The solution to the above technical problem is achieved by the embodiments characterized in the claims.

In particular, disclosed herein is an animal cell line, preferably an insect, avian, or mammalian cell line, more preferably a mammalian, in particular human, cell line that is genetically modified to overexpress a β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1). In one aspect, there is provided a cell line that is genetically modified to overexpress a β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1), which is further genetically modified to overexpress a β-galactoside α-2,3-sialyltransferase 4 (ST3Gal4).

Another aspect relates to a recombinant glycoprotein having GalNAc O-glycans that are sialylated to a degree of at least 80%, wherein said glycoprotein is produced in a cell line as disclosed herein.

Another aspect relates to a method for the expression of recombinant glycoproteins having GalNAc O-glycans that are sialylated to a degree of at least 80%, comprising the step of overexpressing with the recombinant glycoproteins a β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1) and a β-galactoside α-2,3-sialyltransferase 4 (ST3Gal4).

The term "cell line genetically modified to overexpress a ST3Gal1" as used herein, also in the context of other transferases hereinafter, indicates that upon genetic modification, the individual cells of the cell line display a higher activity of the protein, *e.g.* the sialyltransferase, than they did before the genetic modification.

Genetic modifications that allow the overexpression of a given protein are not particularly limited and are known in the art. In a particular example, the cell line comprises an endogenous gene encoding a ST3Gal 1, such as e.g. human cell lines. In such cases, the cells can be genetically modified by inserting a promoter, enhancing element, and/or stabilizing element into the genome of the cells in a

position suitable to cause overexpression of said nucleic acid. This can be done by homologous recombination using TALENS, Zn-finger proteins, CRISPR-CAS9, or other methods known in the art. Thus, in preferred embodiments, the cell line comprises an endogenous gene encoding ST3Gal1 and optionally endogenous genes encoding ST3Gal4 and/or ST6Gal1, and further has at least one genetic element, selected from the group consisting of a promoter, an enhancing element, and a stabilizing element inserted into the genome in one or more position(s) suitable to cause overexpression of ST3Gal1 and optionally ST3Gal4 and/or ST6Gal1. Suitable promoters, enhancing elements and stabilizing elements are not particularly limited and are known in the art. For example, promoters include constitutive promoters, *e.g.* a CMV, EF1alpha, SV40, RSV, UbC, CAG, BOS or PGK promoter, and inducible promoters, *e.g.* tetracycline inducible promoters or other inducible promoters known in the art. Further, enhancing elements (enhancers) include CMV enhancer, ß-globin enhancer, immunoglobulin enhancer, and PGK-enhancer. Furthermore, stabilizing elements (chromatin elements) include matrix

Claims

1. A cell line that is genetically modified to overexpress a β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1), which is further genetically modified to overexpress a β-galactoside α-2,3-sialyltransferase 4 (ST3Gal4).

2. The cell line according to claim 1, which is further genetically modified to overexpress a β-galactoside α-2,6-sialyltransferase 1 (ST6Gal1).

3. The cell line according to claim 1 or claim 2, wherein the cell line comprises endogenous genes encoding ST3Gal1 and ST3Gal4, and optionally an endogenous gene encoding ST6Gal1, and further has at least one genetic element, selected from the group consisting of a promoter, an enhancing element, and a stabilizing element inserted into the genome in one or more position(s) suitable to cause overexpression of ST3Gal1 and ST4Gal4, and optionally ST6Gal1.

4. The cell line according to any one of claims 1 to 3, wherein the cell line comprises exogenous nucleic acids encoding ST3Gal1 and ST3Gal4, and optionally an exogenous nucleic acid encoding ST6Gal1.

5. The cell line according to any one of claims 1 to 4, wherein the cell line is a mammalian cell line, preferably a human cell line.

6. The cell line according to any one of claims 1 to 4, wherein the cell line is derived from a cell line, selected from the group consisting of AGE.CR® cells, Vero cells, MDCK cells, BHK cells, CHO cells, HEK293 cells, HepG2 cells, Huh7 cells, AGE1.HN® cells, NC5T11 cells, Per.C6 cells, HMCLs cells, MM.1 cells, U266 cells, RPMI18226 cells, HKB11 cells, NM cells, NM-F9 cells, and CAP cells.

7. The cell line according to any one of claims 1 to 4, wherein the cell line is derived from human primary amniocytes comprising at least one nucleic acid encoding the gene products of the adenoviral E1 and pIX regions.

8. A recombinant glycoprotein having GalNAc O-glycans that are sialylated to a degree of at least 80%, wherein said glycoprotein is produced in a cell line according to any one of claims 1 to 7.

9. The recombinant glycoprotein according to claim 8 having GalNAc O-glycans that are sialylated to a degree of at least 95%.

10. The recombinant glycoprotein according to claim 8 or claim 9, wherein at least 80% of the GalNAc O-glycans are core 1 GalNAc O-glycans.

11. The recombinant glycoprotein according to claim 10, wherein the core 1 GalNAc O-glycans are disialylated to a degree of at least 25%.

12. The recombinant glycoprotein according to any one of claims 8 to 11, wherein the glycoprotein is selected from the group consisting of growth factors,

peptide hormones, cytokines, enzymes, antibodies, antibody fragments, blood clotting factors, and protease inhibitors.

13. The recombinant glycoprotein according to any one of claims 8 to 12, wherein the glycoprotein is selected from the group consisting of hepatocyte growth factor (HGF), erythropoietin (EPO), Factor VIII (FVIII), Factor IX (FIX), von-Willebrand-Factor (vWF), and C1 esterase inhibitor (C1-inhibitor; C1 Inh).

14. The recombinant glycoprotein according to any one of claims 8 to 13, wherein the glycoprotein is a human glycoprotein.

15. A method for the expression of recombinant glycoproteins having GalNAc O-glycans that are sialylated to a degree of at least 80%, comprising the step of overexpressing with the recombinant glycoproteins a β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1) and a β-galactoside α-2,3-sialyltransferase 4 (ST3Gal4).

16. The method of claim 15, further comprising the step of overexpressing with the recombinant glycoproteins a β-galactoside α-2,6-sialyltransferase 1 (ST6Gal1).