

past, CRISPR/Cas9 recently has been established for versatile and simple targeting of genomic sequences for knockout purposes. For example, miRNA-744 was targeted in CHO cells using CRISPR/Cas9 by using multiple gRNA enabling sequence excision (Raab, N., et al., Biotechnol. J. (2019) 1800477).

5 CN 109 161 545 discloses a microRNA for inhibiting expression of SIRT-1 of chicken, and also discloses a recombinant over-expression plasmid and specific application of the microRNA, and an LMH cell line for constructing stable low-expression SIRT-1 by utilizing over-expression miRNAs.

US 2007/160586 discloses methods for extending the replicative lifespan of cells.

10 US 2011/015272 discloses Sirtuin 1 and the treatment of neurodegenerative diseases. Younghwan, H., et al. disclose the increase of Hspa1a and Hspa1b genes in the resting B cells of SIRT-1 knockout mice (Mol. Biol. Rep. 46 (2019) 4225-4234).

EP 3 308 778 discloses arginine and its use as a t cell modulator.

15 Fischer, S., et al. disclose enhanced protein production by microRNA-30 family in CHO cells is mediated by the modulation of the ubiquitin pathway (J. Biotechnol. 212 (2015) 32-43).

Currently, there is no knockout of a single endogenous gene known that increases productivity. Thus, a single knockout of an endogenous gene is highly desired because of its simplicity to be introduced in host cell lines.

20

Summary of the Invention

25

Herein is reported a method for generating a recombinant mammalian cell expressing a heterologous polypeptide and a method for producing a heterologous polypeptide using said recombinant mammalian cell, wherein in the recombinant mammalian cell the activity or function or expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

30

The ~~invention-disclosure~~ is based, at least in part, on the finding that the knockout of the sirtuin-1 (SIRT-1) gene in mammalian cells, e.g. such as CHO cells, improves on the one hand recombinant productivity, e.g. of standard IgG-type antibodies and especially of complex antibody formats, and reduces on the other hand lactate production by the cells during cultivation. Additionally, it has been found that the viability decline

at the end of a fed-batch cultivation is reduced for recombinant cells according to the current ~~invention~~disclosure, i.e. the timespan with viability above a certain threshold value is increased, compared to cells with fully functional SIRT-1 gene.

~~One independent aspect of the current invention is~~The present disclosure provides a mammalian cell wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

~~One independent aspect of the current invention is~~The present disclosure provides a mammalian cell wherein the expression of the endogenous SIRT-1 gene has been reduced and wherein said mammalian cell has at least one of increased productivity for heterologous polypeptides and/or reduced lactate production during cultivation and/or extended high viability levels during cultivation and/or extended cultivation time compared to a cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

~~One independent aspect of the current invention is~~The present disclosure provides a method for at least one of increasing heterologous polypeptide titer and/or reducing lactate production and/or extended high viability levels during cultivation and/or extension of cultivation time of a recombinant mammalian cell having reduced (endogenous) SIRT-1 expression comprising an exogenous nucleic acid encoding said heterologous polypeptide compared to a cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.
In one aspect, the present disclosure provides a method for increasing heterologous polypeptide titer and/or reducing lactate production of a recombinant mammalian cell comprising an exogenous nucleic acid encoding a heterologous polypeptide by reducing SIRT-1 expression compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

~~One independent aspect of the current invention is~~The present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous SIRT-1 genes in a mammalian cell to reduce the activity of the endogenous SIRT-1 gene, and

b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1 gene has been reduced,

thereby producing a recombinant mammalian cell with increased recombinant productivity and/or reduced lactate production compared to a compared to a cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

In another aspect, the present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous SIRT-1 genes in a mammalian cell to reduce the activity of the endogenous SIRT-1 gene, and

b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1 gene has been reduced,

thereby producing a recombinant mammalian cell with increased recombinant productivity and/or reduced lactate production compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

~~One independent aspect of the current invention is~~ The present disclosure provides
a method for producing a heterologous polypeptide comprising the steps of

- a) cultivating a mammalian cell comprising an exogenous deoxyribonucleic acid encoding the heterologous polypeptide optionally under conditions suitable for the expression of the heterologous polypeptide, and
- b) recovering the heterologous polypeptide from the cell or the cultivation medium,

wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

In another aspect, the present disclosure provides a method for producing a heterologous polypeptide comprising the steps of

- a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the heterologous polypeptide, and
- b) recovering the heterologous polypeptide from the cell or the cultivation medium,

wherein the expression of the endogenous SIRT-1 gene(s) has been reduced.

~~Another independent aspect of the current invention is~~ The present disclosure provides a method for producing a recombinant mammalian cell having/with improved and/or increased recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

- a) applying a nucleic acid or an enzyme or a nuclease-assisted gene targeting system targeting the endogenous SIRT-1 genes to a mammalian cell to reduce or eliminate or diminish or (completely) knock-out the activity or/and function or/and expression of the endogenous SIRT-1 gene, and
- b) selecting a mammalian cell wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out,

thereby producing a recombinant mammalian cell having/with improved and/or increased recombinant productivity and/or reduced lactate production

5 In one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the SIRT-1 gene knockout is a heterozygous knockout or a homozygous knockout.

In one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the productivity of the SIRT-1 knockout cell line is at least 10 %, preferably 15 % or more, most preferred 20 % or more increased compared to a SIRT-1 expressing (parent) mammalian cell.

In one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the reduction or elimination or diminishment or knock-out is mediated by a nuclease-assisted gene targeting system. In one ~~embodiment~~example the nuclease-assisted gene targeting system is selected from the group consisting of CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease and TALEN.

In one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the reduction of SIRT-1 gene expression is mediated by RNA silencing. In one ~~embodiment~~example 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 one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the SIRT-1 knockout is performed before the introduction of the exogenous nucleic acid encoding the heterologous polypeptide or after the introduction of the exogenous nucleic acid encoding the heterologous polypeptide.

In one ~~embodiment~~example of ~~all aspects and embodiments of~~ the current ~~invention~~disclosure the polypeptide is an antibody. In one ~~embodiment~~example the antibody is an antibody comprising two or more different binding sites and optionally a domain exchange. In one ~~embodiment~~example the antibody comprises three or more binding sites or VH/VL-pairs or Fab fragments and optionally a domain exchange. In one ~~embodiment~~example the antibody is a multispecific antibody. In one ~~embodiment~~example the multispecific antibody is selected from the group consisting of

- i) a full-length antibody with domain exchange comprising a first Fab fragment and a second Fab fragment,

wherein in the first Fab fragment

- a) the light chain of the first Fab fragment comprises a VL and a CH1 domain and the heavy chain of the first Fab fragment comprises a VH and a CL domain;
- b) the light chain of the first Fab fragment comprises a VH and a CL domain and the heavy chain of the first Fab fragment comprises a VL and a CH1 domain; or
- c) the light chain of the first Fab fragment comprises a VH and a CH1 domain and the heavy chain of the first Fab fragment comprises a VL and a CL domain;

- a third Fab fragment, wherein the binding site of the third Fab fragment specifically binds to a second antigen, and wherein the third Fab fragment comprises a domain crossover such that the variable light chain domain (VL) and the variable heavy chain domain (VH) are replaced by each other; and
- an Fc-region comprising a first Fc-region polypeptide and a second Fc-region polypeptide;

wherein the first and the second Fab fragment each comprise a heavy chain fragment and a full length light chain,

wherein the C-terminus of the heavy chain fragment of the first Fab fragment is fused to the N-terminus of the first Fc-region polypeptide,

wherein the C-terminus of the heavy chain fragment of the second Fab fragment is fused to the N-terminus of the variable light chain domain of the third Fab fragment and the C-terminus of the CH1 domain of the third Fab fragment is fused to the N-terminus of the second Fc-region polypeptide;

and

- vii) an immunoconjugate comprising a full-length antibody and a non-immunoglobulin moiety conjugated to each other optionally via a peptidic linker.

In addition to the various embodimentsexamples depicted and claimed, the disclosed subject matter is also directed to other embodimentsexamples having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The description of specific embodimentsexamples of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those embodimentsexamples disclosed.