

Claims

1. A cell genetically modified to produce a chimeric polypeptide receptor comprising (i) an extracellular recognition domain, (ii) at least one protease cleavage site, and (iii) an intracellular transcription factor, wherein binding of the extracellular recognition domain to its target induces proteolytic cleavage of the at least one protease cleavage site and endogenous transcription by the intracellular transcription factor of at least one polynucleotide encoding a gene product comprising at least one exosomal polypeptide.
2. The cell according to claim 1, wherein the gene product further comprises a protein of interest.
3. The cell according to claim 2, wherein the protein of interest is an antibody, a single-chain antibody or any other antibody derivative, a bispecific T cell engager (BiTE), a receptor, a cytokine such as an interleukin, an enzyme such as caspase, granzyme, Cas, Cas9, a checkpoint inhibitor, a costimulation inhibitor, an RNA-binding protein, a membrane transporter such as NPC-1, a splicing factor, a protein associated with cellular organelles, a lysosomal enzyme, a transcription factor, a mitochondrial proteins, an intracellular protein, an antiviral protein, an antibacterial protein.
4. The cell according to any one of claims 2-3, wherein when the protein of interest is an RNA-binding protein the cell is further genetically modified to comprise an RNA cargo molecule selected from the group consisting of mRNA, sgRNA, shRNA, miRNA, shRNA, siRNA, lncRNA, ncRNA, piRNA, piwiRNA, circRNA, tRNA, rRNA, crRNA and any combination thereof.
5. The cell according to any one of the preceding claims, wherein the genetic modification is an in vitro or ex vivo genetic modification.
6. The cell according to any one of the preceding claims, wherein the cell is an effector immune cell.
7. The cell according to claim 5 and/or 6, wherein the cell is a T cell, a cytotoxic CD8⁺ T cell, a CD4⁺ T cell, a regulatory T cell, a natural killer (NK) cell, a B cell, a plasma cell, a dendritic cell (DC), a macrophage, a monocyte, a neutrophil, an epithelial cell, an endothelial cell, a microglia, an astrocyte, a neuron, a stem cell, a bone marrow derived mesenchymal stromal cell, a Wharton's jelly derived MSC, or any other cell type.

8. The cell according to any one of the preceding claims, wherein the extracellular recognition domain of the chimeric polypeptide receptor is an antibody, an antibody derivative, a single-chain fragment, a single-chain antibody, a nanobody, a peptide, a ligand for a receptor, an adhesion molecule, a receptor, an interleukin receptor, an extracellular matrix component, or any combination thereof.
9. The cell according to any one of the preceding claims, wherein the at least one protease cleavage site is at least one of an S1, an S2 and/or an S3 cleavage site.
10. The cell according to any one of the preceding claims, wherein the fusion polypeptide is a chimeric Notch polypeptide comprising from N-terminus to C-terminus and in covalent linkage:
 - (i) an extracellular recognition domain that is not naturally present in a Notch receptor polypeptide;
 - (ii) a Notch regulatory region which comprises a Lin 12-Notch repeat, an S2 proteolytic cleavage site, and a transmembrane domain comprising an S3 proteolytic cleavage site;
 - (iii) an intracellular transcription factor that is heterologous to the Notch regulatory region,wherein binding of the extracellular recognition domain to its target induces cleavage at the S2 and S3 protease cleavage sites, thereby releasing the intracellular transcription factor which activates transcription of the polynucleotide.
11. The cell according to claim 10, wherein the Notch regulatory region further comprises a heterodimerization domain comprising the S2 proteolytic cleavage site.
12. The cell according to any one of claims 9-11, wherein the S1 proteolytic cleavage site is a furin-like protease cleavage site comprising the amino acid sequence Arg-X-(Arg/Lys)-Arg, where X is any amino acid.
13. The cell according to any one of the preceding claims, wherein the fusion polypeptide comprises at least one linker.
14. The cell according to any one of the preceding claims, wherein the polynucleotide further comprises a transcriptional control element, responsive to the transcription factor, operably linked to a coding sequence.

15. The cell according to any one of the preceding claims, wherein the cell is genetically modified to produce at least two types of fusion polypeptides, wherein at least one of the (i) extracellular recognition domain, the (ii) protease cleavage site, and the (iii) intracellular transcription factor differ between the fusion polypeptides.
16. The cell according to claim 15, wherein the extracellular recognition domains of the fusion polypeptides are different from one another.
17. An extracellular vesicle (EV) produced by the cell according to any one of claims 1-16.
18. The EV according to claim 16, wherein the EV is an exosome.
19. The EV according to any one of claims 17-18, wherein the EV comprises the gene product.
20. A recombinant expression vector comprising the polynucleotide, encoding for the gene product, of any one of the preceding claims.
21. The recombinant expression vector of claim 20, wherein the recombinant expression vector further comprises a transcriptional control element, responsive to the transcription factor, operably linked to a coding sequence.
22. The recombinant expression vector of any one of claims 20-21, wherein the transcriptional control element is endogenous or heterologous to the cell of any one of claims 1-16.
23. The recombinant expression vector of any one of claims 20-22, wherein the coding sequence of the polynucleotide is endogenous or heterologous to the cell of any one of claims 1-16.
24. A gene product encoded for by the polynucleotide of any one of claims 20-23.
25. A recombinant expression vector encoding for the chimeric polypeptide receptor of any one of the preceding claims.
26. A fusion polypeptide encoded for by the recombinant expression vector of claim 24.
27. A method of exerting a therapeutic effect, the method comprising:
 - (i) introducing a recombinant expression vector according to any one of claims 20-23 and a recombinant expression vector according to claim 25 into a cell ex vivo or in vitro;
 - (ii) administering the genetically modified cell to an individual.

28. A pharmaceutical composition comprising the cell according to any one of claims 1-16.
29. A cell according to any one of claims 1-16 or a pharmaceutical composition according to claim 28 for use in medicine
30. A cell according to any one of claims 1-16 or a pharmaceutical composition according to claim 28 for use in the treatment of cancer, inflammatory disease, autoimmune disease, genetic diseases, infectious diseases, metabolic diseases, CNS diseases, lysosomal storage disorders, and neurodegenerative diseases.

CLAIMS

1. A cell genetically modified to produce a chimeric polypeptide receptor comprising (i) an extracellular recognition domain, (ii) at least one protease cleavage site, and (iii) an intracellular transcription factor, and further genetically modified to comprise a polynucleotide encoding a gene product comprising at least one exosomal polypeptide and a protein of interest, in which binding of the extracellular recognition domain to its target induces proteolytic cleavage of the at least one protease cleavage site and endogenous transcription by the intracellular transcription factor of the polynucleotide encoding the gene product.
2. A cell according to Claim 1, in which the protein of interest is an antibody, a single-chain antibody, a bispecific T cell engager (BiTE), a receptor, a cytokine such as an interleukin, an enzyme such as caspase, granzyme, Cas, Cas9, a checkpoint inhibitor, a costimulation inhibitor, an RNA-binding protein, a membrane transporter such as NPC-1, a splicing factor, a protein associated with cellular organelles, a lysosomal enzyme, a transcription factor, a mitochondrial proteins, an intracellular protein, an antiviral protein, an antibacterial protein.
3. A cell according to Claim 1 or 2, in which when the protein of interest is an RNA-binding protein the cell is further genetically modified to comprise an RNA cargo molecule selected from the group consisting of mRNA, sgRNA, shRNA, miRNA, shRNA, siRNA, lncRNA, ncRNA, piRNA, piwiRNA, circRNA, tRNA, rRNA, crRNA and any combination thereof.
4. A cell according to Claim 1, 2 or 3, in which the cell is an effector immune cell.
5. A cell according to Claim 3 or 4, in which the cell is a T cell, a cytotoxic CD8⁺ T cell, a CD4⁺ T cell, a regulatory T cell, a natural killer (NK) cell, a B cell, a plasma cell, a dendritic cell (DC), a macrophage, a monocyte, a neutrophil, an epithelial cell, an endothelial cell, a microglia, an astrocyte, a neuron, a stem cell, a bone marrow derived mesenchymal stromal cell or a Wharton's jelly derived MSC.
6. A cell according to any preceding claim, in which the extracellular recognition domain of the chimeric polypeptide receptor is an antibody, a single-chain fragment, a single-chain

antibody, a nanobody, a peptide, a ligand for a receptor, an adhesion molecule, a receptor, an interleukin receptor, an extracellular matrix component, or any combination thereof.

7. A cell according to any preceding claim, in which the at least one protease cleavage site is at least one of an S1, an S2 and/or an S3 cleavage site.

5 8. A cell according to any preceding claim, in which the chimeric polypeptide receptor is a chimeric Notch polypeptide comprising from N-terminus to C-terminus and in covalent linkage:

(i) an extracellular recognition domain that is not naturally present in a Notch receptor polypeptide;

10 (ii) a Notch regulatory region which comprises a Lin 12-Notch repeat, an S2 proteolytic cleavage site, and a transmembrane domain comprising an S3 proteolytic cleavage site;

(iii) an intracellular transcription factor that is heterologous to the Notch regulatory region,

15 in which binding of the extracellular recognition domain to its target induces cleavage at the S2 and S3 protease cleavage sites, thereby releasing the intracellular transcription factor which activates transcription of the polynucleotide.

9. A cell according to Claim 8, in which the Notch regulatory region further comprises a heterodimerization domain comprising the S2 proteolytic cleavage site.

20 10. A cell according to Claim 7, in which the S1 proteolytic cleavage site is a furin-like protease cleavage site comprising the amino acid sequence Arg-X-(Arg/Lys)-Arg, where X is any amino acid.

11. A cell according to any preceding claim, in which the chimeric polypeptide receptor comprises at least one linker.

25 12. A cell according to any preceding claim, in which the polynucleotide further comprises a transcriptional control element, responsive to the transcription factor, operably linked to a coding sequence.

13. A cell according to any preceding claim, in which the cell is genetically modified to produce at least two types of fusion polypeptides, in which at least one of the (i) extracellular

recognition domain, the (ii) protease cleavage site, and the (iii) intracellular transcription factor differ between the fusion polypeptides, optionally in which the extracellular recognition domains of the fusion polypeptides are different from one another.

14. An extracellular vesicle (EV) produced by a cell according to any preceding claim,
5 optionally in which the EV is an exosome.
15. A pharmaceutical composition comprising a cell according to any of Claims 1 to 13.
16. A cell according to any of Claims 1 to 13 or a pharmaceutical composition according to Claim 15 for use in medicine.