

certain adjuvant treatments. Thus, there is a significant need to achieve a long-lasting remission, in particular after tumor resection by a more efficient targeting of cancer cells.

Such approaches may be particularly desirable for diseases such as glioblastoma multiforme (GBM), which is the most common and lethal brain cancer in adults with 1-5 cases per 100000 people per year and 12-15 months of median survival. This poor outcome is due to the combination of both the aggressiveness of the disease and the limited efficacy of current therapies that increase the overall survival only marginally. Patients usually undergo surgical resection of the primary tumor mass followed by adjuvant radio- and chemo- (Temozolomide) therapies, although the issues with tumor regrowth described above may lead to recurrence of the cancer.

Attempts have been carried out to restrain cancer (e.g. GBM) development by silencing expression of one or more transcription factors (TFs) with different technologies. For example, TF inactivation has been attempted with several technologies including shRNA, miRNA and TALEN-based epigenetic repressors, but complete and long-term gene silencing has proven challenging. Moreover, cancer cells may rearrange their genetic program to cope with the silencing of a single gene and, thus, maintain unaltered tumorigenic potential.

Accordingly, there remains a significant need for the development of more effective treatments for cancer, in particular treatments that are effective against aggressive cancers such as GBM, and treatments that enable targeting of CSCs

SUMMARY OF THE INVENTION

The present inventors have engineered oncogenic and cancer-associated transcription factors to function as epigenetic repressors, which, for example, enable silencing of downstream tumorigenic networks, thus limiting CSC survival and proliferation.

For example, the inventors engineered the SOX2 transcription factor to generate an epigenetic repressor (referred to as SOX2 Epigenetic Silencer (SES)) that maintains the ability to bind to a large group of its original targets and induce their long-term silencing. By way of example, the inventors deleted the C-terminal domain of SOX2 and fused it with a KRAB domain and the DNA methyltransferase 3A/3L catalytic domains. The inventors found that SES is able to inhibit the SOX2 tumorigenic network (rather than activate it as with the unmodified TF), including genes crucial for tumor maintenance and growth.

The inventors found that SES kills both glioma cell lines and patient-derived CSCs both in vitro and in vivo. Moreover, following in situ viral transduction of GBM xenografts in mice,

SES induced a strong growth inhibition of pre-formed tumor of human origin. The inventors also found that SES is not harmful to neurons and glia, and does not trigger significant transcriptional changes in these cells.

The inventors further validated their approach by successfully utilizing other epigenetic effector domains, such as the chromo shadow and YAF2-RYBP domains, and also by engineering additional transcription factors, such as MYC and TEAD1.

Compared to previous approaches aimed at silencing the expression of endogenous oncogenic transcription factors, the engineered transcription factors developed by the inventors (referred to as epigenetic silencer factors (ESFs)) may bind to the same targets as the unmodified transcription factors and induce their strong and permanent silencing. The inventors' approach may advantageously lead to repression of the transcriptional cascade of the transcription factor, and strongly reduce any issue of gene reactivation that can lead to tumor recurrence.

In one aspect, the ~~invention provides~~ present disclosure refers to an epigenetic silencer factor (ESF) comprising a transcription factor DNA-binding domain operably linked to at least one epigenetic effector domain, wherein the transcription factor is an oncogenic transcription factor or a cancer-associated transcription factor.

In some ~~embodiments~~ examples, the ESF is a polypeptide comprising the transcription factor DNA-binding domain and the at least one epigenetic effector domain. In some examples ~~embodiments~~, the ESF is a fusion protein comprising the transcription factor DNA-binding domain and the at least one epigenetic effector domain.

In preferred examples ~~embodiments~~, the transcription factor is an oncogenic transcription factor. In some ~~embodiments~~ examples, the transcription factor is a cancer-associated transcription factor.

In some examples ~~embodiments~~, the transcription factor is selected from the group consisting of SOX2, MYC, MYCN, TEAD1, TEAD2, TEAD3, TEAD4, FOXA1, FOXA2, ELK1, ELK3, ELK4, SRF, FOXM1, FOXC1, FOXC2, TWIST1, SALL4, ELF1, HIF1A, SOX9, SOX12, SOX18, ETS1, PAX3, PAX8, GLI1, GLI2, GLI3, ETV1, ETV2, ETV3, RUNX1, RUNX2, RUNX3, MAFB, TFAP2C and E2F1.

In some examples ~~embodiments~~, the transcription factor is SOX2.

In some examples ~~embodiments~~, the transcription factor is MYC.

In some examples ~~embodiments~~, the transcription factor is TEAD1.

In preferred ~~examples~~embodiments, the ESF does not comprise a transcription factor activation domain.

In some ~~examples~~embodiments, the at least one epigenetic effector domain is selected from the group consisting of a KRAB domain, a DNMT3A domain, a DNMT3L domain, a ZIM3-KRAB (Z-KRAB) domain, a Chromo Shadow (CS) domain, a YAF2-RYBP (Y-R) domain, an Engrailed Repressor (En-R) domain, a MeCP2 domain, a GLI3RD domain and a MAD1RD domain.

In some ~~examples~~embodiments, the ESF comprises a KRAB domain. In some ~~examples~~embodiments, the ESF comprises a DNMT3A domain. In some ~~examples~~embodiments, the ESF comprises a DNMT3L domain. In preferred ~~examples~~embodiments, the ESF comprises a KRAB domain, a DNMT3A domain and a DNMT3L domain.

In some ~~examples~~embodiments, the KRAB domain is a ZNF10 KRAB domain. In some ~~examples~~embodiments, the DNMT3A domain is the catalytic domain of DNMT3A. In some ~~examples~~embodiments, the CS domain is a CBX5 CS domain.

In some ~~examples~~embodiments, the ESF comprises a ZIM3-KRAB (Z-KRAB) domain. In some ~~examples~~embodiments, the ESF comprises a Chromo Shadow (CS) domain. In some ~~examples~~embodiments, the ESF comprises a YAF2-RYBP (Y-R) domain. In some ~~examples~~embodiments, the ESF comprises an Engrailed Repressor (En-R) domain. In some ~~examples~~embodiments, the ESF comprises a ZIM3-KRAB (Z-KRAB) domain, a Chromo Shadow (CS) domain and a YAF2-RYBP (Y-R) domain. In some ~~examples~~embodiments, the ESF comprises a KRAB domain and a DNMT3A domain. In some ~~examples~~embodiments, the ESF comprises a KRAB domain and an MeCP2 domain. In some ~~examples~~embodiments, the ESF comprises a GLI3RD domain. In some ~~embodiments~~examples, the ESF comprises a MAD1RD domain.

In another aspect, the ~~invention provides~~present disclosure refers to a polynucleotide comprising a nucleic acid sequence encoding the ESF ~~as disclosed herein~~of the invention.

In some ~~examples~~embodiments, the polynucleotide further comprises a promoter operably linked to the nucleic acid sequence encoding the ESF.

In some ~~examples~~embodiments, the promoter is a constitutive promoter. In some ~~embodiments~~examples, the constitutive promoter is an Ef1a promoter.

In some ~~examples~~embodiments, the promoter is a tissue-specific promoter, preferably a cancer cell-specific promoter.

In some ~~examples~~embodiments, the promoter is a proliferating cell-specific promoter.

In some ~~example~~embodiments, the promoter is selected from the group consisting of a Mki67 promoter, a Ccnd1 promoter, a Ccnb2 promoter, a Ccna2 promoter, a Cdc25c promoter, a Cdc2 promoter, a Cks1 promoter, a PCNA promoter, a CDC6 promoter, a POLD1 promoter, a CSK1B promoter, a MCM2 promoter and a PLK1 promoter.

5 In some ~~example~~embodiments, the promoter is a Mki67 promoter.

In another aspect, the ~~invention provides~~present disclosure refers to a vector comprising the polynucleotide ~~as disclosed herein~~of the invention.

10 In some ~~example~~embodiments, the vector is a viral vector. In some ~~example~~embodiments, the vector is a lentiviral vector or adeno-associated viral (AAV) vector. In some ~~example~~embodiments, the vector is a lentiviral vector.

In some ~~example~~embodiments, the vector is an mRNA vector.

15 In some ~~example~~embodiments, the ESF, polynucleotide or vector is comprised in a nanoparticle. In some ~~example~~embodiments, the nanoparticle is a polymeric nanoparticle, inorganic nanoparticle or lipid nanoparticle. In some ~~example~~embodiments, the nanoparticle is a liposome

In another aspect, the ~~invention provides~~present disclosure refers to a cell comprising the ESF, polynucleotide or vector ~~of the invention~~as described herein.

20 In another aspect, the ~~invention provides~~present disclosure refers to a composition comprising the ESF, polynucleotide, vector or cell ~~of the invention~~as described herein. In some ~~example~~embodiments, the composition is a pharmaceutical composition.

The composition may be a hydrogel. In some ~~example~~embodiments, the hydrogel is a poly(ethylene glycol) dimethacrylate (PEG-DMA) hydrogel. In some ~~example~~embodiments, the hydrogel further comprises hydroxyapatite nanoparticles.

25 In another aspect, the ~~invention provides~~present disclosure refers to the ESF, polynucleotide, vector, cell or composition ~~of the invention~~as described herein for use in therapy.

30 In another aspect, the ~~invention provides~~present disclosure refers to use of the ESF, polynucleotide, vector, cell or composition described herein in the manufacture of a medicament for treating cancer. In another example, described herein is the ESF, polynucleotide, vector, cell or composition of the invention as disclosed herein for use in the treatment of cancer. In another aspect, the present disclosure refers to use of the ESF, polynucleotide, vector, cell or composition disclosed herein in the manufacture of a medicament for decreasing transcription and/or expression of at least one target gene in a cell.

35 In some ~~embodiment~~examples, the cancer is glioma, glioblastoma, medulloblastoma, astrocytoma, neuroblastomas, ependymoma, meningioma, retinoblastoma, rhabdomyosarcoma, lung cancer, prostate cancer, breast cancer, liver cancer, pancreatic cancer, bladder cancer,

oropharyngeal cancer or kidney cancer. In some ~~examples~~~~embodiments~~, the cancer is a brain tumour. In some ~~examples~~~~embodiments~~, the cancer is glioblastoma multiforme.

In some ~~examples~~~~embodiments~~, the treatment reduces tumour size.

5 In some ~~examples~~~~embodiments~~, the treatment is as an adjuvant therapy, optionally in combination with surgery. The treatment may reduce the risk of cancer (e.g. GBM) recurrence.

In some ~~embodiments~~~~examples~~, the ESF, polynucleotide, vector, cell or composition (e.g. hydrogel) is administered locally.

10 In another ~~examples~~~~aspect~~, ~~the invention provides~~~~there is disclosed~~ a method of treating cancer in a subject, the method comprising administering the ESF, polynucleotide, vector, cell or composition as disclosed herein ~~of the invention~~ to a subject in need thereof.

In another ~~aspect~~~~example~~, ~~the invention provides~~~~there is disclosed~~ the use of the ESF, polynucleotide, vector, cell or composition of the invention~~as disclosed herein~~ for the manufacture of a medicament for therapy.

15 In another ~~aspect~~~~example~~, ~~the invention provides~~~~there is disclosed~~ the use of the ESF, polynucleotide, vector, cell or composition as disclosed herein ~~of the invention~~ for the manufacture of a medicament for treatment of cancer.

In another ~~examples~~~~aspect~~, ~~the invention provides~~~~there is disclosed~~ the use of the ESF, polynucleotide, vector, cell or composition as disclosed herein ~~of the invention~~ for decreasing transcription and/or expression of at least one target gene in a cell.

20 In another ~~examples~~~~aspect~~, ~~the invention provides~~~~there is disclosed~~ a method of decreasing transcription and/or expression of at least one target gene in a cell, the method comprising introducing the ESF, polynucleotide, vector or composition as disclosed herein ~~of the invention~~ into the cell.

25 In some ~~examples~~~~embodiments~~, the at least one target gene is silenced, preferably permanently silenced.

DESCRIPTION OF THE DRAWINGS

FIGURE 1. Generation and testing of SOX2 epigenetic silencer (SES).

30 (a) Constructs generated based on human SOX2 transcription factor and the epigenetic domain KRAB, DNMT3a (3A) and DNMT3L (3L), V5 was added as tag. (b) Infection efficiency of lentivirus carrying the indicated constructs in SNB19 cells. (c) Left, growth curve of SNB19 cells infected with the indicated constructs show that SES was able to kill the cells after 12 days in culture, ****p < 0.0001; statistically compared with two-way ANOVA; right, microphotographs of the cells at the indicated time points from the infection with either mock

CLAIMS

1. An epigenetic silencer factor (ESF) comprising a transcription factor DNA-binding domain operably linked to at least one epigenetic effector domain, wherein the transcription factor is an oncogenic transcription factor or a cancer-associated transcription factor.
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2. The ESF of claim 1, wherein the transcription factor is selected from the group consisting of SOX2, MYC, MYCN, TEAD1, TEAD2, TEAD3, TEAD4, FOXA1, FOXA2, ELK1, ELK3, ELK4, SRF, FOXM1, FOXC1, FOXC2, TWIST1, SALL4, ELF1, HIF1A, SOX9, SOX12, SOX18, ETS1, PAX3, PAX8, GLI1, GLI2, GLI3, ETV1, ETV2,
10 ETV3, RUNX1, RUNX2, RUNX3, MAFB, TFAP2C and E2F1.
3. The ESF of claim 1 or 2, wherein the transcription factor is Sox2.
4. The ESF of any preceding claim, wherein the at least one epigenetic effector domain is selected from the group consisting of a KRAB domain, a DNMT3A domain, a DNMT3L domain, a ZIM3-KRAB (Z-KRAB) domain, a Chromo Shadow (CS) domain,
15 a YAF2-RYBP (Y-R) domain, an Engrailed Repressor (En-R) domain, a MeCP2 domain, a GLI3RD domain and a MAD1RD domain.
5. The ESF of any preceding claim, wherein the ESF comprises a KRAB domain, a DNMT3A domain and a DNMT3L domain.
6. A polynucleotide comprising a nucleic acid sequence encoding the ESF of any preceding claim.
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7. The polynucleotide of claim 6, wherein the polynucleotide further comprises a promoter operably linked to the nucleic acid sequence encoding the ESF, optionally wherein the promoter is a tissue-specific promoter or a constitutive promoter, optionally a cancer cell-specific promoter.
- 25 8. A vector comprising the polynucleotide of claim 6 or 7, optionally wherein the vector is a viral vector, optionally wherein the vector is a lentiviral vector or adeno-associated viral (AAV) vector.
9. The ESF, polynucleotide or vector of any preceding claim, wherein the ESF, polynucleotide or vector is comprised in a nanoparticle.
- 30 10. A cell comprising the ESF, polynucleotide or vector of any preceding claim.

11. A composition comprising the ESF, polynucleotide, vector or cell of any preceding claim.

12. The ESF, polynucleotide, vector, cell or composition of any preceding claim for use in therapy.

5 13. Use of the ESF, polynucleotide, vector, cell or composition of any one of claims 1-11 in the manufacture of a medicament for treating cancer~~for use in the treatment of cancer.~~

10 14. Use of the ESF, polynucleotide, vector, cell or composition of any one of claims 1-11 in the manufacture of a medicament for decreasing transcription and/or expression of at least one target gene in a cell.

~~15. A method of decreasing transcription and/or expression of at least one target gene in a cell, the method comprising introducing the ESF, polynucleotide, vector or composition of any one of claims 1-9 or 11 into the cell.~~