terminal lysine presence has been described in U.S. Patent Application No. 20130280274. However, as demonstrated in U.S. Patent Application No. 20130280274, optimization of cell culture parameters does not eliminate the heterogeneity of Fc domain C-terminal lysine presence.

[0007] Fc domains of IgG1, IgG2, and IgG4 isotype immunoglobulins contain a conserved basic amino acid lysine at the C-terminus of each Fc domain. Heterogeneity of Fc domain C-terminal lysine presence of these immunoglobulin isotypes is believed to result from proteolysis by endogenous carboxypeptidase(s) during cell culture production. *See*, Harris, *J Chromatogr A*, 705, 1995; and Luo et al., *Biotechnol Bioeng*, 109, 2012. Carboxypeptidases are enzymes that hydrolytically cleave amino acids from the C-termini of proteins and peptides. There are 13 known members of carboxypeptidases found in most mammalian species. *See*, Reznik et al., *Cell Mol Life Sci*, 58, 2001. While different carboxypeptidase proteins and their activities have been detected in CHO cells, a commonly used production host cell, it is not known which carboxypeptidase(s) is responsible for the removal of the Fc domain C-terminal lysine. *See*, Dick et al., *Biotechnol Bioeng*, 100, 2008. It has previously been speculated that one or more carboxypeptidases, including CpB and CpM, may be involved in Fc domain C-terminal lysine processing. *See*, Dick et al., *Biotechnol Bioeng*, 100, 2008.; Harris, *J Chromatogr A*, 705, 1995; and Luo et al., *Biotechnol Bioeng*, 109, 2012.

[0008] 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

[0009] In one aspect, provided herein are host cells for expression of a Fc-containing protein, wherein the CpD gene in the host cell is inactivated, wherein the host cell has at least a 60% reduction of a carboxypeptidase D (CpD) expression level as compared to a host cell comprising a wild type CpD gene without CpD gene inactivation, and wherein the host cell comprises an expression vector comprising a nucleic acid encoding the Fc-containing protein.

[0010] 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 host cell is a Chinese hamster ovary (CHO) cell.

[0011] In some examples according to (or as applied to) any of the examples above, the Fc-containing protein is an antibody. In some examples, the antibody is a monoclonal antibody.

[0012] In some examples according to (or as applied to) any of the examples above, the Fc-containing protein is a Fc-containing fusion protein.

[0013] In some examples according to (or as applied to) any of the examples above, the CpD gene in the host cell is inactivated. In some examples, the CpD gene is inactivated by siRNA. In some examples, the CpD gene is inactivated by shRNA. In some examples, the CpD gene is inactivated by gene deletion. In some examples, the CpD gene is inactivated by gene addition or substitution. In some examples, the CpD gene is inactivated using a clustered, regularly interspaced, short palindromic repeats (CRISPR) system. In some examples, the CpD gene is inactivated using a transcription activator-like effector nuclease (TALEN) system. In some examples, the CpD gene is inactivated using a zinc-finger nuclease (ZFN) system. In some examples, the CpD gene is inactivated using a meganuclease system.

[0014] In some examples according to (or as applied to) any of the examples above, the host cell comprises an expression vector comprising a nucleic acid encoding the Fc-containing protein.

[0015] In one aspect, provided herein are cell culture systems comprising any of the host cells described above.

[0016] In one aspect, provided herein are methods of producing a host cell as described above, wherein the methods comprise inactivating the CpD gene in the host cell, thereby producing the host cell.

[0017] In some examples, the method of producing a host cell comprises inactivating the CpD gene using a siRNA system. In some examples, the method of producing a host cell comprises inactivating the CpD gene using a shRNA system. In some examples, the method of producing a host cell comprises inactivating the CpD gene by gene deletion. In some examples, the method of producing a host cell comprises inactivating the CpD gene by gene addition or substitution. In some examples, the method of producing a host cell comprises inactivating the CpD gene using a CRISPR system. In some examples, the method of producing a host cell comprises inactivating the CpD gene using a TALEN system. In some examples, the method of producing a host cell comprises inactivating the CpD

gene using a ZFN system. In some examples, the method of producing a host cell comprises inactivating the CpD gene using a meganuclease system.

[0018] In one aspect, provided herein are methods of making a Fc-containing protein comprising: a) culturing a host cell as described above, and b) obtaining the Fc-containing protein expressed by the host cell.

[0019] In one aspect, provided herein are pharmaceutical compositions comprising a plurality of Fc-containing proteins, wherein substantially all Fc-containing proteins in the composition have a C-terminal sequence of proline-glycine-lysine on each Fc domain. In some examples, substantially all Fc-containing proteins of the composition have the same amino acid sequence. In some examples, the plurality of Fc-containing proteins are substantially homogeneous in charge state. In some examples, the Fc-containing protein is a Fc-containing fusion protein. In some examples, the Fc-containing protein is an antibody. In some examples, the antibody is a human antibody. In some examples, the antibody is a humanized antibody. In some examples, the antibody comprises two heavy chains, and wherein each heavy chain comprises a C-terminal lysine. In some examples, the Fc-containing protein is conjugated to a drug. In some examples, the Fc-containing proteins in the composition comprise an IgG1 Fc domain. In some examples, the Fc-containing proteins in the composition comprise an IgG2 Fc domain. In some examples, the Fc-containing proteins in the composition comprise an IgG4 Fc domain.

[0020] In some examples according to (or as applied to) any of the examples above, the composition comprising a plurality of Fc-containing proteins is a pharmaceutical composition. In some examples, the pharmaceutical composition comprising a plurality of Fc-containing proteins is a sterile pharmaceutical composition.

[0021] In some examples according to (or as applied to) any of the examples above, the composition comprising a plurality of Fc-containing proteins is a cell culture medium.

[0022] In some examples according to (or as applied to) any of the examples above, the composition comprising a plurality of Fc-containing proteins is a cell lysate.

[0023] In some examples according to (or as applied to) any of the examples above, the composition comprising a plurality of Fc-containing proteins is an eluate from a protein purification column.

[0024] Provided herein are uses of the pharmaceutical composition as described above for the manufacture of a medicament for treating a disease in an individual in need thereof. Also provided herein are methods of treating a disease in an individual in need thereof comprising administering to the individual a pharmaceutical composition or a sterile pharmaceutical composition described in the examples above. In some examples, the method of treating a disease in an individual in need thereof comprises administering the composition parenterally. In some examples, the method of treating a disease in an individual in need thereof comprises administering the composition intravenously or subcutaneously. In some examples, the method of treating a disease in an individual in need thereof comprises administering the composition locally. In some examples, the method of treating a disease in an individual in need thereof comprises administering the composition topically.

[0025] These and other aspects 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 invention.

BRIEF DESCRIPTION OF THE FIGURES

[0026] **FIGS. 1A-1B** shows qPCR mRNA analysis of endogenous carboxypeptidases in cell lines. Each error bar denotes 1 standard deviation. **FIG. 1A** shows relative carboxypeptidase B (CpB), carboxypeptidase E (CpE), carboxypeptidase M (CpM), carboxypeptidase N1 (CpN1), and carboxypeptidase D (CpD) mRNA expression levels in DP12 and CHOK1 host cells. Expression levels of mRNA for each carboxypeptidase were normalized to the housekeeping gene β-2-microglobulin (b2m). White bars represent measurements from the DP12 host and black bars represent measurements from the CHOK1 host. **FIG. 1B** shows relative carboxypeptidase mRNA expression levels in antibody-expressing cell lines. Expression level of mRNA for each carboxypeptidase was normalized to the house keeping gene b2m. White bars represent measurements from the antibody-expressing cell line A and black bars represent measurements from the antibody-expressing cell line B.

[0027] **FIGS. 2A-2B** shows the level of carboxypeptidase mRNA expression following transfection with a carboxypeptidase specific siRNA construct in antibody-expressing cell line A (white bars) and antibody-expressing cell line B (black bars). Each error bar denotes 1 standard deviation. **FIG. 2A** shows relative CpD mRNA expression levels in antibody-expressing cell line A and antibody-expressing cell line

CLAIMS

What is claimed is:

1. A host cell for expression of a Fc-containing protein, wherein the CpD gene in the host cell is inactivated, wherein the host cell has at least a 60% reduction of a carboxypeptidase D (CpD) expression level as compared to a host cell comprising a wild type CpD gene without CpD gene inactivation, and wherein the host cell comprises an expression vector comprising a nucleic acid encoding the Fc-containing protein.

2. The host cell of claim 1, wherein the host cell is a eukaryotic cell.

3. The host cell of claim 1 or 2, wherein the host cell is a Chinese hamster ovary (CHO) cell.

4. The host cell of any one of claims 1-3, wherein the Fc-containing protein is an antibody or a Fc-containing fusion protein.

5. The host cell of any one of claims 1-4, wherein the CpD gene is inactivated by siRNA or shRNA.

6. The host cell of any one of claims 1-4, wherein the CpD gene is inactivated by gene deletion, addition, or substitution.

7. The host cell of claim 6, wherein the CpD gene is inactivated using a clustered, regularly interspaced, short palindromic repeats (CRISPR) system, a transcription activator-like effector nuclease (TALEN) system, a zinc-finger nuclease (ZFN) system, or a meganuclease system.

8. A cell culture system comprising the host cell of any one of claims 1-7.

9. A method of producing a host cell of any one of claims 1-7, wherein the method comprises inactivating the CpD gene in the host cell, thereby producing the host cell.

10. The method of claim 9, wherein the CpD gene is inactivated using a siRNA system or a shRNA system.

11. The method of claim 9, wherein the CpD gene is inactivated by gene deletion, addition, or substitution.

12. The method of claim 11, wherein the CpD gene is inactivated using a CRISPR system, a transcription activator-like effector nuclease (TALEN) system, a zinc-finger nuclease (ZFN) system, or a meganuclease system.

13. A method of making a Fc-containing protein comprising:

    a) culturing the host cell of any one of claims 1-7; and

    b) obtaining the Fc-containing protein expressed by the host cell.

14. A pharmaceutical composition comprising a plurality of Fc-containing proteins, wherein substantially all Fc-containing proteins in the composition have a C-terminal sequence of proline-glycine-lysine on each Fc domain.

15. The composition of claim 14, wherein substantially all Fc-containing proteins of the composition have the same amino acid sequence.

16. The composition of claim 14 or claim 15, wherein the plurality of Fc-containing proteins are substantially homogeneous in charge state.

17. The composition of any one of claims 14-16, wherein the Fc-containing protein is a Fc-containing fusion protein or an antibody.

18. The composition of any one of claims 14-17, wherein the antibody comprises two heavy chains, and wherein each heavy chain comprises a C-terminal lysine.

19. The composition of any one of claims 14-18, wherein the Fc-containing protein is conjugated to a drug.

20. The composition of any one of claims 14-19, wherein Fc-containing proteins in the composition comprise an IgG1 Fc domain, an IgG2 Fc domain, or an IgG4 Fc domain.