Improved methods are needed for producing such antibodies in cells, including antibodies and their fragments that specifically recognize and bind intracellular targets in mammalian cells.

SUMMARY OF ILLUSTRATIVE EMBODIMENTS

[0004a] In one aspect, provided herein is a method of recombinant production of an antibody, the method comprising: (a) expressing a nucleic acid encoding an antibody in a modified cell line, wherein the antibody specifically binds to a target epitope on a target protein that is natively expressed as an intracellular protein in the cell; and wherein the modified cell is engineered to express a variant target protein that comprises a mutation of one or more amino acid residues of the target epitope; and (b) culturing the modified cell line under conditions so that the antibody is produced.

[0004b] In another aspect, provided herein is a method of recombinant production of an antibody, the method comprising: (a) expressing a nucleic acid encoding an antibody in a modified mammalian cell line, wherein the antibody specifically binds a target epitope on a target protein that is natively expressed as an intracellular protein in the mammalian cell line and wherein the modified mammalian cell line is engineered to express a variant target protein that comprises a mutation of one or more amino acid residues of the target epitope; and (b) culturing the modified mammalian cell line under conditions so that the antibody is produced.

[0004c] In another aspect, provided herein is a method of recombinant production of an antibody targeting an epitope on a target protein found intracellularly in a cell line, the method comprising: (a) providing an antibody that binds to an epitope on a target protein that is present intracellularly in a cell line; (b) identifying the epitope of the target protein to which the antibody binds; (c) generating a modified cell line by mutating the epitope of the target protein in the cell line to reduce or abrogate the binding of the antibody to the epitope on the target protein; (d) introducing an expression vector encoding the antibody to the modified cell line; (e) culturing the modified cell line under conditions that allow production of the antibody from the expression vector; and (f) expressing a nucleic acid encoding an antibody in the modified cell line.

[0004d] In another aspect, provided herein is a method of recombinant production of an antibody targeting an epitope on a target protein found intracellularly in a mammalian cell line,

the method comprising: (a) providing an antibody that binds to an epitope on a target protein that is present intracellularly in a mammalian cell line; (b) identifying the epitope of the target protein to which the antibody binds; (c) generating a modified cell line by mutating the epitope of the target protein in the mammalian cell line to reduce or abrogate the binding of the antibody to the epitope on the target protein; (d) introducing an expression vector encoding the antibody to the modified mammalian cell line; (e) culturing the modified mammalian cell line under conditions that allow production of the antibody from the expression vector; and (f) expressing a nucleic acid encoding an antibody in the modified mammalian cell line.

[0004e] In another aspect, provided herein is a method of recombinant production of an antibody targeting GRP78, the method comprising: (a) expressing a nucleic acid encoding an antibody in a modified mammalian cell line, wherein the antibody binds to GRP78 and wherein the modified mammalian cell is engineered with a variant GRP78 that has a mutant epitope comprising a mutation of one or more amino acid residues of the target epitope of the antibody or antigen binding fragment; and (b) culturing the modified mammalian cell line under conditions so that the anti-GRP78 antibody is produced in the culture supernatant.

[0004f] In another aspect, provided herein is a method of recombinant production of an antibody, the method comprising: (a) expressing a nucleic acid encoding an antibody in a modified mammalian cell line, wherein the antibody specifically binds a target epitope on an intracellular signaling protein that is natively expressed in the mammalian cell line and wherein the modified mammalian cell line is engineered to express a variant intracellular signaling protein that comprises a mutation of one or more amino acid residues of the target epitope; and (b) culturing the modified mammalian cell line under conditions so that the antibody is produced.

[0005] The foregoing general description of the illustrative implementations and the following detailed description thereof are merely exemplary aspects of the teachings of this disclosure, and are not restrictive.

[0006] The present disclosure provides, in selected embodiments, methods and cell lines for the manufacture of antibodies that bind to intracellular target proteins. The present disclosure also provides methods of modifying cell lines for the manufacture of antibodies that bind to intracellular target proteins.

[0007] In some embodiments, the disclosure provides methods of recombinant production of an antibody comprising expressing a nucleic acid encoding an antibody in a modified cell line, wherein the antibody specifically binds to a target epitope on a target protein that is natively expressed as an intracellular protein in the cells and wherein the modified cell is engineered to express a variant target protein that comprises an alteration of one or more amino acid residues of the target epitope, and culturing the modified cell line under conditions so that the antibody is produced.

[0008] In some embodiments, the disclosure provides methods of recombinant production of an antibody comprising expressing a nucleic acid encoding an antibody in a modified mammalian cell line, wherein the antibody specifically binds a target epitope on a target protein that is natively expressed as an intracellular protein in the mammalian cell and wherein the modified mammalian cell is engineered to express with a variant target protein that comprises an alteration of one or more amino acid residues of the target epitope, and culturing the modified mammalian cell line under conditions so that the antibody is produced.

[0009] In some embodiments, the target protein that is natively expressed is an intracellular protein in the secretory pathway of the cell line. In some embodiments, the target protein is a stress protein.