Protease Deficient Cho Cell Lines To Prevent Proteolysis of Recombinant Proteins

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BACKGROUND

Chinese hamster ovary (CHO) cells are a family of immortalized cells derived from the epithelial cells of the ovary of the Chinese hamster. They are among the most widely used cell systems in cell biology. The cell line is frequently used in biological and medical research. It's specifically used in the production of recombinant therapeutic proteins. Essentially, the CHO cell is used as a "factory cell," meaning it can be programmed to produce therapeutic proteins, including vaccines and antibodies.

Recombinant protein expression has been used in the development of biologics for many applications including therapeutics and vaccines. However, one challenge in the development of biologics is the unintended proteolysis associated of expressed recombinant proteins in CHO cells. One example of a biologic that faces supoptimal expression in CHO cells is the HIV envelope glycoprotein, gp120, which is one of the main targets for neutralizing antibodies in HIV infection and a prime candidate for component of an HIV vaccine.

When expressed in CHO cells, gp120 undergoes proteolytic clipping by a serine protease at an epitope recognized by neutralizing antibodies. Essentially, the cells produce an enzyme that cuts gp120 into pieces. This proteolysis alters gp120 to the point where it is unrecognizable by the immune system and renders it non-immunogenic.

This issue appears frequently with CHO expression of envelope proteins from clade B HIV. Clade B is the most common genetic subtype present in the US and Europe. While this issue has been observed and attributed to CHO cells, it was previously unknown which enzyme was responsible. Without knowledge of the enzyme responsible, the solutions were based on modifying the protein sequence or adjusting the conditions of production, both of which are suboptimal.

TECHNOLOGY DESCRIPTION

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OTHER INFORMATION

KEYWORDS

CHO, protease deficient cell line, gp120, HIV, HIV vaccine, glycosylation, Viral proteins

CATEGORIZED AS

- Medical
  - Vaccines

RELATED CASES

2018-901-0

Researchers in Phil Berman's group at UC Santa Cruz have identified complement Component 1s (C1s) as the enzyme responsible for gp120 cleavage in CHO cells. C1s is an enzyme in the immune system that is responsible for cutting proteins at specific places to combat bacteria and viruses. Problematically, CHO cells also produce C1s that, in this case, proteolyzed the gp120 product.

The team used CRISPR/Cas9 gene editing to create C1s-deficient CHO cell lines for gp120 expression. These new cells no longer make the C1s enzyme and thus, proteolysis does not occur. Recombinant gp120 expressed in these cells remain intact and antigenically correct, preserving the structure required for recognition by the neutralizing antibodies. The C1s-deficient CHO cells grew just as well as unaltered cells and produced similar yields. This is notable as the removal of C1s did not harm the productivity or viability of the cell line, making it suitable for large-scale manufacturing.

They also removed another gene, MGAT1, which is responsible for the addition of glycans to proteins, called glycosylation. In HIV gp120, the glycan mannose-5 (Man5) is crucial for the recognition of gp120 by the broadly neutralizing monoclonal antibodies (bN-mAbs). These antibody targets are usually obfoscated by MGAT1 and thus, the MGAT1-deficient CHO cells, with simplified sugars, expose the antibody targets. These cells make a fully intact, properly glycosylated gp120 for use in a potential HIV vaccine.

APPLICATIONS

- Production of clade B HIV gp120 proteins
- General biomanufacturing using this more controlled CHO cell line
- Can improve yields and quality of biologics

ADVANTAGES

- Eliminates clipping of gp120
- Exposes neutralizing antibody targets
- No change in recombinant protein expresssion
- Reduces complexity in downstream purification

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
Switzerland	Issued Patent	3866860	01/08/2025	2018-901
Germany	Issued Patent	3866860	01/08/2025	2018-901
France	Issued Patent	3866860	01/08/2025	2018-901
United Kingdom	Issued Patent	3866860	01/08/2025	2018-901
United States Of America	Published Application	2021-031716	10/14/2021	2018-901
European Patent Office	Published Application	3866860	08/25/2021	2018-901

RELATED MATERIALS

- Identification and CRISPR/Cas9 Inactivation of the C1s Protease Responsible for Proteolysis of Recombinant Proteins Produced in CHO Cells - 05/14/2019

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