retroviral infections, there remains a need in the art for RVLP-reduced CHO cells suitable to facilitate the commercial production of recombinant proteins.

SUMMARY

The presently disclosed subject matter relates, in certain examples, to modified CHO cells where, prior to the modification, the CHO cells comprise two or more ERV loci selected from:

(a) CHERV-1b (Genbank Accession No. MN527960, SEQ ID NO. 8);

(b) CHERV-2g (Genbank Accession No. MN527961, SEQ ID NO. 9);

(c) ETC109F (30021-39247 of SEQ ID NO. 10);

(d) CHERV-3g (Genbank Accession No. MN527962, SEQ ID NO. 11); and

(e) an ERV locus comprising a sequence having at least 90% identity to any one of (a)-(d),

where the modification comprises inactivation of two or more of the ERV loci of (a)-(e) present in the CHO cell prior to such modification. In one aspect, the disclosure provides a modified Chinese Hamster Ovary (CHO) cell prior to the modification, the CHO cell comprises two or more endogenous retrovirus (ERV) loci selected from: (a) CHERV-1b (Genbank Accession No. MN527960, SEQ ID NO. 8); (b) CHERV-2g (Genbank Accession No. MN527961, SEQ ID NO. 9); (c) ETC109F (30021-39247 of SEQ ID NO. 10); and (d) CHERV-3g (Genbank Accession No. MN527962, SEQ ID NO. 11); where the modification comprises inactivation of two or more of the ERV loci of (a)-(d). In certain examples, inactivation of the two or more of the ERV loci of (a)-(e) comprises a knock-out of the respective ERV GAG coding sequence. For example, but not by way of limitation, the knock-out of the respective ERV GAG coding sequence can comprise introduction of an indel into the ERV GAG coding sequence. In certain non-limiting examples, the knock-out of the respective ERV GAG coding sequence can comprise introduction of a base edit into the ERV GAG coding sequence. In certain non-limiting examples, the knock-out of the respective ERV GAG coding sequence can comprise introduction of a deletion flanking the ERV GAG coding sequence.

In certain examples, the presently disclosed subject matter is directed to modified CHO cells where, prior to the modification, the CHO cells comprise two or more ERV loci selected from:

(a) CHERV-1b (Genbank Accession No. MN527960, SEQ ID NO. 8);

(b) CHERV-2g (Genbank Accession No. MN527961, SEQ ID NO. 9);

(c) ETC109F (30021-39247 of SEQ ID NO. SEQ ID NO. 10);

(d) CHERV-3g (Genbank Accession No. MN527962, SEQ ID NO. 11); and

(e) an ERV locus comprising a sequence having at least 90% identity to any one of (a)-(d),

where the modification comprises inactivation of two or more of the ERV loci of (a)-(e) present in the CHO cell prior to such modification, and wherein the modified cell

expresses a recombinant product of interest. In certain examples, the modified cell is generated from a recombinant cell that expresses a recombinant product of interest. In certain examples, the recombinant product of interest comprises a recombinant protein. In certain examples, the recombinant protein is antibody or an antigen-binding fragment thereof. In certain examples, the antibody is a multispecific antibody or an antigen-binding fragment thereof. In certain examples, the antibody consists of a single heavy chain sequence and a single light chain sequence or antigen-binding fragments thereof. In certain examples, the antibody is a chimeric antibody, a human antibody or a humanized antibody. In certain examples, the antibody is a monoclonal antibody.

[0001] In certain examples, the recombinant product of interest expressed by a modified CHO cell of the present disclosure is encoded by an exogenous nucleic acid sequence integrated at one or more targeted locations in the cellular genome of the modified CHO cell. In certain examples, the targeted location is at least about 90% homologous to a sequence of a portion of the contig sequence of one of the contigs NW_006874047.1, NW_006884592.1, NW_006881296.1, NW_003616412.1, NW_003615063.1, NW_006882936.1, and NW_003615411.1, or to a sequence selected from SEQ ID Nos. 1-7.

[0002] In certain examples, the modified CHO cell of the present disclosure comprises gene knock-outs selected from: a) BAX; BAK; ICAM-1; GGTA1; CMAH; LPL; LPLA2; and PPT1; b) BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPL, LPLA2; and PPT1; c) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL; LPLA2; and PPT1; d) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPL, LPLA2; PPT1; and LIPA; e) BAX; BAK; ICAM-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA; f) BAX; BAK; ICAM-1; SIRT-1; GGTA1; CMAH; LPLA2; PPT1; and LIPA; g) BAX; BAK; ICAM-1; SIRT-1; MYC; GGTA1; CMAH; LPLA2; PPT1; and LIPA; h) BAX; BAK; ICAM-1; LPL; LPLA2; and PPT1; i) BAX; BAK; ICAM-1; SIRT-1; LPL, LPLA2; and PPT1; j) BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; and PPT1; k) BAX; BAK; ICAM-1; SIRT-1; MYC; LPL; LPLA2; PPT1; and LIPA; l) BAX; BAK; ICAM-1; SIRT-1; and MYC; m) BAX; BAK; ICAM-1; PERK; SIRT-1; and MYC; n) BAX; BAK; ICAM-1; SIRT-1; PERK; MYC; GGTA1; CMAH; LPL, LPLA2; PPT1; and LIPA; o) BAX; BAK; LPL; LPLA2; GGTA1; and CMAH; p) BAX; BAK; PERK; LPL; LPLA2; GGTA1; and CMAH; q) BAX; BAK; MYC; LPL; LPLA2; GGTA1; and CMAH; r) BAX; BAK; MYC; PERK; LPL; LPLA2; GGTA1; and CMAH; s) BAX; BAK; LPL; LPLA2; GGTA1; CMAH; and PPT1; t) BAX; BAK; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1; u) BAX; BAK; MYC;

MYC; PERK; LPL; LPLA2; GGTA1; CMAH; and PPT1; qqqq) BAX; BAK; BCKDHA; BCKDHB; ICAM-1; and SIRT-1; or rrrr) BAX; BAK; BCKDHA; BCKDHB; and ICAM-1.

[0003] In certain examples, the present disclosure is directed to compositions comprising a modified CHO cell as disclosed herein.

[0004] In certain examples, the present disclosure is directed to methods of producing a recombinant product of interest comprising:

a) culturing a modified CHO cell of the present disclosure; and

b) recovering the recombinant product of interest from the cultivation medium or the modified CHO cells.

In one aspect, the disclosure provides a method for producing recombinant product of interest, comprising: (a) culturing a CHO cell as disclosed herein under conditions resulting in the expression of the recombinant product of interest, and (b) recovering the recombinant product of interest from the cultivation medium or the modified CHO cells. In certain examples, the methods of producing a recombinant product of interest of the present disclosure comprise purifying the recombinant product of interest, harvesting the recombinant product of interest, and/or formulating the recombinant product of interest.

In certain examples, the present disclosure is directed to methods of producing a modified CHO cell, comprising:

(a) contacting the cell with a nuclease-assisted gene targeting system and/or nucleic acid targeting at least two endogenous ERVs selected from

(i) CHERV-1b (Genbank Accession No. MN527960, SEQ ID NO. 8);

(ii) CHERV-2g (Genbank Accession No. MN527961, SEQ ID NO. 9);

(iii) ETC109F (30021-39247 of SEQ ID NO. 10);

(iv) CHERV-3g (Genbank Accession No. MN527962, SEQ ID NO. 11); and

(v) an ERV locus comprising a sequence having at least 90% identity to any one of (i)-(iv), and

(b) selecting the modified CHO cell wherein the expression of said ERVs has been reduced or eliminated as compared to an unmodified CHO cell.

In one aspect, the disclosure provides a method for producing a modified CHO cell, comprising: (a) contacting the cell with a nuclease-assisted gene targeting system and/or nucleic acid targeting at least two endogenous ERVs selected from (i) CHERV-1b (Genbank Accession No. MN527960, SEQ ID NO. 8); (ii) CHERV-2g (Genbank Accession No. MN527961, SEQ ID NO. 9); (iii) ETC109F (30021-39247 of SEQ ID NO. 10); and (iv) CHERV-3g (Genbank Accession No. MN527962, SEQ ID NO. 11); (b) selecting the modified CHO cell wherein the expression of said ERVs has been reduced or eliminated as compared to an unmodified CHO cell. In certain examples, the methods of producing a modified CHO cell of the present disclosure comprise introducing an exogenous nucleic acid encoding a recombinant product of interest is into the CHO cell after the modification where the expression of said ERVs is reduced or eliminated as compared to an unmodified CHO cell. In certain examples, the methods of producing a modified CHO cell of the present disclosure comprise introducing an exogenous nucleic acid encoding a recombinant product of interest is into the CHO cell prior to the modification where the expression of said ERVs is reduced or eliminated as compared to an unmodified CHO cell.

In certain examples, the exogenous nucleic acid encoding a recombinant product of interest introduced into the modified cell of the present disclosure, either prior to or after said modification, is integrated in the cellular genome of the CHO cell at one or more targeted locations. In certain examples, the targeted location is at least about 90% homologous to a sequence of a portion of the contig sequence of one of the contigs NW_006874047.1, NW_006884592.1, NW_006881296.1, NW_003616412.1, NW_003615063.1, NW_006882936.1, and NW_003615411.1 or to a sequence selected from SEQ ID Nos. 1-7.

In certain examples, the exogenous nucleic acid encoding a recombinant product of interest introduced into the modified cell of the present disclosure, either prior to or after said modification, is randomly integrated in the cellular genome of the modified CHO cells.

In certain examples, the recombinant product of interest encoded by the exogenous nucleic acid encoding introduced into the modified cell of the present disclosure, either prior to or after said modification, comprises a recombinant protein. In certain examples, the recombinant protein is an antibody or an antigen-binding fragment thereof. In certain examples, antibody is a multispecific antibody or an antigen-binding fragment thereof. In certain examples, the antibody consists of a single heavy chain sequence and a single light chain sequence or antigen-binding fragments thereof. In certain examples, the antibody is a chimeric antibody, a human antibody or a humanized antibody. In certain examples, the antibody is a monoclonal antibody.

In certain examples, the nucleic acid sequence encoding the recombinant product of interest introduced into the modified cell of the present disclosure, either prior to or after said modification, is introduced using a transposase-mediated gene integration system.

In certain examples, the nuclease-assisted gene targeting system employed for the reduction of ERV expression in the modified CHO cells of the present disclosure is selected from the group consisting of CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease, TALEN or meganuclease.

In certain examples, the reduction of ERV expression in the modified CHO cells of the present disclosure is mediated by RNA silencing. In certain examples, the RNA silencing is selected from the group consisting of siRNA gene targeting and knock-down, shRNA gene targeting and knock-down, and miRNA gene targeting and knock-down.

In certain examples, the methods of producing a modified CHO cell of the present disclosure comprise use of a CHO cells comprising gene knock-outs selected from: