Methods for Producing Recombinant Glycoproteins with Modified Glycosylation

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/954,337, filed on March 17, 2014, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Glycosylation is important to the structures and functions of glycoproteins. For example, glycosylation is suggested to affect protein folding (and thus stability) and/or bioactivities of glycoproteins. The demand of therapeutic recombinant glycoproteins, especially monoclonal antibodies, robustly grows in the recent two decades. Previous studies reveal that minor differences in glycan structures of recombinant glycoproteins may impact on the biological activities and pharmacokinetics of the glycoproteins. For example, Darbepoetin alfa is a hyper-glycosylated analog of recombinant human erythropoietin (EPO) with two extra N-linked glycosylation sites. The extra N-glycosylation increases the percentage of the molecular mass in carbohydrates and significantly extends the serum half-life of Darbepoetin alfa, as compared to endogenous and recombinant EPO. In addition, for a therapeutic antibody whose efficacy mainly relies on antibody-dependent cell cytotoxicity (ADCC), both chemo-enzymatic and genetic approaches to remove the core fucose residue on the Fc portion have been developed to increase the potency of the ADCC effect induced by that antibody.

However, currently available methods for remodeling glycosylation often require multiple enzymes and/or multiple steps, resulting in high costs for manufacturing glyco-engineered recombinant proteins.

SUMMARY

In one aspect, the present invention refers to a genetically engineered host animal cell, which expresses an exogenous fucosidase, and an exogenous endoglycosidase, wherein the exogenous fucosidase is a mammalian fucosidase or a bacterial fucosidase, and wherein the

engineered host animal cell produces glycoproteins having modified glycosylation. In another aspect, the present invention refers to a method for producing a defucosylated glycoprotein, comprising: providing a genetically engineered host animal cell expressing (a) a glycoprotein, and (b) an exogenous fucosidase, and an exogenous endoglycosidase; wherein the exogenous fucosidase is a mammalian fucosidase or a bacterial fucosidase; culturing the host animal cell under conditions allowing for producing the glycoprotein, the fucosidase, and the endoglycosidase; and collecting the host animal cell or the culture supernatant for isolating the glycoprotein. In still another aspect, the present invention refers to a method for preparing the genetically engineered host animal cell of the present invention, comprising introducing into an animal cell one or more expression vectors, which collectively encode the exogenous fucosidase, and the exogenous endoglycosidase. The present disclosure is based on the development of genetically engineered host animal cells capable of producing glycoproteins such as antibodies having modified glycosylation, including defucosylation and monoglycosylation. Such host animal cells were

engineered to overly express one or more of fucosidases, endoglycosidases, or both. Unexpectedly, changes to the cellular glycosylation machinery in the host animal cells did not result in adverse effects in relation to glycoprotein synthesis and host cell growth.

Accordingly, the present disclosure describes a genetically engineered host animal cell (*e.g.*, a mammalian cell), which overly expresses a fucosidase, an endoglycosidase, or both, wherein the host animal cell produces glycoproteins having modified glycosylation as compared with the wild-type couterpart. In some examples, the fucosidase can be a mammalian fucosidase or a bacterial fucosidase, for example, human FUCA1, human FUCA2, *Cricetulus griseus* fucosidase, alpha-L-1 *Chryseobacterium meningosepticum* α1,6-fucosidase, or bacterial fucosidase BF3242. Alternatively or in addition, the endoglycosidase can be an Endo S enzyme, *e.g.*, an enzyme comprising the amino acid sequence of SEQ ID NO:11. In some examples, the genetically engineered host animal cell expresses (i) human FUCA1, human FUCA2, *Cricetulus griseus* fucosidase, alpha-L-1 *Chryseobacterium meningosepticum* α1,6-fucosidase, or bacterial fucosidase BF3242, and (ii) an Endo S (such as SEQ ID NO:11).

The genetically engineered host animal cell described herein may further express a glycoprotein, which can be exogenous (not expressed in the native animal cell of the same type). Examples include, but are not limited to, an antibody, an Fc-fusion protein, a cytokine, a hormone, a growth factor, or an enzyme.

In some examples, the genetically engineered host animal cell is a mammalian cell, *e.g.*, a Chinese hamster ovary (CHO) cell, a rat myeloma cell, a baby hamster kidney (BHK) cell, a hybridoma cell, a Namalwa cell, an embryonic stem cell, or a fertilized egg.

Also described herein are methods for producing glycoproteins having modified glycosylation patterns (*e.g.*, defucosylated or mono-glycosylated) using any of the genetically engineered host animal cells described herein. The method may comprise (i) providing a host animal cell expressing (a) a glycoprotein, and (b) a fucosidase, an endoglycosidase, or both; culturing the host animal cell under conditions allowing for producing the glycoprotein and the fucosidase, the endoglycosidase, or both; (ii) collecting the host animal cell or the culturing supernatant for isolating the glycoprotein, and optionally (iii) isolating the glycoprotein. The method may further comprise (iv) analyzing the glycosylation pattern of

the glycoprotein.

Further, the present disclosure features a method for preparing any of the genetically engineered host animal cells described herein. The method may comprise (i) introducing into an animal cell one or more expression vectors, which collectively encode a fucosidase, an endoglycosidase, or both, and optionally (ii) introducing into the animal cell an expression vector encoding a glycoprotein. The method may further comprise selecting transformed cells expressing the fucosidase, the endoglycosidase, and the glycoprotein.

The details of one or more examples are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration showing the structures of various N-glycans. A: typical N-linked glycans of glycoproteins. B: defucosylated N-glycans. C: N-glycans having a mono-Nacetylglucosamine reducing sugar (monoglycosylated).

Figure 2 is a schematic illustration of an exemplary expression vector for producing a fucosidase. A: map of an exemplary plasmid that carries a fucosidase gene. B: an exemplary expression cassette for expressing a fucosidase or an endoglycosidase S.

Figure 3 includes diagrams showing the production of homogenous afucosylated mono-sugar (GlcNAc) antibody h4B12 by transient expression of the antibody in host cells engineered to express a fucosidase and/or an endoglycosidase. A: a schematic illustration of producing homogenous afucosylated mono-sugar (GlcNAc) antibodies. B: a chart showing the glycosylation of antibody 4B12 produced in host cells engineered to express human FUCA1, human FUCA2, *C. griseus* fucosidase, alpha-L-1, or *C. meningosepticum* α1,6-fucosidase, as determined by LC/MS/MS. C: a diagram showing the transient expression of a fucosidase or an endoglycosidase as indicated in CHO cells detected by Western blot. D: a chart showing antibody h4B12 produced in CHO cells expressing a fucodisase or an endoglycosidase as indicated, the antibody having homogenous fucose-free mono-sugar (GlcNAc) glycoform as determined by LC/MS/MS. A homogenous N-glycan refers to the

What is claimed is:

1. A genetically engineered host animal cell, which expresses an exogenous fucosidase, and an exogenous endoglycosidase, wherein the exogenous fucosidase is a mammalian fucosidase or a bacterial fucosidase, and wherein the engineered host animal cell produces glycoproteins having modified glycosylation.

2. The genetically engineered host animal cell of claim 1, wherein the endoglycosidase is an Endo S enzyme.

3. The genetically engineered host animal cell of claim 1 or 2, which further expresses a glycoprotein.

4. The genetically engineered host animal cell of claim 3, wherein the glycoprotein is exogenous.

5. The genetically engineered host animal cell of claim 3 or claim 4, wherein the glycoprotein is an antibody, an Fc-fusion protein, a cytokine, a hormone, a growth factor, or an enzyme.

6. The genetically engineered host animal cell of claim 5, wherein the glycoprotein is an antibody.

7. The genetically engineered host animal cell of any of claims 1-6, wherein the animal cell is a mammalian cell.

8. The genetically engineered host animal cell of claim 7, wherein the mammalian cell is selected from the group consisting of a Chinese hamster ovary (CHO) cell, a rat myeloma cell, a baby hamster kidney (BHK) cell, a hybridoma cell, and a Namalwa cell.

9. The genetically engineered host animal cell of any of claims 1-8, which expresses (i) human FUCA1, human FUCA2, *Cricetulus griseus* fucosidase, alpha-L-1 *Chryseobacterium meningosepticum* α1,6-fucosidase, or bacterial fucosidase BF3242, and (ii) an Endo S.

10. A method for producing a defucosylated glycoprotein, comprising:

providing a genetically engineered host animal cell expressing (a) a glycoprotein, and (b) an exogenous fucosidase, and an exogenous endoglycosidase; wherein the exogenous fucosidase is a mammalian fucosidase or a bacterial fucosidase;

culturing the host animal cell under conditions allowing for producing the glycoprotein, the fucosidase, and the endoglycosidase; and

collecting the host animal cell or the culture supernatant for isolating the glycoprotein.

11. The method of claim 10, wherein the endoglycosidase is an Endo S enzyme.

12. The method of claim 10 or 11, wherein the glycoprotein is exogenous.

13. The method of claim 12, wherein the glycoprotein is an antibody, an Fc-fusion protein, a cytokine, a hormone, a growth factor, or an enzyme.

14. The method of claim 13, wherein the glycoprotein is an antibody.

15. The method of any of claims 10-14, wherein the genetically engineered host animal cell is a mammalian cell.

16. The method of claim 15, wherein the mammalian cell is selected from the group consisting of a Chinese hamster ovary (CHO) cell, a rat myeloma cell, a baby hamster kidney (BHK) cell, a hybridoma cell, and a Namalwa cell.

17. The method of any of claims 10-16, further comprising isolating the glycoprotein.

18. The method of any of claims 10-17, further comprising analyzing the glycosylation pattern of the glycoprotein.

19. A method for preparing the genetically engineered host animal cell of claim 1, comprising introducing into an animal cell one or more expression vectors, which collectively encode the exogenous fucosidase, and the exogenous endoglycosidase.

20. The method of claim 19, further comprising introducing into the animal cell an expression vector encoding a glycoprotein.