Antibody therapy

The search for new therapies to treat cancer and other diseases has resulted in the development of human and humanized antibodies capable of inhibiting receptor function. International patent publication WO 2006/023491 provides a method of RNA interference, which comprises contacting the cell with a fusion protein-double stranded RNA complex, the complex comprising the double stranded RNA segment containing a double stranded RNA of interest and a fusion protein which is an antibody Fab fragment- protamine fusion protein.

SUMMARY OF THE ~~INVENTION~~DISCLOSURE

The present disclosure~~invention~~ is based in part on the identification of isolated molecules that specifically bind the ENDO180 polypeptide on a cell surface. In some embodiments the molecules bind the extracellular domain of the ENDO180 polypeptide and are internalized into the cell by the polypeptide, thereby providing a vehicle useful for delivery of therapeutic and diagnostic cargo to a cell expressing the ENDO180 polypeptide. Accordingly, in some embodiments the present ~~invention~~ disclosure provides a conjugate comprising a molecule that specifically binds the ENDO180 polypeptide and a therapeutic agent useful for the delivery of the therapeutic agent into the cell. In some embodiments the ENDO180 polypeptide is substantially identical to an amino acid sequence set forth in SEQ ID NO:2, encoded by a polynucleotide substantially identical to a nucleic acid sequence set forth in SEQ ID NO:1.

In one aspect the present ~~invention~~ disclosure provides an anti-ENDO180 antibody which is produced by hybridoma cell line designated E3-8D8 (BCCM Accession Number LMBP 7203CB), or a fragment of the antibody, which binds to ENDO180 receptor on the surface of a cell. In some embodiments binding of the antibody to the receptor results in internalization of the antibody into the cell. Also provided is the E3-8D8 hybridoma cell line.

In some embodiments the antibody or fragment thereof is humanized or a chimeric antibody or fragment thereof.

The ~~invention~~ disclosure provides a composition comprising at least one anti-ENDO180 antibody or fragment thereof, the antibody produced by the E3-8D8 hybridoma or a humanized molecule thereof a chimeric antibody or fragment thereof, together with a carrier.

In some embodiments the isolated antibody is selected from the group consisting of a full IgG, a Fab fragment, a Fab' fragment, an F(ab')2 fragment, the variable portion of the heavy and/or light chains thereof, Fab miniantibodies, and a scFv. In some embodiments the antibody is a recombinant polypeptide comprising a heavy chain CDR3 domain having an amino acid sequence set forth in SEQ ID NO:7 or a variant thereof which retains the ability to specifically bind ENDO180. In some embodiments the antibody further comprises a light chain CDR3 domain having an amino acid sequence set forth in SEQ ID NO:8 or a variant thereof which retains the ability to specifically bind ENDO180.

In some embodiments the antibody is a scFv recombinant polypeptide comprising an amino acid sequence set forth in SEQ ID NO:6 or a variant thereof, which retains the ability to specifically bind ENDO180. In specific embodiments the antibody exhibiting binding affinity to ENDO180 receptor and comprising CDR3 domains set forth in SEQ ID NOS 7 and 8 is internalized by the receptor into the cell expressing ENDO180 upon contact of the antibody to the receptor.

The ~~invention~~ disclosure further provides a composition comprising at least one anti-ENDO180 antibody or fragment thereof, as described above, and a moiety including a radioisotope, a therapeutic agent, a cytotoxic agent, or a detectable label. In some embodiments the moiety is attached (or linked, or conjugated), either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds, to the antibody.

In some embodiments provided is an anti-ENDO180 antibody or antigen-binding fragment thereof selected from

a) the monoclonal antibody produced by the hybridoma cell line E3-8D8 (BCCM Accession Number LMBP 7203CB);

b) an antibody or fragment thereof that binds to the same epitope as the antibody in (a);

c) a humanized antibody of (a) or (b);

d) a fragment of an antibody comprising a polypeptide substantially similar to SEQ ID NO: 6; and

e) a recombinant polypeptide comprising CDR3 with an amino acid sequence substantially similar to amino acid sequences set forth in SEQ ID NO:7 and 8.

Further provided is a composition comprising an anti-ENDO180 antibody or antigen-binding fragment thereof selected from

a) the monoclonal antibody produced by the hybridoma cell line E3-8D8 (BCCM Accession Number LMBP 7203CB);

b) an antibody or fragment thereof that binds to the same epitope as the antibody in (a);

c) a humanized antibody of (a) or (b);

d) a fragment of an antibody comprising a polypeptide substantially similar to SEQ ID NO: 6; and

e) a recombinant polypeptide comprising CDRs having an amino acid sequence substantially similar to amino acid sequences set forth in SEQ ID NO:7 and 8.

In some embodiments the composition further comprises a moiety including a radioisotope, a therapeutic agent, a cytotoxic agent, or a detectable label.

The present ~~invention~~ disclosure also provides a method of treating a subject afflicted with a proliferative disorder comprising administering to the subject a composition comprising an anti-ENDO180 antibody or antigen-binding fragment thereof selected from

a) the monoclonal antibody produced by the hybridoma cell line E3-8D8 (BCCM Accession Number LMBP 7203CB);

b) an antibody or fragment thereof that binds to the same epitope as the antibody in (a);

c) a humanized antibody of (a) or (b);

d) a fragment of an antibody comprising a polypeptide substantially similar to SEQ ID NO: 6; and

e) a recombinant polypeptide comprising CDRs having an amino acid sequence substantially similar to amino acid sequences set forth in SEQ ID NO:7 and 8.

In some embodiments the proliferative disorder is selected from a solid tumor, a hematopoietic tumor, metastases, fibrosis and a macrophage associated disorder.

In some embodiments the tumor is an ovarian tumor, a breast tumor, osteoblastic/osteocytic cancer, prostate cancer, head and neck cancer, leukemia, renal cell carcinoma, or transitional cell carcinoma.

In some embodiments the fibrosis is liver fibrosis, myelofibrosis, kidney fibrosis for any reason (CKD including end-stage renal disease, ESRD); lung fibrosis (including interstitial lung fibrosis ILF); abnormal scarring (keloids) associated with all possible types of skin injury accidental and jatrogenic (operations); scleroderma; cardiofibrosis, failure of glaucoma filtering operation; intestinal adhesions.

In some embodiments the macrophage-associated disorder is inflammation or atherosclerosis.

In one aspect the present ~~invention~~ disclosure provides a conjugate comprising:

a) an antibody or an antigen binding portion thereof which specifically binds to the extracellular domain of the ENDO180 polypeptide on the surface of a cell;

b) a moiety including a radioisotope, a therapeutic agent, a cytotoxic agent, or a detectable label.; and

c) optionally a linking moiety which links (a) to (b).

In some embodiments the moiety is a therapeutic agent selected from an oligonucleotide agent and a non-oligonucleotide agent. In some embodiments the therapeutic agent is an oligonucleotide therapeutic agent, including an inhibitory oligonucleotide. Accordingly, in various embodiments the therapeutic agent is selected from an antisense compound, a chemically modified siRNA compound, an unmodified siRNA compound, a chemically modified shRNA compound, an unmodified shRNA compound, a chemically modified miRNA compound, and an unmodified miRNA compound. In various preferred embodiments the therapeutic agent is chemically modified siRNA. In some embodiments the chemically modified siRNA compound inhibits expression of a target gene associated with cancer, fibrosis or macrophage associated disease. In some embodiments the target gene is selected from any one of the target genes set forth in Table A, hereinbelow.

In certain embodiments the therapeutic agent is attached to the antibody via a nucleotide or non-nucleotide linking moiety.

In yet another aspect the present ~~invention~~ disclosure provides a pharmaceutical composition comprising the conjugate of the present ~~invention~~ disclosure.

In yet another aspect the present ~~invention~~ disclosure provides a method of treating a subject suffering from a proliferative disease comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds ENDO180

polypeptide and is internalized by the ENDO180 polypeptide, wherein the antibody is covalently or non-covalently bound to a therapeutic agent.

In some embodiments the proliferative disease is selected from malignant and benign proliferative disease. In some embodiments proliferative disease is cancer. In other embodiments proliferative disease is fibrosis. Non-limiting examples of diseases and disorders for use of the present ~~invention~~ disclosure include

1. soft tissue sarcomas in which ENDO180 is expressed in the tumor and tumor stroma cells (activated myofibroblasts, neovasculature and infliltrating cells of macrophage-monocyte lineage);

2. carcinomas in which ENDO180 is expressed in the tumor stroma cells (activated myofibroblasts, neovasculature and infliltrating cells of macrophage-monocyte lineage);

3. carcinoma that express ENDO180 and have undergone epithelial-mesenchymal transition thus acquiring high metastatic potential;

4. leukemia expressing ENDO180 for example, from macrophage-monocyte lineage;

5. fibrotic diseases, for example of kidney, lung and liver with activated myofibroblasts;

6. diseases and disorders associated with macrophage including atherosclerosis and chronic inflammation.

In one aspect, there is provided an anti-ENDO180 antibody or antigen-binding fragment thereof selected from:

a. the monoclonal antibody produced by the hybridoma cell line E3-8D8 (BCCM Accession Number LMBP 7203CB) or an antigen-binding fragment thereof;

b. a chimeric antibody of (a) or an antigen-binding fragment thereof;

c. a humanized antibody of (a) or an antigen-binding fragment thereof; and

d. a recombinant polypeptide comprising amino acid sequences set forth in SEQ ID NO: 6 or an antigen-binding fragment thereof;

wherein upon contact with a cell expressing ENDO180 on its surface the antibody or antigen binding fragment thereof is internalized into the cell.

In one aspect, there is provided a composition comprising the anti-ENDO180 antibody or antigen binding fragment thereof as disclosed herein, and a pharmaceutically acceptable carrier.

In one aspect, there is provided the use of a composition as disclosed herein comprising a moiety selected from a detectable label, a cytotoxic agent and a therapeutic agent for the manufacture of a medicament for delivering a moiety to an ENDO180 expressing cell in a subject.

In one aspect, there is provided the use of a composition as disclosed herein comprising a moiety that is a therapeutic agent, for the manufacture of a medicament for treating a subject afflicted with cancer, fibrosis or macrophage associated disease.

In one aspect, there is provided the use of a composition as disclosed herein comprising a moiety that is a therapeutic agent, for the manufacture of a medicament for inhibiting growth of a cancer cell.

In one aspect, there is provided the hybridoma cell line E3-8D8, deposited with the BCCM with Accession Number LMBP 7203CB.

In one aspect, there is provided an isolated anti-ENDO180 antibody or antigen-binding fragment thereof secreted by the hybridoma cell line as disclosed herein.

In one aspect, there is provided an antibody or antigen-binding fragment thereof that binds to the same epitope as the antibody as disclosed herein.

In one aspect, there is provided a conjugate comprising a) the antibody or antibody or antigen-binding fragment of as disclosed herein; b) a moiety selected from a detectable label, a cytotoxic agent and a therapeutic agent; and c) optionally a linker which links a) to b).

In one aspect, there is provided a composition comprising the conjugate as disclosed herein; and a carrier.

In one aspect, there is provided the use of a conjugate as disclosed herein or the composition comprising the conjugate as disclosed herein; and a carrier for the manufacture of a medicament for delivering a moiety to an ENDO180 expressing cell in a subject.

In one aspect, there is provided the use of the composition comprising the conjugate as disclosed herein; and a carrier, for the manufacture of a medicament for treating a subject afflicted with cancer, fibrosis or macrophage associated disease.

In one aspect, there is provided an anti-ENDO180 antibody or antigen-binding fragment comprising a polypeptide sequence set forth in SEQ ID NO:6.

In one aspect, there is provided an isolated polynucleotide having SEQ ID NO:5 which encodes the polypeptide as disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A-1J provides polynucleotide and amino acid sequences of various compounds according to the present invention. Fig. 1A: human ENDO180 mRNA (SEQ ID NO:1); Fig 1B:. human ENDO180 polypeptide (SEQ ID NO:2); Fig. 1C: SEQ ID NO:3 polynucleotide sequence of extracellular domain of human ENDO180 (amino acids 1-522) with FLAG sequence, FLAG domain underlined (pcDNA3-5'hendo180-FLAG construct, SEQ D NO:3); Fig. 1D polypeptide sequence of SEQ ID NO:3 (SEQ ID NO:4); Fig. 1E: polynucleotide sequence of scFv clone G7V (SEQ ID NO:5); Fig. 1F: polypeptide sequence of scFv clone G7V (SEQ ID NO:6); Fig. 1G. heavy chain CDR3 of G7V (SEQ ID NO:7); Fig. 1H. light chain CDR3 of G7V (SEQ ID NO:8); Fig. 1J: polypeptide 1-522 of the extracellular domain of human ENDO180.

Figures 2A-2H. Internalization of CypHer5E fluorophore anti-ENDO180 mAbs to ENDO180 expressing cells.