deficient in glycoprotein alpha-1,3-galactosyltransferase-1 (Ggta1).

23. The non-human mammalian cell line of 22, wherein the cell line comprises an inactivated chromosomal sequence encoding Ggta1.

24. The non-human mammalian cell line of claim 23, wherein the inactivated chromosomal sequence encoding Ggta1comprises a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.

25. The non-human mammalian cell line of either claim 23 or claim 24, wherein the chromosomal sequence is inactivated with a targeting endonuclease.

26. The non-human mammalian cell line of claim 25, wherein the targeting endonuclease is a zinc finger nuclease.

27. The non-human mammalian cell line of any one of claims 23-26, wherein the inactivated chromosomal sequence encoding Ggta1 comprises no exogenously introduced sequence.

28. The non-human mammalian cell line of any one of claims 23-27, wherein the inactivated chromosomal sequence encoding Ggta1 is monoallelic and the cell line produces a reduced amount of Ggta1.

29. The non-human mammalian cell line of any one of claims 23-27, wherein the inactivated chromosomal sequence encoding Ggta1 is biallelic and the cell line produces no Ggta1.

30. The non-human mammalian cell line of any one of claims 22-29, wherein the cell line produces at least one protein having a reduced content of or no N-glycolylneuraminic acid (Neu5Gc) residues.

31. The non-human mammalian cell line of any one of claims 13-30, wherein the cell line is a Chinese hamster ovary (CHO) cell line.

32. The non-human mammalian cell line of claim 13, wherein the cell line is a CHO cell line, all copies of the chromosomal sequence encoding Cmah are inactivated, and the cell line produces no Cmah.

33. The non-human mammalian cell line of claim 22, wherein the cell line is a CHO cell line, all copies of the chromosomal sequence encoding Cmah and all copies of the chromosomal sequence encoding Ggta1 are inactivated, and the cell line produces no Cmah or Ggta1.

CLAIMS

1. A Chinese hamster ovary (CHO) cell line deficient in glutamine synthetase (GS), cytidine monophosphate-N-acetylneuraminic acid hydroxylase (Cmah), and glycoprotein alpha-1,3-galactosyltransferase-1 (Ggta1).

2. The CHO cell line of claim 1, wherein the cell line comprises an inactivated chromosomal sequence encoding Cmah and an inactivated chromosomal sequence encoding Ggta1.

3. The CHO cell line of claim 2, wherein the inactivated chromosomal sequences encoding Cmah and Ggta1 comprise a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.

4. The CHO cell line of claim 2, wherein the chromosomal sequences are inactivated with a targeting endonuclease.

5. The CHO cell line of claim 4, wherein the targeting endonuclease is a zinc finger nuclease.

6. The CHO cell line of claim 2 wherein the inactivated chromosomal sequences encoding Cmah and Ggta1 comprise no exogenously introduced sequence.

7. The CHO cell line of claim 2, wherein the inactivated chromosomal sequences encoding Cmah and Ggta1 are monoallelic and the cell line produces a reduced amount of Cmah and Ggta1.

8. The CHO cell line of claim 2, wherein the inactivated chromosomal sequences encoding Cmah and Ggta1 are biallelic and the cell line produces no Cmah or Ggta1.

9. The CHO cell line of claim 1, wherein the cell line produces at least one protein having a reduced content of or no N-glycolylneuraminic acid (Neu5Gc) residues.

10. The CHO cell line of claim 2, wherein the inactivated chromosomal sequence encoding Cmah comprises a deletion of 17 or 31 base pairs, and the inactivated chromosomal sequence encoding Ggta1 comprises a deletion of 3, 4, or 23 base pairs.

11. A Chinese hamster ovary (CHO) cell line comprising inactivated chromosomal sequences encoding glutamine synthetase (GS), cytidine monophosphate-N-acetylneuraminic acid hydroxylase (Cmah), and glycoprotein alpha-1,3-galactosyltransferase-1 (Ggta1).

12. The CHO cell line of claim 11, wherein the inactivated chromosomal sequences encoding GS, Cmah, and Ggta1 are biallelic and the cell line produces no GS, Cmah, or Ggta1.