

FUT8 KO hosts have growth issues and may require longer suspension adaptation time, increasing the CLD timelines with possible implications for lower titers (Malphettes *et al.*, *Biotechnol Bioeng*, 106, **2010**, 774-783).

[0019] Thus, there exists a need in the art for a cell line, and methods of culturing said cell line, capable of producing afucosylated and fucosylated forms of a protein at a desired ratio, and compositions comprising the protein thereof.

[0020] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY

[0021] The present application in some aspects refers to methods of producing a protein, wherein the protein is produced in fucosylated and afucosylated forms at a predetermined ratio, the methods comprising: culturing a host cell engineered to express the protein in a culture medium, wherein GDP-keto-6-deoxymannose-3,5-epimerase,4-reductase (FX) activity in the host cell is inactivated, wherein the host cell is a FX knockout host cell having FX knocked out in all alleles, and wherein the culture medium comprises a fucose source in an amount sufficient to produce the fucosylated and afucosylated forms of the protein at the predetermined ratio. In some examples, the predetermined ratio is about 50:1 to about 1:50 (including, for example, about 50:5.5, about 50:12.5, about 50:21.5, about 50:33.5, about 50:50, about 50:75, about 50:116.5, about 50:200, or about 50:450).

[0022] In some aspects, the present disclosure refers to methods of producing a protein having an enhanced ADCC function mediated both by NK cells and PMN cells, the methods comprising: culturing a host cell engineered to express the protein in a culture medium, wherein FX activity in the host cell is inactivated, wherein the host cell is a FX knockout host cell having FX knocked out in all alleles, and wherein the culture medium comprises a fucose source in an amount sufficient to produce fucosylated and afucosylated forms of the protein at a ratio that provides the enhanced ADCC function. In some examples, the ADCC function is determined by an ADCC assay. In some examples, the ADCC function is determined by a FC γ RIII binding assay.

[0023] In some aspects, the present disclosure refers to methods of adjusting a level of fucosylation of a protein produced by a host cell engineered to produce the protein, the methods comprising: (a) culturing the host cell in an initial culture medium, wherein

FX activity in the host cell is inactivated, wherein the host cell is a FX knockout host cell having FX knocked out in all alleles, and wherein the culture medium comprises a fucose source; (b) determining the level of fucosylation of the protein; and (c) adjusting the amount of the fucose source in the culture medium such that the protein is produced at a predetermined level of fucosylation. In some examples, determining the level of fucosylation of the protein comprises determining the fucosylated form of the protein. In some examples, determining the level of fucosylation of the protein comprises determining the afucosylated form of the protein. In some examples, adjusting the amount of the fucose source comprises increasing amount of the fucose source when the level of fucosylation is low. In some examples, adjusting the amount of the fucose source comprises decreasing amount of the fucose source when the level of fucosylation is high.

[0024] In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture medium free of the fucose source prior to culturing the host cell in the culture medium comprising the fucose source. In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture medium, wherein the fucose source is present in the culture medium at the beginning of the culturing step. In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture medium, wherein the fucose source is added to the culture medium during the culturing step. In some examples, the fucose source is added to the culture medium via bolus addition. In some examples, the fucose source is added to the culture medium via continuous feeding.

[0025] In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture medium, wherein the amount of the fucose source in the culture medium during the culturing step is between about 0.01 mM and about 1 mM.

[0026] In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture medium, wherein the culturing step is carried out at lower than about 37° C.

[0027] In some examples according to (or as applied to) any of the examples above, the method comprises culturing the host cell in a culture

medium, wherein the culture medium further comprises a glucose source or a mannose source.

[0028] In some examples according to (or as applied to) any of the examples above, the method further comprises isolating the protein from the cell culture.

[0029] In some examples according to (or as applied to) any of the examples above, the fucose source is a fucose. In some examples, the fucose is L-fucose. In some examples, the fucose is L-fucose-1-phosphate. In some examples according to (or as applied to) any of the examples above, the fucose source is GDP-fucose.

[0030] In some aspects, the present disclosure refers to methods of characterizing a protein in an afucosylated form, the methods comprising: comparing the biological activity of the afucosylated protein with a fucosylated form of the protein, wherein the afucosylated and fucosylated forms of the protein are produced by the same host cell engineered to produce the protein, and wherein FX activity in the host cell is inactivated, and wherein the host cell is a FX knockout host cell having FX knocked out in all alleles. In some examples, the afucosylated form of the protein is produced by the host cell in a first cell culture free of a fucose source. In some examples, the fucosylated form of the protein is produced by the host cell in a second cell culture comprising a fucose source.

[0031] In some examples, the method of characterizing a protein in an afucosylated form comprises comparing the biological activity *in vitro*. In some examples, the method of characterizing a protein in an afucosylated form comprises comparing the biological activity *in vivo*. In some examples, the ADCC function is determined by an ADCC assay. In some examples, the ADCC function is determined by a FCγRIII binding assay.

[0032] In some examples according to (or as applied to) any of the examples above, the fucosylated form of the protein is determined at a glycan structure level. In some examples, PNGase F is used to cleave a glycan structure from the protein. In some examples, the fucosylated form of the protein is determined at a glycan structure level, wherein the fucosylated form of the protein is determined by capillary electrophoresis (CE).

[0033] In some examples according to (or as applied to) any of the examples above, the fucosylated form of the protein is determined at a protein level. In some examples, the fucosylated form of the protein is determined at a

protein level, wherein the fucosylated form of the protein is determined by mass spectrometry (MS).

[0034] In some examples according to (or as applied to) any of the examples above, the protein is an Fc-containing protein. In some examples, the Fc-containing protein comprises an antibody heavy chain or a fragment thereof. In some examples, the Fc-containing protein is a full length antibody. In some examples, the full length antibody is a monoclonal antibody. In some examples, the Fc-containing protein is a Fc-containing fusion protein. In some examples, the Fc-containing fusion protein is an immunoadhesin.

[0035] In some examples according to (or as applied to) any of the examples above, the host cell is a eukaryotic cell. In some examples, the host cell is a mammalian cell. In some examples, the mammalian cell is a Chinese hamster ovary (CHO) cell.

[0036] In some examples according to (or as applied to) any of the examples above, the host cell comprises no more than 20% FX or GMD activity as compared to a host cell comprising a wild type FX or GMD gene, respectively, without inactivation.

[0037] In some examples according to (or as applied to) any of the examples above, the FX or GMD gene in the host cell is inactivated. In some examples, the FX or GMD gene is inactivated by siRNA. In some examples, the FX or GMD gene is inactivated by shRNA. In some examples, there is provided a method wherein the FX or GMD gene is inactivated by a sequence deletion. In some examples, there is provided a method wherein the FX or GMD gene is inactivated by a sequence addition or substitution. In some examples, the FX or GMD gene is inactivated using a clustered, regularly interspaced, short palindromic repeats (CRISPR) system. In some examples, the FX or GMD gene is inactivated using a transcription activator-like effector nuclease (TALEN) system. In some examples, the FX or GMD gene is inactivated using a zinc-finger nuclease (ZFN) system. In some examples, the FX or GMD gene is inactivated using a meganuclease system.

[0038] In some examples according to (or as applied to) any of the examples above, the host cell comprises substantially no FX activity.

[0039] In some examples according to (or as applied to) any of the examples above, the fucose source is a fucose. In some examples, the fucose

is L-fucose. In some examples, the fucose is L-fucose-1-phosphate. In some examples according to (or as applied to) any of the examples above, the fucose source is GDP-fucose.

[0040] In some examples, provided herein are compositions comprising the protein produced by any one of the methods described herein.

[0041] In some examples, there are provided compositions comprising a protein having an enhanced ADCC function mediated both by NK cells and PMN cells, wherein the composition comprise afucosylated and fucosylated forms of the protein at a ratio that provides the enhanced ADCC function.

[0042] In some examples, the composition comprises a fucosylated form of the protein comprising fucose at the reducing end of a glycan structure. In some examples, the composition is a pharmaceutical composition. In some examples, the composition is a cell culture medium. In some examples, the composition is a cell lysate. In some examples, the composition is a eluate from a protein purification column.

[0043] In some aspects, provided herein are cell cultures comprising: (a) a host cell engineered to express a protein, wherein FX activity in the host cell is inactivated, and wherein the host cell is a FX knockout host cell having FX knocked out in all alleles; and a culture medium comprising a fucose source at about 0.01mM to about 1 mM.

[0044] Also provided herein are methods of treating a disease in an individual in need thereof comprising: administering to the individual a pharmaceutical composition described herein. In one example, there is provided use of the composition described herein in the manufacture of a medicament for treating a disease in an individual in need thereof. In some examples, the composition is administered parenterally. In some examples, the composition is administered intravenously or subcutaneously. In some examples, the composition is administered locally. In some examples, the composition is administered topically.

[0045] These and other examples and advantages of the present disclosure will become apparent from the subsequent detailed description and the appended claims. It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0046] **FIG. 1** shows an overview of the fucosylation pathway including the de novo and salvage pathways for generating GDP-fucose. GDP-keto-6-deoxymannose-

CLAIMS

What is claimed is:

1. A method of producing a protein, wherein the protein is produced in fucosylated and afucosylated forms at a predetermined ratio, the method comprising:
 - culturing a host cell engineered to express the protein in a culture medium,
 - wherein GDP-keto-6-deoxymannose-3,5-epimerase,4-reductase (FX) activity in the host cell is inactivated,
 - wherein the host cell is a FX knockout host cell having FX knocked out in all alleles,
 - and
 - wherein the culture medium comprises a fucose source in an amount sufficient to produce the fucosylated and afucosylated forms of the protein at the predetermined ratio.
2. The method of claim 1, wherein the predetermined ratio is about 50:1 to about 1:50.
3. A method of producing a protein having an enhanced ADCC function mediated both by NK cells and PMN cells, the method comprising:
 - culturing a host cell engineered to express the protein in a culture medium,
 - wherein FX activity in the host cell is inactivated,
 - wherein the host cell is a FX knockout host cell having FX knocked out in all alleles,
 - and
 - wherein the culture medium comprises a fucose source in an amount sufficient to produce fucosylated and afucosylated forms of the protein at a ratio that provides the enhanced ADCC function.
4. The method of claim 3, wherein the ADCC function is determined by an ADCC assay or by a FC γ RIII binding assay.
5. A method of adjusting a level of fucosylation of a protein produced by a host cell engineered to produce the protein, the method comprising:
 - (a) culturing the host cell in an initial culture medium,
 - wherein FX activity in the host cell is inactivated,
 - wherein the host cell is a FX knockout host cell having FX knocked out in all alleles,
 - and
 - wherein the culture medium comprises a fucose source;
 - (b) determining the level of fucosylation of the protein; and
 - (c) adjusting the amount of the fucose source in the culture medium such that the protein is produced at a predetermined level of fucosylation.
6. The method of claim 5, wherein adjusting the amount of the fucose source comprises increasing amount of the fucose source when the level of fucosylation is low.

7. The method of claim 5 or 6, wherein adjusting the amount of the fucose source comprises decreasing amount of the fucose source when the level of fucosylation is high.
8. The method of any one of claims 1-7, wherein the method comprises culturing the host cell in a culture medium free of the fucose source prior to culturing the host cell in the culture medium comprising the fucose source.
9. The method of claim 8, wherein the fucose source is present in the culture medium at the beginning of the culturing step.
10. The method of any one of claims 1-9, wherein the amount of the fucose source in the culture medium during the culturing step is between about 0.01 mM and about 1 mM.
11. The method of any one of claims 1-10, wherein the culturing step is carried out at lower than about 37° C.
12. The method of any one of claims 1-11, wherein the fucose source is a fucose.
13. The method of any one of claims 1-12, wherein the fucose source is GDP-fucose.
14. The method of any one of claims 1-13, wherein the fucose source is L-fucose.
15. A method of characterizing a protein in an afucosylated form, the method comprising:
 - comparing the biological activity of the afucosylated protein with a fucosylated form of the protein,
 - wherein the afucosylated and fucosylated forms of the protein are produced by the same host cell engineered to produce the protein, and
 - wherein FX activity in the host cell is inactivated, and
 - wherein the host cell is a FX knockout host cell having FX knocked out in all alleles.
16. The method of any one of claims 1-15, wherein the fucosylated form of the protein is determined at a glycan structure level or at a protein level.
17. The method of any one of claims 1-16, wherein the protein is an Fc-containing protein.
18. The method of claim 17, wherein the Fc-containing protein is a full length antibody.
19. The method of any one of claims 1-18, wherein the host cell is a mammalian cell.
20. The method of claim 19, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

21. The method of any one of claims 1-20, wherein the FX gene in the host cell is inactivated by a sequence deletion or by a sequence addition or substitution.
22. The method of any one of claims 1-21, wherein the FX gene is inactivated using:
 - (a) a clustered, regularly interspaced, short palindromic repeats (CRISPR) system;
 - (b) a transcription activator-like effector nuclease (TALEN) system;
 - (c) a zinc-finger nuclease (ZFN) system; or
 - (d) a meganuclease system.
23. A cell culture comprising:
 - (a) a host cell engineered to express a protein,
wherein FX activity in the host cell is inactivated, and
wherein the host cell is a FX knockout host cell having FX knocked out in all alleles;
and
 - (b) a culture medium comprising a fucose source at about 0.01mM to about 1 mM.