USE OF SEMAPHORIN-4D BINDING MOLECULES TO PROMOTE NEUROGENESIS FOLLOWING STROKE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to U.S. Nonprovisional Appl. No. 13/842,523, filed on March 15, 2013 and U.S. Provisional Appl. No. 61/646,119, filed on May 11, 2012, the content of each is hereby incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in ASCII text file (Name: "1843072PC01_SequenceListing.txt"; Size: 7,562 bytes; and Date of Creation: May 10, 2013) filed herewith is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Semaphorin 4D (SEMA4D), also known as CD100, is a transmembrane protein (*e.g.*, SEQ ID NO: 1 (human); SEQ ID NO: 2 (murine)) that belongs to the semaphorin gene family. SEMA4D is expressed on the cell surface as a homodimer, but upon cell activation SEMA4D can be released from the cell surface via proteolytic cleavage to generate sSEMA4D, a soluble form of the protein, which is also biologically active. See Suzuki *et al.*, *Nature Rev. Immunol. 3*:159-167 (2003); Kikutani *et al.*, *Nature Immunol. 9*:17-23 (2008).

[0004] SEMA4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, SEMA4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). Its expression, however, is upregulated in these cells following activation by various stimuli. The release of soluble SEMA4D from immune cells is also increased by cell activation.

[0005] SEMA4D has been implicated in the development of autoimmune demyelinating diseases such as multiple sclerosis and certain cancers. The failure of the mammalian nervous system to completely regenerate after injury is a major clinical problem. Neural damage as a result of stroke or trauma to the brain, as well as neurodegenerative diseases such as Alzheimer's disease, are a leading cause of death and disability. While the role of SEMA4D signaling through its receptors, e.g., Plexin-B1, on angiogenesis is well-recognized, the effect of SEMA4D signaling on promoting neurogenesis remains unclear. There remains, therefore, a need to provide a solution to the unmet medical need for therapeutic means of neuroregeneration.

BRIEF SUMMARY

[0006] Methods for using Semaphorin-4D binding molecules for promotion of neurogenesis are disclosed herein. Evidence is presented demonstrating that SEMA4D can compromise the ability of neural cells to regenerate. According to embodiments of the disclosure illustrated herein, there is provided a method for promoting neurogenesis in neural tissue of a patient exhibiting at least one symptom of a central nervous system disorder, the method comprising administering to a subject in need thereof an effective amount of an isolated binding molecule which specifically binds to and/or inhibits semaphorin-4D (SEMA4D).

[0007] According to embodiments of the disclosure illustrated herein, there is provided a method for increasing the number of progenitor cells in a subject with a disorder of the central nervous system comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorm-4D (SEMA4D), wherein the binding to SEMA4D acts to increase the number of progenitor cells.

[0007a] In one ~~aspect~~embodiment, there is provided the use of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the manufacture of a medicament for increasing the number of progenitor cells in a subject with a central nervous system disorder.

[0007b] In one aspect, there is provided the use of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for treating stroke, wherein administration of an effective amount of the antibody or fragment thereof can promote neurogenesis in neural tissue of a subject following stroke injury, and wherein administration of an effective amount of the antibody or fragment thereof to the subject can increase one or more of proliferation, differentiation, or migration of neural progenitor cells.

[0008] According to embodiments of the disclosure illustrated herein, there is provided a method for promoting neurogenesis in neural tissue of a patient exhibiting at least one symptom of a central nervous system disorder, comprising administering to a subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D.

[0008a] In one ~~aspect~~embodiment, there is provided the use of an isolated binding molecule which specifically binds semaphorin-4D (SEMA4D) in the manufacture of a medicament for promoting neurogenesis in neural tissue of a patient exhibiting at least one symptom of a central nervous system disorder.

[0009] According to embodiments of the disclosure illustrated herein, there is provided a method for alleviating a symptom of a disease or disorder of the central nervous system, such as stroke, in a patient comprising administering to the subject an effective amount of an

isolated binding molecule which specifically binds to and/or inhibits to semaphorin-4D (SEMA4D), wherein the binding to SEMA4D acts to alleviate the symptom.

[0009a] In one ~~aspect~~embodiment, there is provided the use of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the manufacture of a medicament for treating stroke in a patient

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0010] FIGURE 1: Schematic of experimental protocol for middle cerebral artery (MCA) occlusion described in the Examples.

[0011] FIGURES 2A-2E: Show neural stem/precursor cells in brain samples stained for Nestin/Sox2 in the areas indicated in 2E following occlusion of the middle cerebral artery (MCAO) and treatment with either isotype control ("Control IgG", 2A and 2C) or MAb 67-2 ("Anti-Sema4D-Ab", 2B and 2D). The brain samples were prepared 7 days after MCAO.

[0012] FIGURES 3A-3D: Show (A) mRNA expression by RT-PCR with quantitative analysis for (B) Sox2, (C) nestin, and (D) PLP relative to actin control in brain tissue of MCAO mice treated with isotype control ("Control IgG") or MAb 67-2 ("Anti-SEMA4D"). Brain tissue was isolated 7 days after MCAO. Nestin and Sox2 are markers of neural stem precursor cells. PLP is a marker of myelination.

[0013] FIGURES 4A-4D: Show relative total volume of brain and striatum in treated and untreated hemispheres in MCAO mice treated with isotype control or MAb 67-2. Measurements were taken 30 days after MCAO. FIGS. 4A-4B show brain sections stained with NeuN from (A) 1 representative isotype control ("Control IgG") mouse and (B) 1 representative anti-SEMA4D antibody-treated ("Anti-SEMA4D") mouse. Brain volume is represented by the volume of the striatum (solid line) and the hemisphere (dotted line). FIGS. 4C-4D show the calculated ratio (L/R) of striatum and hemispheric volume, respectively, for isotype control ("IgG") mouse and anti-SEMA4D antibody-treated ("SEMA") mouse.

[0014] FIGURES 5A-5B: Show expression of the mature neuronal cell marker, NeuN, in MCAO mice (A) MAb 67-2 ("Sema4D-Ab") or (B) isotype control ("Control IgG") at 30 days after MCAO. The striatum at the border of cerebral infarction is indicated with arrows.

[0015] FIGURES 6A-C: Shows light and dark phase open-field locomotor activity of mice following MCAO and treatment with isotype control ("IgG") or MAb 67-2 ("SEMA4D")

immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. See, *e.g.*, Hamers-Casterman *et al.*, *Nature 363*:446-448 (1993).

[0039] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable domain by one of ordinary skill in the art, since they have been precisely defined (see below).

[0040] In the case where there are two or more definitions of a term that is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat et al. (1983) U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" and by Chothia and Lesk, *J. Mol. Biol. 196*:901-917 (1987), ~~which are incorporated herein by reference,~~ where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues that encompass the CDRs as defined by each of the above cited references are set forth below in **Table 1** as a comparison. The exact residue numbers that encompass a particular CDR will vary

known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable domain to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site.

[0061] It is further recognized that the framework regions within the variable domain in a heavy or light chain, or both, of a humanized antibody may comprise solely residues of human origin, in which case these framework regions of the humanized antibody are referred to as "fully human framework regions" (for example, MAb VX15/2503, disclosed in U.S. Patent Appl. Publication No. US 2010/0285036 A1 as MAb 2503, ~~incorporated herein by reference in its entirety~~). Alternatively, one or more residues of the framework region(s) of the donor variable domain can be engineered within the corresponding position of the human framework region(s) of a variable domain in a heavy or light chain, or both, of a humanized antibody if necessary to maintain proper binding or to enhance binding to the SEMA4D antigen. A human framework region that has been engineered in this manner would thus comprise a mixture of human and donor framework residues, and is referred to herein as a "partially human framework region."

[0062] For example, humanization of an anti-SEMA4D antibody can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature 321*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-327 (1988); Verhoeyen *et al.*, *Science 239*:1534-1536 (1988)), by substituting rodent or mutant rodent CDRs or CDR sequences for the corresponding sequences of a human anti-SEMA4D antibody. See also U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205; ~~herein incorporated by reference~~. The resulting humanized anti-SEMA4D antibody would comprise at least one rodent or mutant rodent CDR within the fully human framework regions of the variable domain of the heavy and/or light chain of the humanized antibody. In some instances, residues within the framework regions of one or more variable domains of the humanized anti-SEMA4D antibody are replaced by corresponding non-human (for example, rodent) residues (see, for example, U.S. Pat. Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370), in which case the resulting humanized anti-SEMA4D antibody would comprise partially human framework regions within the variable domain of the heavy and/or light chain.

[0063] Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (*e.g.*, to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.*, *Nature 331*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-329 (1988); and Presta, *Curr. Op. Struct. Biol. 2*:593-596 (1992); herein incorporated by reference. Accordingly, such "humanized" antibodies may include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies. See, for example, U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Pat. No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.

II. Neural Stem/ Precursor Cells or Neural Stem/ Progenitor Cells (NSPCs)

[0064] Neurogenesis refers generally to the production of new neurons. Traditionally, neurogenesis was thought to occur only during the embryonic and early postnatal periods and to have no significant role in the adult brain. In recent years, however, neurogenesis has been postulated to occur in selected regions of adult mammalian brain and may be stimulated in these as well as other regions in response to injury.

[0065] During development of the central nervous system and peripheral nervous system, neural stem cells proliferate and divide into progenitor cells that eventually differentiate into cell types that compose the adult brain. Cells derived from the neural tube give rise to neurons and glia of the CNS, while cells derived from the neural crest give rise to the cells of the peripheral nervous system (PNS).

[0066] Neural stem/progenitor cells and their therapeutic use have been described in the art, for example, see U.S. Pat. No. 6,638,501, Bjornson et al.; U.S. Pat. No. 6,541,255, Snyder et al.; U.S. Pat. No. 6,498,018, Carpenter; U.S. Patent Application 20020012903, Goldman et al.; Palmer et al. (2001) Nature 411(6833):42-3; Palmer et al. (1997) Mol Cell Neurosci. 8(6):389-404; Svendsen et al. (1997) Exp. Neurol. 148(1):135-46 and Shihabuddin (1999) Mol Med Today. 5(11):474-80. Methods for isolation and culture of neural stem cells are also known in the art, see U.S. Pat. No. 6,777,233; U.S. Pat. No. 6,497,872, and US Patent application 20030143737A1. ~~All these references are specifically incorporated by reference.~~

[0067] Neural stem and precusor cells participate in normal development through migration along well-established migratory pathways to disseminated CNS regions, differentiation into cell types in response to microenvironmental cues, and interspersion with host progenitors and their progeny. Human neural stem cells are capable of expressing foreign transgenes in vivo in these disseminated locations. As such, these cells have the potential to be used to treat a variety of conditions affecting the CNS, including degenerative disorders (e.g. Alzheimer's and Parkinson's), acute brain injury (e.g. stroke, head injury, cerebral palsy) and a large number of CNS dysfunctions (e.g. depression, epilepsy, and schizophrenia).

[0068] In recent years neurodegenerative disease has become an important concern due to the expanding elderly population which is at greatest risk for these disorders. These diseases, which include Alzheimer's Disease, Huntington's Disease, Amyotrophic Lateral Sclerosis, and Parkinson's Disease, have been linked to the degeneration of neural cells in particular locations of the CNS, leading to the inability of these cells or the brain region to carry out their intended function.

[0069] Degeneration in a brain region known as the basal ganglia can lead to diseases with various cognitive and motor symptoms, depending on the exact location. The basal ganglia consists of many separate regions, including the striatum (which consists of the caudate and putamen), the globus pallidus, the substantia nigra, substantia innominate, ventral palladium, nucleus basalis of Meynert, ventral tegmental area and the subthalamic nucleus.

[0070] In Alzheimer's disease, for instance, there is cellular degeneration of the forebrain and cerebral cortex. In addition, there appears to be localized degeneration in an area of the

13:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); and Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001)).

[0079] SEMA4D knock out (SEMA4D-/-) mice have provided additional evidence that SEMA4D plays an important role in both humoral and cellular immune responses. There are no known abnormalities of non-lymphoid tissues in SEMA4D-/- mice. Dendritic cells (DCs) from the SEMA4D-/- mice have poor allostimulatory ability and show defects in expression of costimulatory molecules, which can be rescued by the addition of sSEMA4D. Mice deficient in SEMA4D (SEMA4D-/-) fail to develop experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein peptide, because myelin oligodendrocyte glycoprotein-specific T cells are poorly generated in the absence of SEMA4D (Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002)). A significant amount of soluble SEMA4D is also detected in the sera of autoimmunity-prone MRL/lpr mice (model of systemic autoimmune diseases such as SLE), but not in normal mice. Further, the levels of sSEMA4D correlate with levels of auto-antibodies and increase with age (Wang *et al.*, *Blood 97*:3498-3504 (2001)). Soluble SEMA4D has also been shown to accumulate in the cerebral spinal fluid and sera of patients with demyelinating disease, and sSEMA4D induces apoptosis of human pluripotent neural precursors (Dev cells), and both inhibits process extension and induces apoptosis of rat oligodendrocytes *in vitro* (Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004)). This apoptosis was blocked by an anti-SEMA4D MAb.

IV. Anti-SEMA4D Antibodies

[0080] Antibodies that bind SEMA4D have been described in the art. See, for example, US Publ. Nos. 2008/0219971 A1, US 2010/0285036 A1, and US 2006/0233793 A1, International Patent Applications WO 93/14125, WO 2008/100995, and WO 2010/129917, and Herold *et al.*, *Int. Immunol.* 7(1): 1-8 (1995), each of which is herein incorporated in its entirety by reference.

[0081] The invention generally relates to a method of promoting neurogenesis in a subject having neurodegenerative disorders, neuroinflammatory disorders, acute brain injuries, and certain CNS dysfunctions e.g., a human patient, comprising administration of an antibody which specifically binds to SEMA4D, or an antigen-binding fragment, variant, or derivative thereof. In certain embodiments, the antibody blocks the interaction of SEMA4D with one or more of its receptors, e.g., Plexin-B1. Anti-SEMA4D antibodies

[0093] Also included for use in the methods provided herein are polypeptides encoding anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof as described herein, polynucleotides encoding such polypeptides, vectors comprising such polynucleotides, and host cells comprising such vectors or polynucleotides, all for producing anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof for use in the methods described herein.

[0094] Suitable biologically active variants of the anti-SEMA4D antibodies of the invention can be used in the methods of the present invention. Such variants will retain the desired binding properties of the parent anti-SEMA4D antibody. Methods for making antibody variants are generally available in the art.

[0095] Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York); Kunkel, Proc. *Natl. Acad. Sci. USA 82*:488-492 (1985); Kunkel *et al.*, *Methods Enzymol. 154*:367-382 (1987); Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest may be found in the model of Dayhoff *et al.* (1978) in Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.), pp. 345-352, herein incorporated by reference in its entirety. The model of Dayhoff *et al.* uses the Point Accepted Mutation (PAM) amino acid similarity matrix (PAM 250 matrix) to determine suitable conservative amino acid substitutions. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. Examples of conservative amino acid substitutions as taught by the PAM 250 matrix of the Dayhoff *et al.* model include, but are not limited to, Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln, and Phe↔Trp↔Tyr.

[0096] In constructing variants of the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof, polypeptides of interest, modifications are made such that variants continue to possess the desired properties, *e.g.*, being capable of specifically binding to a SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D, *e.g.*, expressed on the surface of or secreted by a cell and having SEMA4D blocking activity, as described herein. Obviously, any mutations made in the DNA encoding the variant

polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

[0097] Methods for measuring anti-SEMA4D binding molecule, e.g., an antibody or antigen-binding fragment, variant, or derivative thereof, binding specificity include, but are not limited to, standard competitive binding assays, assays for monitoring immunoglobulin secretion by T cells or B cells, T cell proliferation assays, apoptosis assays, ELISA assays, and the like. See, for example, such assays disclosed in WO 93/14125; Shi *et al.*, *Immunity 13*:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001); Wang *et al.*, *Blood 97*:3498-3504 (2001); and Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004), all of which are herein incorporated by reference.

[0098] When the issue is discussed herein whether any particular polypeptide, including the constant regions, CDRs, VH domains, or VL domains disclosed herein, is at least about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or even about 100% identical to another polypeptide, the % identity can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). BESTFIT uses the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482-489, to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0099] For purposes of the present invention, percent sequence identity may be determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) Adv.

blocking activity" can include activity which modulates one or more of the following activities associated with SEMA4D: B cell activation, aggregation and survival; CD40-induced proliferation and antibody production; antibody response to T cell dependent antigens; T cell or other immune cell proliferation; dendritic cell maturation; demyelination and axonal degeneration; apoptosis of pluripotent neural precursors and/or oligodendrocytes; induction of endothelial cell migration; inhibition of spontaneous monocyte migration; binding to cell surface Plexin-B1 or other receptor, or any other activity association with soluble SEMA4D or SEMA4D that is expressed on the surface of SEMA4D+ cells. Anti-SEMA4D activity can also be attributed to a decrease in incidence or severity of diseases associated with SEMA4D expression, including, but not limited to, certain types of cancers including lymphomas, autoimmune diseases, inflammatory diseases including central nervous system (CNS) and peripheral nervous system (PNS) inflammatory diseases, transplant rejections, and invasive angiogenesis. Examples of optimized antibodies based on murine anti-SEMA4D MAbs BD16 and BB18, were described in US Publ. No. 2008/0219971 A1, International Patent Application WO 93/14125 and Herold *et al.*, *Int. Immunol.* 7(1): 1-8 (1995), each of which are herein incorporated by reference in their entirety. The modifications may involve replacement of amino acid residues within the CDR such that an anti-SEMA4D antibody retains specificity for the SEMA4D antigen and has improved binding affinity and/or improved anti-SEMA4D activity.

[0106] In certain embodiments, the binding molecule of the application, *e.g.*, an anti-SEMA4D antibody or antigen binding fragment thereof, inhibits SEMA4D activity and/or the interaction of SEMA4D with a SEMA4D receptor or portion thereof. In certain embodiments, the inhibited SEMA4D activity includes one or more of the following: B cell activation, aggregation and/or survival; CD40-induced proliferation and/or antibody production; antibody response to T cell dependent antigens; T cell or other immune cell proliferation; dendritic cell maturation; demyelination and axonal degeneration; apoptosis of pluripotent neural precursors and/or oligodendrocytes; induction of endothelial cell migration; inhibition of spontaneous monocyte migration; SEMA4D dimerization; binding to cell surface Plexin-B1 or other receptor, or any other activity association with soluble SEMA4D or SEMA4D that is expressed on the surface of SEMA4D+ cells.

Lewin (2003) Genes VIII (Prentice Hall2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0134] ~~All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.~~

[0134] ~~[0135]~~ The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Experimental Design

[0135] ~~[0136]~~ The basic experimental design is shown in FIG. 1. Male, 6 week-old, CB-17/lcr-1/1Jcl mice ("CB-17 mice") were used to evaluate the effect of anti-SEMA4D antibodies on neurogenesis following stroke injury. The mice were subjected to middle cerebral artery (MCA) occlusion (MCAO). In brief, animals were placed under deep anesthesia induced with 4% halothane in a 30% oxygen/70% nitrous oxide mixture, and the anesthetic plane was maintained with 1-2% halothane. Permanent focal cerebral ischemia was produced by ligation and disconnection of the distal portion of the left middle cerebral artery (MCA) as described elsewhere (Taguchi et al., 2004, 2007). The left MCA was isolated, electrocauterized, and disconnected just distal to crossing the olfactory tract (distal M1 portion) with animals under halothane anesthesia. Cerebral blood flow (CBF) in the MCA area was monitored as described previously (Matsushita et al., 1998). Cerebral infarction produced in this mouse strain is highly reproducible and limited to the ipsilateral cerebral cortex (Taguchi et al., 2004, 2007).

[0136] ~~[0137]~~ Following the occlusion, the mice were divided into two groups: the treatment group was injected with anti-SEMA4D antibody MAb 67-2 and the control group was injected with IgG isotype control MAb 2B8. Mice received 0.6 mg/kg of monoclonal antibody via intraperitoneal (IP) injection at 1 hour, 3 hours, 3 days, 7 days, 14 days and 21 days after MCAO.

[0137] ~~[0138]~~ At day 7 following MCAO, a subset of animals from each antibody-injected cohort was sacrificed, brains extracted, and tissues analyzed by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR).

[0138] ~~[0139]~~ For immunohistochemical analyses of brain sections, animals were perfused with a 2% paraformaldehyde-lysine-periodate (PLP) fixative (0.75 M lysine-HCl/0.01 M

periodate/0.075 M phosphate buffer), and the brains were cut into 20-μm-thick serial sections on a cryostat. Brain sections were treated with 0.1% H_2O_2 for quenching, washed in phosphate-buffered saline (PBS), and incubated overnight with one or more of the following primary antibodies: anti-NeuN for neurons, anti-Nestin and anti-Sox2 for neural stem/precursor cells in dilution buffer (0.3% Triton-X-100/5% horse serum/PBS). After being washed three times in PBS, the sections were incubated with the appropriate secondary antibodies for 3 hours, and for NeuN staining, were then visualized by the ABC reaction using the peroxidase ABC Elite Kit (Vector Laboratories, Burlingame, CA) with diaminobenzidine (DAB; Sigma). For Nestin/Sox2 double immunocytochemistry, FITC- (Sox2) and Cy3-labeled (Nestin) secondary antibodies were used. Fluorescent photomicrographs were obtained with a laser confocal microscope. The results are shown in Figure 2A-E and discussed in detail below.

[0139] **[0140]** For RT-PCR analyses, total RNA was isolated from microdissected brain tissue at day 7 post-MCAO and cDNA prepared. cDNA was PCR-amplified under the following conditions: 15 s at 94°C, 30 s at 56°C, and 1 min at 68°C (40 cycles). Primer sequences were as follows: nestin forward, CACTAGAAAGCAGGAACCAG (SEQ ID NO:11) and nestin reverse, AGATGGTTCACAATCCTCTG (SEQ ID NO:12) (amplicon size, 307 bp); Sox2 forward, TTGGGAGGGGTGCAAAAAGA (SEQ ID NO:13) and Sox2 reverse, CCTGCGAAGCGCCTAACGTA (SEQ ID NO:14) (amplicon size, 312 bp); proteolipid protein (PLP) forward, TGAGCGCAACGGTAACAGG (SEQ ID NO:15) and PLP reverse, GGGAGAACACCATACATTCTGG (SEQ ID NO:16) (amplicon size, 295 bp); and β-actin forward,GCTCGTCGTCGACAAGGGCTC (SEQ ID NO:17) and β-actin reverse,CAAACATGATCTGGGTCATCTTCTC (SEQ ID NO:18) (amplicon size, 353 bp). The results are shown in Figure 3A-D and discussed in detail below.

[0140] **[0141]** Behavioral assessments were performed on the remaining cohorts of mice on days 14 and 30 following MCAO. To assess cortical function, mice were analyzed by behavioral testing using the open field task. Briefly, animals were allowed to search freely in a square acrylic box (30 x 3 x 30 cm) for 20 minutes. A light source on the ceiling of the enclosure was on during the first 10 min (light period) and off during a subsequent 10-min period (dark period). On the X- and Y-banks of the open field, two infrared beams were mounted 2 cm above the floor, spaced with at 10-cm intervals, forming a flip–flop circuit between them. The total number of beam crossings by the animal was counted and

scored as traveling behavior (locomotion). The results are shown in Figure 6A-C and discussed in detail below.

[0141] [0142] On day 30 following MCAO, the remaining cohorts of mice were sacrificed and brains processed for NeuN immunohistochemistry. Mice were perfused transcardially with 4% paraformaldehyde, brains were removed, and coronal sections (14 μm) were stained with mouse antibody to NeuN, followed by reaction with biotinylated goat anti-mouse IgG (Chemicon, Temecula, CA; 1/500), ABC Elite Kit (Vector Laboratories, Burlingame, CA), and DAB (Sigma) as chromogen. The area of the ipsilateral and contralateral cerebral hemisphere occupied by the neuronal nuclear marker NeuN was measured using Image J. Ipsilateral and contralateral cerebral hemisphere volume was calculated by integrating coronally oriented ipsilateral and contralateral cerebral hemisphere area. Involution of ipsilateral cerebral hemisphere volume was calculated as (ipsilateral/contralateral cerebral hemisphere volume). The results are shown in Figure 4A-D and Figure 5A-B and discussed in detail below.

Example 2: Effect of Anti-SEMA4D Antibody on the Presence of Neural Stem/Precursor Cells in MCAO Brain

[0142] [0143] At 7 days after MCAO, the effect of anti-SEMA4D antibody on the presence of neural stem/precursor cells was examined. FIG. 2 shows images of immunocytochemical staining of ischemic tissue from 2 representative areas in the border of infarction (symbolized in Figure 2E) of isotype control (left panels, A and C) and anti-SEMA4D antibody-treated mice (right panels, B and D). These results show that mice treated with anti-SEMA4D antibody have increased expression of neural stem/progenitor cell marker proteins as compared to mice treated with control antibody. These data suggests that anti-SEMA4D antibody may protect neural stem/precursor cell populations and/or induce neurogenesis in the ischemic brain.

[0143] [0144] The mRNA expression of neural stem/precursor cells in mice treated with anti-SEMA4D antibody and isotype control was detected by conventional RT-PCR in the ischemic tissue on day 7 after MCAO. These results are shown in FIGS. 3A-D. The results show a significant increase ($p<0.05$) in expression of Sox2, nestin and PLP relative to the housekeeping gene, β-actin, in mice treated with anti-SEMA4D antibody as compared to those treated with isotype control antibody. Since Sox2 and nestin are markers for neural

stem/precursor cells, increased expression of these markers following treatment with anti-SEMA4D antibody suggests that anti-SEMA4D antibody may promote neurogenesis. An increase in neurogenesis observed after anti-SEMA4D treatment in the ischemic brain could be a result of increased proliferation of neural stem/precursor cells, and/or increased survival of newly formed neural stem/precursor cells.

Example 3: Effect of Anti-SEMA4D Antibody on Brain Volume After Cerebral Infarction

[0144][0145] At 30 days after MCAO, the effect of anti-SEMA4D antibody on the volume of the brain was examined. FIGS. 4A-4B show brain sections stained with an antibody specific for the mature neuronal marker, NeuN, from 1 representative isotype control mouse (left, 4A) and 1 representative anti-SEMA4D antibody-treated mouse (right, 4B). Brain volume is represented by the volume of the striatum (solid line) and the hemisphere (dotted line). FIGS. 4C-4D show the calculated ratio (L/R) of striatum and hemisphere volume, respectively, for each group of treated mice. While there is no statistically significant difference in the total hemispheric volume of the brains (dashed line) between mice treated with anti-SEMA4D antibody and those treated with isotype control, mice treated with anti-SEMA4D antibody have significantly larger striatal volume (solid line) than mice treated with control antibody. These data suggest that striatal regions proximal to the focal ischemic insult may be protected by anti-SEMA4D antibody treatment.

Example 4: Effect of Anti-SEMA4D Antibody on the Presence of Neurons in MCAO Brain

[0145][0146] At 30 days after MCAO, the effect of anti-SEMA4D antibody on the presence of neurons in the brain was examined. FIG. 5 shows NeuN-stained images of ischemic brain tissue from 1 representative isotype control mouse (bottom panels, 5B) and 1 representative anti-SEMA4D antibody-treated mouse (top panels, 5A). These results show that mice treated with anti-SEMA4D antibody have increased expression of the mature neuronal cell marker, NeuN, in the striatum at the border of cerebral infarction (arrows), when compared to mice treated with control antibody. These data suggest that anti-SEMA4D antibody treatment promotes the development of mature neurons, potentially by

protecting neural stem/precursor cell populations and/or inducing neurogenesis in the ischemic brain.

Example 5: Effect of Anti-SEMA4D Antibody on Behavioral Activity of MCAO Mice

[0146] [0147] At 30 days after MCAO, the effect of anti-SEMA4D antibody on behavioral activity was examined. Mice treated with anti-SEMA4D antibody and control IgG were subjected to an open-field test for 10 minutes under the light and dark environment. FIGS. 6A-6B show how mice undergoing MCAO (both control IgG and anti-SEMA4D antibody treated mice) have significantly higher activity in light phase ($p<0.05$) compared to sham-treated mice, but not in dark phase. Mice usually have increased activity when they are put into the dark from the light environment. FIG. 6C shows that the ratio of dark/light phase locomotion activity was significantly improved in mice treated with anti-SEMA4D antibody, comparable to sham-treated mice, in contrast to mice treated with control antibody ($p<0.05$). The dark/light phase ratios were not significantly different between the anti-SEMA4D and sham surgery group. These results suggest that anti-SEMA4D antibody treatment of MCAO-lesioned mice may act to normalize their responses to dark/light stimuli, while the mice receiving control IgG appear to maintain abnormal dark/light activity typically exhibited by MCAO-lesioned mice.

Example 6: Effect of Anti-SEMA4D Antibody on Blood Brain Barrier Integrity and Neurogenesis

[0147] [0148] Another study will be conducted to assess the effect(s) of anti-SEMA4D on blood-brain barrier (BBB integrity) and neurogenesis in a rat model of permanent middle cerebral artery occlusion (MCAO).

[0148] [0149] The basic experimental design is shown in FIG. 7. The rats will be subjected to middle cerebral artery (MCA) occlusion (MCAO). Following the occlusion, the rats will be divided into three groups of 15 rats: a treatment group will be injected with anti-SEMA4D antibody MAb 67-2, a control group will be injected with IgG isotype control MAb 2B8, and a non-MCAO group will be injected with IgG isotype control MAb 2B8. Rats will receive 15.0 mg/kg of monoclonal antibody via intraperitoneal (IP) injection at 1 hour, 3 days, 7 days, 14 days and 21 days after MCAO. Blood samples will be taken

periodically for later assessment of drug levels. Rats will receive scheduled antibody injections and will undergo behavioral testing (cylinder test and limb placing test) over a 4-week timeframe.

[0149] [0150] Magnetic resonance imaging (MRI) will be used to assess the BBB integrity of 5 randomly selected rats from each treatment group. Another subset of rats (i.e., 5 rats) will receive i.p. BrdU injections on Days 4-6 to label neuroprogenitor cells. At termination, the subset of rats receiving BrdU will be perfused with 4% paraformaldehyde and brains extracted. The third subset of rats will be perfused with saline, brains microdissected, and tissue fresh-frozen. Extracted brains/microdissected tissues will undergo immunohistochemical and biochemical assessment of microglial activation and neurogenesis.

[0150] [0151] Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims and list of embodiments disclosed herein. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

WHAT IS CLAIMED IS:

1. Use of an isolated binding molecule which specifically binds semaphorin-4D (SEMA4D) in the manufacture of a medicament for promoting neurogenesis in neural tissue of a patient exhibiting at least one symptom of a central nervous system disorder.

2. The use of claim 1, wherein the isolated binding molecule inhibits SEMA4D activity.

3. The use of claim 1, wherein the inhibited SEMA4D activity is dimerization.

4. The use of claim 1, wherein the isolated binding molecule inhibits SEMA4D interaction with a receptor or a portion of the receptor.

5. The use of claim 4, wherein the receptor is selected from the group consisting of Plexin-B1, Plexin-B2 or CD72.

6. The use of claim 1, wherein the isolated binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D.

7. The use of claim 1, wherein the isolated binding molecule specifically binds to the same SEMA4D epitope as a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67.

8. The use of claim 1, wherein the isolated binding molecule comprises an antibody or antigen-binding fragment thereof.

9. The use of claim 8, wherein the antibody or antigen-binding fragment thereof is or is derived from monoclonal antibody VX15/2503 or 67.

10. The use of claim 8, wherein the antibody is chimeric, human or humanized.

1 1. The use of claim 8, wherein the antibody comprises the six CDRs from monoclonal antibody VX15/2503 or 67.

12. The use of claim 1 wherein the central nervous system disorder is selected from the group consisting of a neurodegenerative disorder, acute brain injury, and certain CNS dysfunctions.

13. The use of claim 12 wherein the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Huntington's Disease, Amyotrophic Lateral Sclerosis, Parkinson's Disease, and multiple sclerosis.

14. The use of claim 12 wherein the CNS dysfunction is selected from the group consisting of depression, epilepsy, and schizophrenia.

15. The use of claim 1, wherein the method increases the number of, enhances differentiation of, and/or increases survival of progenitor cells.

16. Use of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the manufacture of a medicament for increasing the number of progenitor cells in a subject with a central nervous system disorder.

17. The use of claim 16, wherein the method reduces the burden of the central nervous system disorder in the subject.

18. Use of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the manufacture of a medicament for treating stroke in a patient.

19. The use of claim 18, wherein the isolated binding molecule specifically binds to SEMA4D.

20. The use of claim 18, wherein the isolated binding molecule inhibits SEMA4D interaction with a receptor or a portion of the receptor.

21. The use of claim 18, wherein the isolated binding molecule is an antibody or an antigen-binding fragment thereof.

22. The use of claim 17, wherein the an antibody or an antigen-binding fragment thereof that specifically binds to SEMA4D comprises a variable heavy domain having the sequence set forth in SEQ ID NO: 1 or 3 and a variable light domain having the sequence set forth in SEQ ID NO: 2 or 4.

23. The use of claim 12 wherein the acute brain injury is selected from the group consisting of stroke, head injury, cerebral palsy, cerebral infarction, spinal cord injury, and traumatic injury.

WHAT IS CLAIMED IS:

1. Use of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for treating stroke, wherein administration of an effective amount of the antibody or fragment thereof can promote neurogenesis in neural tissue of a subject following stroke injury, and wherein administration of an effective amount of the antibody or fragment thereof to the subject can increase one or more of proliferation, differentiation, or migration of neural progenitor cells.

2. The use of claim 1, wherein the antibody or fragment thereof can inhibit SEMA4D interaction with a receptor or a portion of the receptor.

3. The use of claim 2, wherein the receptor is selected from the group consisting of Plexin-Bl, Plexin-B2, and CD72.

4. The use of claim 1, wherein the antibody or fragment thereof:

a. competitively inhibits a reference monoclonal antibody comprising: a variable domain of a heavy chain (VH) comprising the amino acid sequence SEQ ID NO: 1, and a variable domain of a light chain (VL) comprising the amino acid sequence SEQ ID NO: 2; or

b. binds to the same epitope as a reference monoclonal antibody comprising: a VH comprising the amino acid sequence SEQ ID NO: 1, and a VL comprising the amino acid sequence SEQ ID NO: 2.

5. The use of claim 4, wherein the antibody or fragment thereof comprises: a VH comprising the VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences SEQ ID NOs: 5, 6, and 7, respectively; and a VL comprising the VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences SEQ ID NOs: 8, 9, and 10, respectively.

6. The use of claim 5, wherein the antibody or fragment thereof comprises a VH comprising the amino acid sequence SEQ ID NO: 1 and a VL comprising the amino acid sequence SEQ ID NO: 2.

7. The use of claim 5, wherein the antibody or fragment thereof comprises a VH comprising the amino acid sequence SEQ ID NO: 3 and a VL comprising the amino acid sequence SEQ ID NO: 4.

8. The use of any one of claims 1 to 7, wherein the antibody or fragment thereof is to be administered to the blood side of the blood brain barrier.

9. The use of any one of claims 1 to 7, wherein the antibody or fragment thereof is to be administered to the brain side of the blood brain barrier.

10. The use of any one of claims 1 to 7, wherein the subject exhibits an increase in differentiation of neural progenitor cells.

11. The use of any one of claims 1 to 7, wherein the subject is human.

12. The use of any one of claims 1 to 7, wherein administration of an effective amount of the antibody or fragment thereof to the subject results in an increase in expression of a neural precursor or progenitor cell marker in the subject.

13. The use of claim 12, wherein the marker is Sox2 and/or nestin.

14. The use of any one of claims 1 to 7, wherein the antibody or fragment thereof is an IgG isotype.