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Genome engineering of CHO cells by multiplex and sequential CRISPR modification: from apoptosis resistance to the production of afucosylated antibodies

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A method for generation of Chinese hamster ovary (CHO) cells with multiple gene knockouts and overexpressions using a minimal number of editing and cloning cycles will enable the creation of platforms for producing therapeutic protein producers with high specific productivity, extended cultivation time, and controlled glycoform composition of target proteins. The development of new cell lines involves the knockout of genes regulating various programmed cell death pathways, stress response, post-translational modifications, and other regulatory and metabolic cellular processes. The practical efficiency of simultaneous multiplex genome editing combined with the introduction of additional copies of housekeeping genes into the CHO genome remains insufficiently studied, but potentially opens the way to engineering cells with radically altered properties for bioreactor cultivation.

As a result of two rounds of CRISPR editing based on the CHO S line, a derivative line CHO 4BGD was obtained with knockouts of the BAK1, BAX, DHFR, and GLUL (GS) genes and overexpression of Bcl-2 and Beclin-1 proteins. This clonal line is characterized by high cell density during prolonged cultivation (up to 20 days), resistance to apoptosis induction, enhanced macroautophagy, and the ability to maintain multistage amplification of plasmids carrying target proteins genes [1]. It was confirmed that derivative producer lines efficiently secrete biosimilar antibodies, Fc-fusion proteins and hormones [2] while retaining these cellular properties.

To generate producers of afucosylated antibodies, the CHO 4BGD line was further modified by knockout of the FUT8 gene using a pair of guide RNAs and selection with lentil lectin. The resulting clones retained key metabolic properties and demonstrated suitability for producing afucosylated glycoproteins. The CHO 4BGD line has been deposited in a public microorganism collection and is freely distributed.

References

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