

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number
WO 02/00709 A2

(51) International Patent Classification⁷: **C07K 14/47**

(21) International Application Number: PCT/US01/14843

(22) International Filing Date: 11 June 2001 (11.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/213,944 23 June 2000 (23.06.2000) US

(71) Applicant (*for all designated States except US*): **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **SU, Eric, Wen** [US/US]; 13447 Dunes Drive, Carmel, IN 46032 (US).

(74) Agents: **KELEHER, Gerald, P.** et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- entirely in electronic form (except for this front page) and available upon request from the International Bureau

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CEREBELLIN HOMOLOGOUS POLYPEPTIDES AND THERAPEUTIC USES THEREOF

(57) Abstract: The present invention provides nucleic acid sequences encoding novel human cerebellin-like protein. These novel nucleic acids are useful for constructing the claimed DNA vectors and host cells of the invention and for preparing the claimed recombinant proteins and antibodies.



WO 02/00709 A2

-1-

**CEREBELLIN HOMOLOGOUS POLYPEPTIDES
AND THERAPEUTIC USES THEREOF**

FIELD OF THE INVENTION

The present invention relates to the identification and isolation of novel DNA, therapeutic uses and the recombinant production of novel polypeptides having sequence homology to human cerebellum, designated herein as LP232 polypeptide. The present invention also relates to vectors, host cells, and antibodies directed to LP232 polypeptides.

BACKGROUND OF THE INVENTION

The cerebellum contains a hexadecapeptide, termed cerebellin. Three independent, overlapping cDNA clones were isolated from a human cerebellum cDNA library that encode the cerebellin sequence. The longest clone codes for a protein of 193 amino acids that has been named precerebellin. *Proc Natl Acad Sci USA*: **88**(3):1069-73; Feb 1(1991). This protein has a significant similarity (31.3% identity, 52.2% similarity) to the globular region of the B chain of human complement component C1q. The region of relatedness extends over approximately 145 amino acids located in the carboxyl terminus of both proteins.

The precerebellin amino terminus contains three N-linked glycosylation sites but does not show a classical signal-peptide motif. No other obvious membrane-spanning domains were predicted from the cDNA sequence. The cDNA predicts that cerebellin is flanked by Val-Arg and Glu-Pro residues. Therefore, cerebellin is liberated from precerebellin by some means other than the classical dibasic amino acid proteolytic-cleavage mechanism seen in many neuropeptide precursors.

-2-

Precerebellin transcripts are abundant in the cerebellum but are present at either very low or undetectable levels in other brain areas. During rat development, it was shown that precerebellin transcripts mirror the level of cerebellin. Low levels of precerebellin mRNA are seen at birth. Levels increase modestly from postpartum day 1 to 8, then increase more dramatically between day 5 and 15. Eventually, they reach peak values between day 21 and 56. *Proc Natl Acad Sci USA*: **88**(3):1069-73; Feb 1(1991). Moreover, cerebellin-like immunoreactivity has been shown to be associated with Purkinje cell postsynaptic structures which suggests that the cerebellin precursor may be involved in synaptic physiology.

A murine homolog of precerebellin, Cbln1, and a closely related gene, Cbln2 have been cloned. *Brain Res Mol Brain Res*; **27**(1):152-6 (1994). Amino acid comparison of Cbln1 with Cbln2 showed that Cbln2 is 88% identical to the carboxy terminal region of Cbln1. Southern blot analysis and genome mapping confirmed that these are independent genes.

Recently, cerebellin-2 was described in WO9942576-A1 and is about 90% homologous to LP232. Cerebellin-2 was indicated as useful in treating or preventing neurological disorders associated with the inappropriate expression of cerebellin-2 proteins and disruption of the synapse function. Owing to its homology to cerebellin-2, LP232 is useful for treating neurologic disorders that include Parkinson's disease, Alzheimer's disease, bipolar and unipolar affective disorders, schizophrenia, olivopontocerebellar atrophy, Shy-Dager syndrome and other disorders caused by disruption of synapse function. LP232 is also useful as an antigen in vaccine and antibody production as well as in assays to identify agonists and antagonists of LP232 function.

-3-

In general, all novel proteins are of interest. Extracellular proteins play an important role in the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Secreted proteins have various industrial applications, including pharmaceuticals, diagnostics, biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins. Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents.

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., *Proc. Natl. Acad. Sci., B*: 7108-13 (1996); U.S. Patent No. 5,536,637)]. The results of such efforts are presented herein.

-4-

SUMMARY OF THE INVENTION

The present invention provides nucleic acid sequences encoding the novel human LP232. These novel nucleic acids are useful for constructing the claimed DNA vectors and host cells of the invention and for preparing the claimed recombinant proteins and antibodies. In particular, a human EST cDNA clone is disclosed that contains an open reading frame encoding a 287 amino acid homologue of human cerebellin.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Applicants have identified a cDNA clone that encodes a novel polypeptide having sequence identity with cerebellin, wherein the polypeptide is designated in the present application as "LP232". In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding the LP232 polypeptide. In another aspect, the isolated nucleic acid comprises DNA encoding the LP232 polypeptide having amino acid residues from about 1 through 287 of SEQ ID NO: 1, or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, high stringency conditions.

**MSGRRALSAVPAVLLVLTLPGLPVWAQNDTEPIVLEGKCLVVCD
SNPATDSKGSSSSPLGISVRAANSKVAFSAVRSTNHEPSEMSNKT
RIIYFDQVRPGGKRAPRGWAVSRHLSTRLPAQAKLVRLGN GESCK
ALAFPNMPGSETSTVPLPTPLTSPGSHFFRAPKRGCCGGGGEAWE
PRARQREL RASFEEQQSVFVAPRKG IYSFSFHVIKVYQSQT IQVNL
MLNGKPVISAFAGDKDVTREAA TNGVLLYLDKEDKVYLKLEKGNL
VGGWQYSTFSGFLVFPL**

(SEQ ID NO: 1)

-5-

In another embodiment, the isolated nucleic acid comprises DNA having at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) a DNA molecule encoding an LP232 polypeptide comprising the sequence of amino acid residues from 1 or about 27 to 287, inclusive, of SEQ ID NO:1 or (b) the complement of the DNA molecule of (a). Alternatively, the isolated nucleic acid comprises DNA encoding the LP232 polypeptide having the sequence of amino acid residues from about 1 to about 287, inclusive, of SEQ ID NO:1.

In another aspect, the invention concerns an isolated nucleic acid molecule encoding the LP232 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about base pairs: (a) 178 to about 259, inclusive, of SEQ ID NO:2 and (b) 178 to about 1039, inclusive, of SEQ ID NO:2.

```

1  GGA GAC TTT GAC TTC AAG CCA CAG AAT TGG TGG AAG TGT GCG CGC CGC CGC CGT CGC
   CCT CTG AAA CTG AAG TTC GGT GTC TTA ACC ACC TTC ACA CGC GCG GCG GCG GCA GCG

61  TCC TGC AGC GCT GTC GAC CTA GCC GCT AGC ATC TTC CCG AGC ACC GGG ATC CCG GGG TAG
   AGG ACG TCG CGA CAG CTG GAT CGG CGA TCG TAG AAG GGC TCG TGG CCC TAG GGC CCC ATC

+1
121 GAG GCG ACG CGG GCG AGC ACC AGC GCC AGC CGG CTG CGG CTG CCC ACA CGG CTC ACC ATG
   CTC CGC TGC GCC CGC TCG TGG TCG CGG TCG GCC GAC GCC GAC GGG TGT GCC GAG TGG TAC

```

Met

-6-

+2	Gly	Ser	Gly	Arg	Arg	Ala	Leu	Ser	Ala	Val	Pro	Ala	Val	Leu	Leu	Val	Leu	Thr	Leu	Pro
181	GGC	TCC	GGG	CGC	CGG	GCG	CTG	TCC	GCG	GTG	CCG	GCC	GTG	CTG	CTG	GTC	CTC	ACG	CTG	CCG
	CCG	AGG	CCC	GCG	GCC	CGC	GAC	AGG	CGC	CAC	GGC	CGG	CAC	GAC	GAC	CAG	GAG	TGC	GAC	GGC
+22	Gly	Leu	Pro	Val	Trp	Ala	Gln	Asn	Asp	Thr	Glu	Pro	Ile	Val	Leu	Glu	Gly	Lys	Cys	Leu
241	GGG	CTG	CCC	GTC	TGG	GCA	CAG	AAC	GAC	ACG	GAG	CCC	ATC	GTG	CTG	GAG	GGC	AAG	TGT	CTG
	CCC	GAC	GGG	CAG	ACC	CGT	GTC	TTG	CTG	TGC	CTC	GGG	TAG	CAC	GAC	CTC	CCG	TTC	ACA	GAC
+42	Val	Val	Cys	Asp	Ser	Asn	Pro	Ala	Thr	Asp	Ser	Lys	Gly	Ser	Ser	Ser	Ser	Pro	Leu	Gly
301	GTG	GTG	TGC	GAC	TCG	AAC	CCG	GCC	ACG	GAC	TCC	AAG	GGC	TCC	TCT	TCC	TCC	CCG	CTG	GGG
	CAC	CAC	ACG	CTG	AGC	TTG	GGC	CGG	TGC	CTG	AGG	TTC	CCG	AGG	AGA	AGG	AGG	GGC	GAC	CCC
+62	Ile	Ser	Val	Arg	Ala	Ala	Asn	Ser	Lys	Val	Ala	Phe	Ser	Ala	Val	Arg	Ser	Thr	Asn	His
361	ATA	TCG	GTC	CGG	GCG	GCC	AAC	TCC	AAG	GTC	GCC	TTC	TCG	GCG	GTG	CGG	AGC	ACC	AAC	CAC
	TAT	AGC	CAG	GCC	CGC	CGG	TTG	AGG	TTC	CAG	CGG	AAG	AGC	CGC	CAC	GCC	TCG	TGG	TTG	GTG
+82	Glu	Pro	Ser	Glu	Met	Ser	Asn	Lys	Thr	Arg	Ile	Ile	Tyr	Phe	Asp	Gln	Val	Arg	Pro	Gly
421	GAG	CCA	TCC	GAG	ATG	AGC	AAC	AAG	ACG	CGC	ATC	ATT	TAC	TTC	GAT	CAG	GTC	AGA	CCC	GGG
	CTC	GGT	AGG	CTC	TAC	TCG	TTG	TTC	TGC	GCG	TAG	TAA	ATG	AAG	CTA	GTC	CAG	TCT	GGG	CCC
+102	Gly	Lys	Arg	Ala	Pro	Arg	Gly	Trp	Ala	Val	Ser	Arg	His	Leu	Ser	Thr	Arg	Leu	Pro	Ala
481	GGG	AAG	CGA	GCA	CCT	AGG	GGG	TGG	GCG	GTC	TCC	AGG	CAC	CTC	AGC	ACG	AGG	CTG	CCT	GCC
	CCC	TTC	GCT	CGT	GGA	TCC	CCC	ACC	CGC	CAG	AGG	TCC	GTG	GAG	TCG	TGC	TCC	GAC	GGA	CGG
+122	Gln	Ala	Lys	Leu	Val	Arg	Leu	Gly	Asn	Gly	Glu	Ser	Cys	Lys	Ala	Leu	Ala	Phe	Pro	Asn
541	CAG	GCT	AAG	CTG	GTC	CGA	TTG	GGA	AAT	GGG	GAA	TCA	TGT	AAA	GCA	CTC	GCC	TTC	CCA	AAT
	GTC	CGA	TTC	GAC	CAG	GCT	AAC	CCT	TTA	CCC	CTT	AGT	ACA	TTT	CGT	GAG	CGG	AAG	GGT	TTA
+142	Met	Pro	Gly	Ser	Glu	Thr	Ser	Thr	Val	Pro	Leu	Pro	Thr	Pro	Leu	Thr	Ser	Pro	Gly	Ser
601	ATG	CCT	GGG	TCT	GAA	ACT	TCT	ACC	GTC	CCT	CTT	CCT	ACC	CCT	CTC	ACC	AGC	CCC	GGC	TCC
	TAC	GGA	CCC	AGA	CTT	TGA	AGA	TGG	CAG	GGA	GAA	GGA	TGG	GGA	GAG	TGG	TCG	GGG	CCG	AGG
+162	His	Phe	Phe	Arg	Ala	Pro	Lys	Arg	Gly	Cys	Cys	Gly	Gly	Gly	Gly	Glu	Ala	Trp	Glu	Pro
661	CAT	TTC	TTC	CGG	GCC	CCT	AAA	AGA	GGC	TGC	TGC	GGC	GGC	GGG	GGA	GAG	GCT	TGG	GAG	CCC
	GTA	AAG	AAG	GCC	CGG	GGA	TTT	TCT	CCG	ACG	ACG	CCG	CCG	CCC	CCT	CTC	CGA	ACC	CTC	GGG
+182	Arg	Ala	Arg	Gln	Arg	Glu	Leu	Arg	Ala	Ser	Phe	Glu	Gln	Gln	Ser	Val	Phe	Val	Ala	Pro
721	AGA	GCC	CGG	CAG	CGA	GAG	CTC	AGG	GCA	AGT	TTC	GAG	CAA	CAG	TCT	GTC	TTT	GTA	GCA	CCA
	TCT	CGG	GCC	GTC	GCT	CTC	GAG	TCC	CGT	TCA	AAG	CTC	GTT	GTC	AGA	CAG	AAA	CAT	CGT	GGT
+202	Arg	Lys	Gly	Ile	Tyr	Ser	Phe	Ser	Phe	His	Val	Ile	Lys	Val	Tyr	Gln	Ser	Gln	Thr	Ile
781	AGA	AAA	GGA	ATT	TAC	AGT	TTC	AGT	TTT	CAC	GTG	ATT	AAA	GTC	TAC	CAG	AGC	CAA	ACT	ATC
	TCT	TTT	CCT	TAA	ATG	TCA	AAG	TCA	AAA	GTG	CAC	TAA	TTT	CAG	ATG	GTC	TCG	GTT	TGA	TAG
+222	Gln	Val	Asn	Leu	Met	Leu	Asn	Gly	Lys	Pro	Val	Ile	Ser	Ala	Phe	Ala	Gly	Asp	Lys	Asp
841	CAG	GTT	AAC	TTG	ATG	TTA	AAT	GGA	AAA	CCA	GTA	ATA	TCT	GCC	TTT	GCG	GGG	GAC	AAA	GAT
	GTC	CAA	TTG	AAC	TAC	AAT	TTA	CCT	TTT	GGT	CAT	TAT	AGA	CGG	AAA	CGC	CCC	CTG	TTT	CTA
+242	Val	Thr	Arg	Glu	Ala	Ala	Thr	Asn	Gly	Val	Leu	Leu	Tyr	Leu	Asp	Lys	Glu	Asp	Lys	Val
901	GTT	ACT	CGT	GAA	GCT	GCC	ACG	AAT	GGT	GTC	CTG	CTC	TAC	CTA	GAT	AAA	GAG	GAT	AAG	GTT
	CAA	TGA	GCA	CTT	CGA	CGG	TGC	TTA	CCA	CAG	GAC	GAG	ATG	GAT	CTA	TTT	CTC	CTA	TTC	CAA
+262	Tyr	Leu	Lys	Leu	Glu	Lys	Gly	Asn	Leu	Val	Gly	Gly	Trp	Gln	Tyr	Ser	Thr	Phe	Ser	Gly
961	TAC	CTA	AAA	CTG	GAG	AAA	GGT	AAT	TTG	GTT	GGA	GGC	TGG	CAG	TAT	TCC	ACG	TTT	TCT	GGC
	ATG	GAT	TTT	GAC	CTC	TTT	CCA	TTA	AAC	CAA	CCT	CCG	ACC	GTC	ATA	AGG	TGC	AAA	AGA	CCG

-7-

+282 Phe Leu Val Phe Pro Leu ***

```
1021 TTT CTG GTG TTC CCC CTA TAG GAT TCA ATT TCT CCA TGA TGT TCA TCC AGG TGA GGG ATG
    AAA GAC CAC AAG GGG GAT ATC CTA AGT TAA AGA GGT ACT ACA AGT AGG TCC ACT CCC TAC

1081 ACC CAC TCC TGA GTT ATT GGA AGA TCA TTT TTT CAT CAT TGG ATT GAT GTC TTT TAT TGG
    TGG GTG AGG ACT CAA TAA CCT TCT AGT AAA AAA GTA GTA ACC TAA CTA CAG AAA ATA ACC

1141 TTT CTC ATG GGT GGA TAT GGA TCT AAG GAT TCT AGC CTG TCT GAA CCA ATA CAA AAT TTC
    AAA GAG TAC CCA CCT ATA CCT AGA TTC CTA AGA TCG GAC AGA CTT GGT TAT GTT TTA AAG

1201 ACC AGA TTA TTT GTG GTG TGT CTG TTT CAG TAA ATT TGG ATT GGG GAC TCT AAG CAG ATA
    TGG TCT AAT AAA CAC CAC ACA GAC AAA GTC ATT TAA ACC TAA CCC CTG AGA TTC GTC TAT

1261 AAT ACC TAA TGG CTT AAA TGG AAC AG
    TTA TGG ATT ACC GAA TTT ACC TTG TC
```

(SEQ ID NO:2)

In another aspect, the invention concerns an isolated nucleic acid molecule encoding an active LP232 polypeptide comprising a nucleotide sequence that hybridizes to the complement of a nucleic acid sequence that encodes amino acids (a) 1 or about 27 to about 287, inclusive, of SEQ ID NO:1 or (b) about 27 to about 287, inclusive, of SEQ ID NO:1. Preferably, hybridization occurs under stringent hybridization and wash conditions.

In a further aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity, to: (a) DNA molecule comprising the sequence of nucleotides from about 1 or about 27 to about 287,

-8-

inclusive, of SEQ ID NO:2 or (b) the complement of the DNA molecule of (a).

In another aspect, the isolated nucleic acid molecule comprises: (a) the nucleotide sequence from about 1 or about 27 to about 287, inclusive, of SEQ ID NO:2 or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule produced by hybridizing a test DNA molecule under stringent conditions with: (a) a DNA molecule encoding an LP232 polypeptide having the sequence of amino acid residues from about 1 or about 27 to about 287.

inclusive, of SEQ ID NO:1, or (b) the complement of the DNA molecule of (a), and if the DNA molecule has at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) or (b), and isolating the test DNA molecule.

In yet a further aspect, the invention concerns an isolated nucleic acid molecule comprising: (a) DNA encoding a polypeptide scoring at least about 91% positives, yet more preferably at least about 92% positives, yet more preferably at least about 93% positives, yet more preferably at least about 94% positives, yet more preferably at least about 95% positives, yet more preferably at least about 96% positives, yet more preferably at least about 97% positives, yet more preferably at least about 98% positives, yet more preferably

-9-

at least about 99% positives, when compared with the amino acid sequence of residues about 27 to about 287 inclusive, of SEQ ID NO:1, or (b) the complement of the DNA of (a). In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding an LP232 polypeptide without the N-terminal signal sequence and/or initiating methionine, or is complementary to such encoding nucleic acid molecule. The signal peptide has been presumptively identified as extending from about amino acid residue 1 to about amino acid residue 27, inclusive, in SEQ ID NO:1. It is noted, however, that the C-terminal boundary of the signal peptide may vary, but likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art [Nielsen et al., *Prot. Engin.* 10(1): 1-6 (1997); von Heijne et al., *Nucl. Acid Res* 14(11): 4683-4690 (1986)]. Moreover, it is also recognized that, in some cases, cleavage of the signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding them, are contemplated by the present invention.

Another embodiment is directed to fragments of a LP232-encoding sequence that may find use as, for example, hybridization probes or for encoding fragments of an LP232 polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-LP232 antibody. Such nucleic acids fragments are usually at least about 20 nucleotides in length, preferably at least about 30 nucleotides in length, more preferable at least about 40 nucleotides in length, yet more preferably at least about 50

-10-

nucleotides in length, yet more preferably at least about 60
nucleotides in length, yet more preferably at least about 70
nucleotides in length, yet more preferably at least about 80
nucleotides in length, yet more preferably at least about 90
nucleotides in length, yet more preferably at least about 100
nucleotides in length, yet more preferably at least about 110
nucleotides in length, yet more preferably at least about 120
nucleotides in length, yet more preferably at least about 130
nucleotides in length, yet more preferably at least about 140
nucleotides in length, yet more preferably at least about 150
nucleotides in length, yet more preferably at least about 160
nucleotides in length, yet more preferably at least about 170
nucleotides in length, yet more preferably at least about 180
nucleotides in length, yet more preferably at least about 190
nucleotides in length, yet more preferably at least about 200
nucleotides in length, yet more preferably at least about 250
nucleotides in length, yet more preferably at least about 300
nucleotides in length, yet more preferably at least about 350
nucleotides in length, yet more preferably at least about 400
nucleotides in length, yet more preferably at least about 450
nucleotides in length, yet more preferably at least about 500
nucleotides in length, yet more preferably at least about 600
nucleotides in length, yet more preferably at least about 700
nucleotides in length, yet more preferably at least about 800
nucleotides in length, yet more preferably at least about 900
nucleotides in length, yet more preferably at least about 1000
nucleotides in length, wherein in this context "about" means
the referenced nucleotide sequence length plus or minus 10% of
that referenced length. In a preferred embodiment, the
nucleotide sequence fragment is derived from any coding region
of the nucleotide sequence shown in SEQ ID NO:2.

-11-

In another embodiment, the invention provides a vector comprising DNA encoding an LP232 polypeptide or its variants. The vector may comprise any of the isolated nucleic acid molecules hereinabove described.

In another embodiment, the invention provides a host cell comprising the above vector. By way of example, the host cells may be CHO cells, *Escherichia coli*, or yeast. A process for producing LP232 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of LP232 polypeptides and recovering LP232 polypeptides from the cell culture.

In another embodiment, the invention provides isolated LP232 polypeptides encoded by any of the isolated nucleic acid sequences hereinabove defined.

In another aspect, the invention concerns an isolated LP232 polypeptide, comprising an amino acid sequence having at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to the sequence of amino acid residues about 1 or about 27 to about 287, inclusive, of SEQ ID NO:1. In a preferred aspect, the polypeptide comprises amino acid residues about 1 or about 27 to about 287 inclusive, of SEQ ID NO:1.

In a further aspect, the invention concerns an isolated LP232 polypeptide comprising an amino acid sequence scoring at least about 91% positives, yet more preferably at least

-12-

about 92% positives, yet more preferably at least about 93% positives, yet more preferably at least about 94% positives, yet more preferably at least about 95% positives, yet more preferably at least about 96% positives, yet more preferably at least about 97% positives, yet more preferably at least about 98% positives, yet more preferably at least about 99% positives, when compared with the amino acid sequence of residues from about 1 or about 27 to about 287, inclusive, of SEQ ID NO:1.

In a specific aspect, the invention provides an isolated LP232 polypeptide without the N-terminal signal sequence and/or initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of LP232 polypeptide and recovering the LP232 polypeptide from the cell culture.

In still a further aspect, the invention provides a polypeptide produced by: (1) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding an LP232 polypeptide having the sequence of amino acid residues from about 27 to about 287, inclusive, of SEQ ID NO:1, or (b) the complement of the DNA molecule of (a); and if the test DNA molecule has at least about an 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more

-13-

preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) or (b); (2) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (3) recovering the polypeptide from the cell culture.

In yet another aspect, the invention concerns an isolated LP232 polypeptide comprising the sequence of amino acid residues from about 1 or about 27 to about 287, inclusive, of SEQ ID NO:1, or a fragment thereof which is biologically active or sufficient to provide a binding site for an anti-LP232 antibody, wherein the identification of an LP232 polypeptide or fragments thereof that possess biological activity or provide a binding site for an anti-LP232 antibody may be accomplished in a routine manner using techniques which are well known in the art.

In another embodiment, the invention provides chimeric molecules comprising an LP232 polypeptide fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises an LP232 polypeptide fused to an epitope tag sequence or a histidine purification handle, or an Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody that specifically binds to an LP232 polypeptide or fragment thereof. Optionally, the antibody is a monoclonal antibody, an antibody fragment or a single chain antibody.

In yet another embodiment, the invention concerns agonists and antagonists of a native LP232 polypeptide. In a particular aspect, the agonist or antagonist is an anti-LP232 antibody or a small molecule.

In yet another embodiment, the invention concerns a method of identifying agonists or antagonists of a native LP232 polypeptide by contacting the native LP232 polypeptide

-14-

with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In still a further embodiment, the invention concerns a composition comprising an LP232 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a carrier. Preferably, the carrier is pharmaceutically acceptable.

In still a further embodiment, the invention concerns the use of an LP232 polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-LP232 antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the LP232 polypeptide or an agonist or antagonist thereof (e.g., anti-LP232 antibody). In a particular aspect, the invention concerns the use of an LP232 polypeptide, or an agonist or antagonist thereof in a method for treating a neurologic disorder.

In still a further embodiment, the invention relates to a method of treating a neurologic disorder by administration of a therapeutically effective amount of an LP232 polypeptide, agonist, or antagonist thereof to a mammal suffering from said disorder.

In still a further embodiment, the invention relates to a method of diagnosing a neurologic disorder by (1) culturing test cells or tissues expressing LP232; (2) administering a compound which can inhibit LP232-modulated signaling; and (3) measuring the LP232 mediated phenotypic effects in the test cells.

In still a further embodiment, the invention relates to LP232 antagonists and/or agonist molecules. In one aspect, the invention provides a method of screening compounds that mimic LP232 (agonists) or diminish the effect of LP232 (antagonists).

-15-

In still a further embodiment, the invention relates to a therapeutic composition comprising a therapeutically effective amount of LP232 polypeptide, antagonist or agonist thereof in combination with a pharmaceutically-acceptable carrier.

In still a further embodiment, the invention relates to an article of manufacture comprising a container, label and therapeutically effective amount of LP232 polypeptide, antagonist or agonist thereof in combination with a pharmaceutically-acceptable carrier.

I. Definitions

The terms "LP232 polypeptide" and "LP232" when used herein encompass native sequence LP232 polypeptide and polypeptide variants thereof (which are further defined herein). The LP232 polypeptides may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence LP232 polypeptide" comprises a polypeptide having the same amino acid sequence as an LP232 polypeptide derived from nature. Such native sequence LP232 polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence LP232 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of an LP232 polypeptide, (e.g., soluble forms containing, for instance, an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of an LP232 polypeptide.

In one embodiment of the invention, the native sequence LP232 polypeptide is a full-length or mature native sequence LP232 polypeptide comprising amino acids 1 or 27 through 287

-16-

of SEQ ID NO:1. Also, while the LP232 polypeptides disclosed in SEQ ID NO:1 are shown to begin with a methionine residue designated as amino acid position 1, it is conceivable and possible that another methionine residue located either upstream or downstream from amino acid position 1 may be employed as the starting amino acid residue.

"LP232 variant" means an "active" LP232 polypeptide as defined below, having at least about 80% amino acid sequence identity with the LP232 polypeptide, having the deduced amino acid sequence of residues 1 or about 27 to about 287 shown in SEQ ID NO:1, for a full-length or mature native sequence LP232 polypeptide. Such LP232 polypeptide variants include, for instance, LP232, wherein one or more amino acid residues are added, substituted or deleted, at the N- or C-terminus or within the sequence of SEQ ID NO:1. Ordinarily, an LP232 polypeptide variant will have at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% amino acid sequence identity with the amino acid sequence of SEQ ID NO:1 with or without the signal peptide (e.g., with signal peptide amino acid residues 1 to 27 of SEQ ID NO:1, without signal peptide about 28 to 287 of SEQ ID NO:1). The variants provided herein exclude native sequence LP232 as well the polypeptides and nucleic acids described herein with which the LP232 polypeptides share 100% identity and/or which are already known in the art.

-17-

"Percent (%) amino acid sequence identity" with respect to the LP232 amino acid sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in an LP232 polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, ALIGN-2, Megalign (DNASTAR) or BLAST (e.g., Blast, Blast-2, WU-Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the % identity values used herein are generated using WU-BLAST-2 [Altschul et al., *Methods in Enzymology* 266: 460-480 (1996)]. Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM 62. For purposes herein, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the LP232 polypeptide of interest and the comparison amino acid sequence of interest (i.e., the sequence against which the LP232 polypeptide of interest is being compared) as determined by WU-BLAST-2, by (b) the total number of amino acid residues of the LP232 polypeptide of interest, respectively.

-18-

A "LP232 variant polynucleotide" or "LP232 variant nucleic acid sequence" means an active LP232 polypeptide-encoding nucleic acid molecule as defined below having at least about 85% nucleic acid sequence identity with the nucleotide acid sequence of nucleotides about 259 to about 1039 of the LP232-encoding nucleotide sequence shown in SEQ ID NO:2. Ordinarily, an LP232 polypeptide will have at least about 85% nucleic acid sequence identity, more preferably at least about 90% nucleic acid sequence identity, yet more preferably at least about 91% nucleic acid sequence identity, yet more preferably at least about 92% nucleic acid sequence identity, yet more preferably at least about 93% nucleic acid sequence identity, yet more preferably at least about 94% nucleic acid sequence identity, yet more preferably at least about 95% nucleic acid sequence identity, yet more preferably at least about 96% nucleic acid sequence identity, yet more preferably at least about 97% nucleic acid sequence identity, yet more preferably at least about 98% nucleic acid sequence identity, yet more preferably at least about 99% nucleic acid sequence identity with the nucleic acid sequence of nucleotides about 178 or about 259 to about 1039 of the LP232-encoding nucleotide sequence shown in SEQ ID NO:2. Variants specifically exclude or do not encompass the native nucleotide sequence, as well as those prior art sequences that share 100% identity with the nucleotide sequences of the invention.

"Percent (%) nucleic acid sequence identity" with respect to the LP232 sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the LP232 sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity

-19-

can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, Align-2, Megalign (DNASTAR), or BLAST (e.g., Blast, Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % nucleic acid identity values are generated using the WU-BLAST-2 (BlastN module) program (Altschul et al., *Methods in Enzymology* 266: 460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1; overlap fraction = 0.125; word threshold (T) = 11; and scoring matrix = BLOSUM62. For purposes herein, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the LP232 polypeptide-encoding nucleic acid molecule of interest and the comparison nucleic acid molecule of interest (i.e., the sequence against which the LP232 polypeptide-encoding nucleic acid molecule of interest is being compared) as determined by WU-BLAST-2, by (b) the total number of nucleotides of the LP232 polypeptide-encoding nucleic acid molecule of interest.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g., as a result of conservative substitutions). The % identity value of positives is determined by the fraction of residues scoring a positive value in the BLOSUM 62 matrix. This value is determined by dividing (a) the number of amino acid residues scoring a positive value in the

-20-

BLOSUM62 matrix of WU-BLAST-2 between the LP232 polypeptide amino acid sequence of interest and the comparison amino acid sequence (i.e., the amino acid sequence against which the LP232 polypeptide sequence is being compared) as determined by WU-BLAST-2, by (b) the total number of amino acid residues of the LP232 polypeptide of interest.

"Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the LP232 polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" LP232 polypeptide-encoding nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the LP232 polypeptide-encoding nucleic acid. An isolated

-21-

LP232 polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated LP232 polypeptide-encoding nucleic acid molecules therefore are distinguished from the LP232 polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated LP232 polypeptide-encoding nucleic acid molecule includes LP232 polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express LP232 polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If

-22-

such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes required higher temperatures for proper annealing, while short probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reactions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, 1995.

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that (1) employ low ionic strength and high temperature for washing, for example, 15 mM sodium chloride/1.5 mM sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride/75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5X SSC (750 mM sodium chloride, 75 mM sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's

-23-

solution, sonicated salmon sperm DNA (50 $\mu\text{g/ml}$), 0.1% SDS, and 10% dextran sulfate at 42°C with washes at 42°C in 0.2X SSC (30 mM sodium chloride/3 mM sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1X SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al. [*Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, (1989)], and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5X SSC (750 mM sodium chloride, 75 mM sodium citrate), 50 mM sodium phosphate at pH 7.6, 5X Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1X SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc., as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" where used herein refers to a chimeric polypeptide comprising an LP232 polypeptide, or domain sequence thereof, fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody may be made, or which can be identified by some other agent, yet is short enough such that it does not interfere with the activity of the LP232 polypeptide. The tag polypeptide preferably is also fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8

-24-

to about 50 amino acid residues (preferably, between about 10 to about 20 residues).

As used herein, the term "immunoadhesion" designates antibody-like molecules that combine the binding specificity of a heterologous protein (an "adhesion") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesions comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesion part of an immunoadhesion molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesion may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

The term "antibody" is used in the broadest sense and specifically covers single anti-LP232 polypeptide monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-LP232 antibody compositions with polyepitopic specificity, single-chain anti-LP232 antibodies, and fragments of anti-LP232 antibodies. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Active" or "activity" for the purposes herein refers to form(s) of LP232 which retain the biologic and/or immunologic activities of native or naturally-occurring LP232 polypeptide. Elaborating further, "biological" activity refers to a

-25-

biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring LP232 polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring LP232 polypeptide. An "immunological" activity refers only to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring LP232 polypeptide.

The term "antagonist" is used in the broadest sense and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native LP232 polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native LP232 polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native LP232 polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists or antagonists of an LP232 polypeptide may comprise contacting an LP232 polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the LP232 polypeptide.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense

-26-

and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

The terms "treating", "treatment" and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventive therapy. An example of "preventive therapy" is the prevention or lessened targeted pathological condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption but, rather, is cyclic in nature.

The term "mammal" as used herein refers to any mammal, classified as a mammal, including humans, domestic and farm animals, and zoo, sports or pet animals, such as cattle (e.g., cows), horses, dogs, sheep, pigs, rabbits, goats, cats, etc. In a preferred embodiment of the invention, the mammal is a human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

A "therapeutically-effective amount" is the minimal amount of active agent (e.g., an LP232 polypeptide, antagonist or agonist thereof) which is necessary to impart therapeutic benefit to a mammal. For example, a "therapeutically-effective amount" to a mammal suffering or prone to suffering

-27-

or to prevent it from suffering from a neurologic disorder is such an amount which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression, physiological conditions associated with or resistance to succumbing to a disorder principally characterized by synaptic dysfunction

"Carriers" as used herein include pharmaceutically-acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically-acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecule weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEENTM, polyethylene glycol (PEG), and PLURONICTM.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (Zapata et al., *Protein Engin.* 8 (10): 1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

-28-

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fv fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly

-29-

distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domain, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun, *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore, eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404.097, WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may

-30-

include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue, or preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alternation of a substrate compound or composition which is detectable.

"Solid phase" is meant to be a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of

-31-

discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an LP232 polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecule weight below about 500 daltons.

The term "modulate" means to affect (e.g., either upregulate, downregulate or otherwise control) the level of a signaling pathway. Cellular processes under the control of signal transduction include, but are not limited to, transcription of specific genes, normal cellular functions, such as metabolism, proliferation, differentiation, adhesion, apoptosis and survival, as well as abnormal processes, such as transformation, blocking of differentiation and metastasis.

Of course, a polynucleotide which hybridizes only to polyA⁺ sequences (such as any 3' terminal polyA⁺ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a polyA⁺ stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The LP232 polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the LP232 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA,

-32-

and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the LP232 polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. LP232 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

LP232 polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the gene-encoded amino acids. The LP232 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the LP232 polypeptides, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given LP232 polypeptide. Also, a given LP232 polypeptide may contain many types of modifications. LP232 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic LP232

-33-

polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, Creighton, *Proteins - Structure and Molecular Properties*, 2nd Ed., W. H. Freeman and Company, New York (1993); Johnson, *Posttranslational Covalent Modification of Proteins*, Academic Press, New York, pp. 1-12 (1983); Seifter et al., *Meth. Enzymol.* 182: 626-46 (1990); Rattan et al., *Ann. NY Acad. Sci.* 663: 48-62 (1992).

II. Compositions and Methods of the Invention

A. Full-length LP232 polynucleotide of the present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as LP232. In particular, applicants have identified and isolated cDNA encoding an LP232 (e.g., LP232-A, SEQ ID NO:1) polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of

-34-

the LP232 polypeptide have sequence identity with human cerebellin. Accordingly, it is presently believed that LP232 polypeptide disclosed in the present application are newly identified members of the cerebellin family and, thus, may be involved in synaptic function.

B. LP232 Variants

In addition to the full-length native sequence LP232 polypeptides described herein, it is contemplated that LP232 variants can be prepared. LP232 variants can be prepared by introducing appropriate nucleotide changes into the LP232-encoding DNA or by synthesis of the desired LP232 polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the LP232 polypeptide, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence LP232 or in various domains of the LP232 polypeptide described herein can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent NO. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding LP232 polypeptide that results in a change in the amino acid sequence of the LP232 polypeptide as compared with the native sequence LP232 polypeptide. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the LP232 polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the LP232 polypeptide with that of homologous known protein molecules and minimizing the number

-35-

of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

LP232 polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length or native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the LP232 polypeptide.

LP232 fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating LP232 fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, LP232 polypeptide fragments share at least one biological and/or immunological activity with the native LP232 polypeptide shown in SEQ ID NO:1.

-36-

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 1
Conservative Substitutions

Original Residue	Example Substitutions	Preferred Substitutions
Ala (A)	val, leu, ile	val
Arg (R)	lys, gln, asn	lys
Asn (N)		gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro, ala	ala
His (H)	asn, gln, lys, arg	arg
Ile (I)	leu, val, met, ala, phe, norleucine	leu
Leu (L)	norleucine, ile, val, met, ala, phe	ile
Lys (K)	arg, gln, asn,	arg
Met (M)	leu, phe, ile	leu
Phe (F)	leu, val, ile, ala, tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr, phe	tyr
Tyr (Y)	trp, phe, thr, ser	phe

-37-

Val (V)	ile, leu, met, phe, ala norleucine	leu
---------	---------------------------------------	-----

Substantial modifications in function or immunological identity of the LP232 polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: sys, ser, thr;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites, or more preferably, into the remaining (non-conserved) sites. The variations can be made using methods known in the art such as oligonucleotides-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., *Nucl. Acids Res.* 13(12): 4331-43 (1985); Zoller et al., *Nucl. Acids Res.* 10(20): 6487-500 (1982)], cassette mutagenesis [Wells et al., *Gene* 34(2-3): 315-23 (1985)], restriction selection mutagenesis [Wells et al., *Philos. Trans. R. Soc. London Ser. A* 317: 415 (1986)] or other

-38-

known techniques can be performed on the cloned DNA to produce the LP232-encoding variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, W.H. Freeman & Co., NY; Chothia, *J. Mol. Biol.* 105(1): 1-12 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

C. Modifications of LP232

Covalent modifications of LP232 polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of an LP232 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of an LP232 polypeptide. Derivatization with bifunctional agents is useful, for instance, for cross-linking LP232 to a water-insoluble support matrix or surface for use in the method for purifying anti-LP232 antibodies, and vice-versa. Commonly used cross-linking agents include, e.g., 1,1-bis(diazo-acetyl)-2-phenylethane, glutaraldehyde, N-hydroxy-succinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis-

-39-

(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)-dithiolpropionimide].

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains [T.E. Creighton. *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the LP232 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence LP232 polypeptide and/or adding one or more glycosylation sites that are not present in the native sequence LP232 polypeptide. Additionally, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to LP232 polypeptides may be accomplished by altering the amino acid sequence thereof. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence LP232 polypeptide (for O-linked glycosylation sites). The LP232 amino acid sequence may optionally be altered through changes at the DNA level,

-40-

particularly by mutating the DNA encoding the LP232 polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the LP232 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330, published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the LP232 polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Sojar et al., *Arch. Biochem. Biophys.* 259: 52-7 (1987) and by Edge et al., *Anal. Biochem.* 118: 131-7 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., *Meth. Enzymol.* 138: 350-9 (1987).

Another type of covalent modification of LP232 comprises linking the LP232 polypeptide to one of a variety of nonproteinaceous 20 polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

LP232 polypeptides of the present invention may also be modified in a way to form chimeric molecules comprising an LP232 polypeptide fused to another heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of an LP232 polypeptide with a tag polypeptide which provides an epitope to which an anti-tag

-41-

antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the LP232 polypeptide. The presence of such epitope-tagged forms of an LP232 polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the LP232 polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA.5 [Field et al., *Mol. Cell. Biol.* 8(5): 2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., *Mol. Cell. Biol.* 5(12): 3610-16 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., *Prot. Engin.* 3(6): 547-53 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., *Bio/Technology*, 6:120410 (1988)]; the KT3 epitope peptide [Martin et al., *Science* 255(5041): 192-4 (1992)]; a cr-tubulin epitope peptide [Skinner et al., *J. Biol. Chem.*, 266(22): 14163-6 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., *Proc. Natl. Acad. Sci. USA* 87(16): 6393-7 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of an LP232 polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble transmembrane domain deleted or inactivated form of an LP232 polypeptide in place of at least one variable region within an Ig molecule.

-42-

In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3 or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions, see also U.S. Patent 5,428,130, issued June 27, 1995.

In yet a further embodiment, the LP232 polypeptides of the present invention may also be modified in a way to form a chimeric molecule comprising an LP232 polypeptide fused to a leucine zipper. Various leucine zipper polypeptides have been described in the art. See, e.g., Landschulz et al., *Science* 240(4860): 1759-64 (1988); WO 94/10308; Hoppe et al., *FEBS Letters* 344(2-3): 191-5 (1994); Abel et al., *Nature* 341(6237): 24-5 (1989). It is believed that use of a leucine zipper fused to an LP232 polypeptide may be desirable to assist in dimerizing or trimerizing soluble LP232 polypeptide in solution. Those skilled in the art will appreciate that zipper may be fused at either the N- or C-terminal end of the LP232 molecule.

D. Preparation of LP232

The description below relates primarily to production of LP232 by culturing cells transformed or transfected with a vector containing LP232 polypeptide encoding nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare LP232 polypeptides. For instance, the LP232 sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.* 85: 2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for

-43-

instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of LP232 polypeptides may be chemically synthesized separately and combined using chemical or enzymatic methods to produce a full-length LP232 polypeptide.

1. Isolation of DNA Encoding LP232

DNA encoding an LP232 polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the LP232 mRNA and to express it at a detectable level. Accordingly, human LP232-encoding DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The LP232-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated synthetic procedures, oligonucleotide synthesis).

Libraries can be screened with probes (such as antibodies to an LP232 polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY (1989). An alternative means to isolate the gene encoding LP232 is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., *PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY (1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be

-44-

detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art and include the use of radiolabels like ^{32}P -labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., supra.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein (e.g., through sequence alignment using computer software programs such as ALIGN, DNASTAR, BLAST, BLAST-2, INHERIT and ALIGN-2 which employ various algorithms to measure homology).

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for LP232 polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general,

-45-

principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al. *Gene* 23(3): 315-30 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology* 52(2): 456-67 (1973) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of van Solingen et al., *J Bact.* 130(2): 946-7 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. USA* 76(8): 3829-33 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene or polyomithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., *Methods in Enzymology* 185: 527-37 (1990) and Mansour et al., *Nature* 336(6197): 348-52 (1988).

Suitable host cells for cloning or expressing the nucleic acid (e.g., DNA) in the vectors herein include prokaryote,

-46-

yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 3 1.446); *E. coli* X1 776 (ATCC 3 1.537); *E. coli* strain W3 110 (ATCC 27.325) and K5 772 (ATCC 53.635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescens*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 4 1 P disclosed in DD266,7 10, published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3 110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *ronA*; *E. coli* W3 110 strain 9E4, which has the complete genotype *ton4 ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA, ptr3 phoA E15 (argF-lac) I69 degP ompT /can'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vivo* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

-47-

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for LP232 vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* [Beach and Nurse, *Nature* 290: 140-3 (1981); EP 139,383 published 2 May 1995]; *Muyveromyces* hosts [U.S. Patent No. 4,943,529; Fleer et al., *Bio/Technology* 9(10): 968-75 (1991)] such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574) [de Louvencourt et al., *J. Bacteriol.* 154(2): 737-42 (1983)]; *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906) [Van den Berg et al., *Bio/Technology* 8(2): 135-9 (1990)]; *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070) [Sreekrishna et al., *J. Basic Microbiol.* 28(4): 265-78 (1988)]; *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa* [Case et al., *Proc. Natl. Acad. Sci. USA* 76(10): 5259-63 (1979)]; *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* [Ballance et al., *Biochem. Biophys. Res. Comm.* 112(1): 284-9 (1983)]; Tilburn et al., *Gene* 26(2-3): 205-21 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA* 81(5): 1470-4 (1984)] and *A. niger* [Kelly and Hynes, *EMBO J.* 4(2): 475-9 (1985)]. Methylophilic yeasts are selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeast may be found in C. Antony, *The Biochemistry of Methylophilic Yeasts* 269 (1982).

-48-

Suitable host cells for the expression of glycosylated LP232 are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sp, *Spodoptera* high5 as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line [293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.*, 36(1): 59-74 (1977)]; Chinese hamster ovary cells/-DHFR [CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77(7): 4216-20 (1980)]; mouse sertoli cells [TM4, Mather, *Biol. Reprod.* 23(1):243-52 (1980)]; human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding the desired LP232 polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

-49-

Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The LP232 polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the LP232-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* cc-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2u plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

-50-

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement autotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the LP232-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77(7): 4216-20 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., *Nature* 282(5734): 39-43 (1979); Kingsman et al., *Gene* 7(2): 141-52 (1979); Tschumper et al., *Gene* 10(2): 157-66 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEPC1 [Jones, *Genetics* 85: 23-33 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the LP232-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the P-lactamase and lactose promoter systems [Chang et al., *Nature* 275(5681): 617-24 (1978); Goeddel et al., *Nature* 281(5732): 544-8 (1979)], alkaline phosphatase, a tryptophan (*up*) promoter system [Goeddel, *Nucleic Acids Res.* 8(18): 4057-74 (1980); EP 36,776 published 30 September 1981], and hybrid promoters such as the

-51-

tat promoter [deBoer et al., *Proc. Natl. Acad. Sci. USA* 80(1): 21-5 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the LP232 polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., *J. Biol. Chem.* 255(24): 12073-80 (1980)] or other glycolytic enzymes [Hess et al., *J. Adv. Enzyme Reg.* 7: 149 (1968); Holland, *Biochemistry* 17(23): 4900-7 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. LP232 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters,

-52-

provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding an LP232 polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -ketoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the LP232 coding sequence but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding LP232 polypeptide.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of LP232 polypeptides in recombinant vertebrate cell culture are described in Gething et al., *Nature* 293(5834): 620-5 (1981); Mantei et al., *Nature* 281(5726): 40-6 (1979); EP 117,060; and EP 117,058.

-53-

4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA* 77(9): 5201-5 (1980)], dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native provided herein or against exogenous sequence fused to LP232-encoding DNA and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of LP232 may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g., Triton-X 100) or by enzymatic cleavage. Cells employed in

-54-

expression of LP232 polypeptides can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify LP232 from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reversed-phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the LP232 polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, *Methods in Enzymology* 182: 83-9 (1990) and Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, NY (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular LP232 polypeptide produced.

E. Uses for LP232

Nucleotide sequences (or their complement) encoding LP232 polypeptides have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of antisense RNA and DNA. LP232-encoding nucleic acid will also be useful for the preparation of LP232 polypeptides by the recombinant techniques described herein.

The full-length LP232 nucleotide sequence (SEQ ID NO:2) or the full-length native sequence LP232 nucleotide-encoding sequence, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length LP232

-55-

gene or to isolate still other genes (for instance, those encoding naturally-occurring variants of LP232, or the same from other species) which have a desired sequence identity to the LP232 nucleotide sequence disclosed in SEQ ID NO:2. Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the sequence of SEQ ID NO:2, or from genomic sequences including promoters, enhancer elements and introns of native sequence LP232-encoding DNA. By way of example, a screening method will comprise isolating the coding region of the LP232 gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as ³²P or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the LP232 gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine members of such libraries the probe hybridizes. Hybridization techniques are described in further detail in the Examples below.

Any EST sequence (or fragment thereof) disclosed in the present application may similarly be employed as probes, using the methods disclosed herein. Other useful fragments of the LP232 nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target LP232 mRNA (sense) or LP232 DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of LP232 DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense

-56-

oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res* 48(10): 2659-68 (1988) and van der Krol et al., *Bio/Techniques* 6(10): 958-76 (1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of LP232 proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such poly-L-lysine. Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO₄-mediated

-57-

DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MSV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated CDTSA, CTSB and DCTSC (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related LP232 sequences.

-58-

Nucleotide sequences encoding an LP232 polypeptide can also be used to construct hybridization probes for mapping the gene which encodes that LP232 polypeptide and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as in situ hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequences for LP232 encode a protein which binds to another protein (for example, where the LP232 polypeptide functions as a receptor), the LP232 polypeptide can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor LP232 polypeptide can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native LP232 or a receptor for LP232. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode LP232 polypeptide or any of its modified forms can also be used to generate either

-59-

transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding LP232 polypeptide can be used to clone genomic DNA encoding LP232 in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding LP232. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for LP232 transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding LP232 introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding LP232 polypeptide. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of LP232 can be used to construct an LP232 "knock out" animal which has a defective or altered gene encoding LP232 polypeptide as a result of

-60-

homologous recombination between the endogenous gene encoding LP232 polypeptide and altered genomic DNA encoding LP232 polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding LP232 polypeptide can be used to clone genomic DNA encoding LP232 polypeptide in accordance with established techniques. A portion of the genomic DNA encoding LP232 polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see, e.g., Thomas and Capecchi, *Cell* 51(3): 503-12 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation), and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see, e.g., Li et al., *Cell* 69(6): 915-26 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see, e.g., Bradley, *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized, for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the LP232 polypeptide.

-61-

"Gene therapy" includes both conventional gene therapy, where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane [Zamecnik et al., *Proc. Natl. Acad. Sci. USA* 83(12): 4143-6 (1986)]. The oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups with uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cell *in vitro* or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically, retroviral) vectors and viral coat protein-liposome mediated transfection [Dzau et al., *Trends in Biotechnology* 11(5): 205-10 (1993)]. In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cells, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may by

-62-

used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example by Wu et al., *J. Biol. Chem.* 262(10): 4429-32 (1987); and Wagner et al., *Proc. Natl. Acad. Sci. USA* 87(9): 3410-4 (1990). For a review of gene marking and gene therapy protocols, see Anderson, *Science* 256(5058): 808-13 (1992).

The LP232 polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes.

The nucleic acid molecule encoding the LP232 polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data, are presently available. Each LP232 nucleic acid molecule of the present invention can be used as a chromosome marker.

The LP232 polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the LP232 polypeptides of the present invention may be differentially expressed in one tissue as compared to another. LP232 nucleic acid molecules will find use for generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

LP232 polypeptides of the present invention which possess biological activity related to that of cerebellin may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to

-63-

employ the LP232 polypeptides of the present invention for such purposes.

F. Anti-LP232 Antibodies

The present invention further provides anti-LP232 polypeptide antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

1. Polyclonal Antibodies

The anti-LP232 antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the LP232 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

-64-

2. Monoclonal Antibodies

The anti-LP232 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature* 256(5517): 495-7 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the LP232 polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used, if cells of human origin are desired, or spleen cells or lymph node cells are used, if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.

-65-

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California, and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.* 133(6): 3001-5 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., NY, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against an LP232 polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Rodbard, *Anal. Biochem.* 107(1): 220-39 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

-66-

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA* 81(21): 6851-5 (1984)] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

-67-

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

3. Humanized Antibodies

The anti-LP232 antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise

-68-

substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature* 321(6069): 522-5 (1986); Riechmann et al., *Nature* 332(6162): 323-7 (1988); and Presta, *Curr. Op. Struct. Biol.* 2: 593-6 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., *Nature* 321(6069): 522-5 (1986); Riechmann et al., *Nature* 332(6162): 323-7 (1988); Verhoeyen et al., *Science* 239(4847): 1534-6 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) 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 FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.* 227(2): 381-8 (1992);

-69-

Marks et al., *J. Mol. Biol.* 222(3): 581-97 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.* 147(1): 86-95 (1991)]. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or complete inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Biotechnology* 10(7): 779-83 (1992); Lonberg et al., *Nature* 368(6474): 856-9 (1994); Morrison, *Nature* 368(6474): 812-3 (1994); Fishwild et al., *Nature Biotechnology* 14(7): 845-51 (1996); Neuberger, *Nature Biotechnology* 14(7): 826 (1996); Lonberg and Huszar, *Int. Rev. Immunol.* 13(1): 65-93 (1995).

4. Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U. S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in

-70-

such as way so as to convert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, glycosidase, glucose oxidase, human lysozyme, human glucuronidase, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases (e.g., carboxypeptidase G2 and carboxypeptidase A) and cathepsins (such as cathepsins B and L). that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as alpha-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; p-lactamase useful for converting drugs derivatized with p-lactams into free drugs; and penicillin amidases, such as penicillin Vamidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes" can be used to convert the prodrugs of the invention into free active drugs [see, e.g., Massey, Nature 328(6129): 457-8 (1987)]. Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-LP232 antibodies by techniques well known in the art such as the use of the heterobifunctional cross-linking agents

-71-

discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of the antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art [see, e.g., Neuberger et al., *Nature* 312(5995): 604-8 (1984)].

5. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an LP232 polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature* 305(5934):537-9 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., *EMBO J* 10(12): 3655-9 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant

-72-

domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology* 121: 210-28 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using chemical linkage. Brennan et al. [*Science* 229(4708): 81-3 (1985)] describe a procedure wherein intact

-73-

antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab' -TNB derivatives is then reconverted to the Fab' thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab' -TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al. [*J. Exp. Med.* 175(1): 217-25 (1992)] describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers [Kostelny et al., *J. Immunol.* 148(5): 1547-53 (1992)], wherein the leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody

-74-

heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Holliger et al, [*Proc. Natl. Acad Sci. USA* 90(14): 6444-8 (1993)] has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_H and V_L domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.* 152(11): 5368-74 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared [Tutt et al., *J Immunol.* 147(1): 60-9 (1991)].

Exemplary bispecific antibodies may bind to two different epitopes on a given "LP" protein herein. Alternatively, an anti-"LP" protein arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g., CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular "LP" protein. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular "LP" polypeptide. These antibodies possess an "LP"-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of

-75-

interest binds the "LP232" polypeptide and further binds tissue factor (TF).

6. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/20373]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

7. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody. For example, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp Med.* 176(4): 1191-5 (1992) and Shopes, *J. Immunol.* 148(9): 2918-22 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as

-76-

described in Wolff et al., *Cancer Res.* 53(11): 2560-5 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., *AntiCancer Drug Design* 3(4): 219-30 (1989).

8. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof, or a small molecule toxin), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active protein toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, cholera toxin, botulinus toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites jbrdii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, cretin, sapaonaria officinalis inhibitor, gelonin, saporin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. Small molecule toxins include, for example, calicheamicins, maytansinoids, palytoxin and CC 1065. A variety of radionuclides are available for the production of radioconjugated antibodies.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters

-77-

(such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds, hexanediamine), and bis-diazonium derivatives. For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238(4830): 1098-104 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent, and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

9. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Eppstein et al., *Proc. Natl. Acad. Sci. USA* 82: 3688-92 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA* 77(7): 4030-4 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the

-78-

antibody of the present invention can be conjugated to the liposomes as described in Martin et al., *J. Biol. Chem.* 257(1): 286-8 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon et al., *J. National Cancer Inst.* 81(19): 484-8 (1989).

10. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding an LP232 polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If an LP232 polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco et al., *Proc. Natl. Acad. Sci. USA* 90(16): 7889-93 (1993).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent,

-79-

cytokines, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or felatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, supra.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid γ -ethyl-L-glutamate, non-degradable ethylene-vinylacetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long

-80-

time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanisms involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thiosulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

G. Uses for anti-LP232 Antibodies

The anti-LP232 antibodies of the present invention have various utilities. For example, anti-LP232 antibodies may be used in diagnostic assays for LP232 polypeptides, e.g., detecting expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including

-81-

those methods described by Hunter et al., *Nature* 144: 945 (1962); David et al., *Biochemistry* 13(5): 1014-21 (1974); Pain et al., *J Immunol. Meth.*, 40(2): 219-30 (1981); and Nygren, *J. Histochem. Cytochem.* 30(5): 407-12 (1982).

Anti-LP232 antibodies also are useful for the affinity purification of LP232 polypeptides from recombinant cell culture or natural sources. In this process, the antibodies against an LP232 polypeptide are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is then contacted with a sample containing the LP232 polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the LP232 polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the LP232 polypeptide from the antibody.

H. LP232 and Cerebellin Antagonists/Agonists

This invention encompasses methods of screening compounds to identify those that mimic the LP232 or cerebellin (agonists) or prevent the effect of the LP232 or cerebellin (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the LP232 or cerebellins encoded by the genes identified herein or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

-82-

The assays can be performed in a variety of formats including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art. In binding assays, the interaction is binding, and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the LP232 or cerebellin encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the LP232 or cerebellin and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the LP232 or cerebellin to be immobilized can be used to anchor it to solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular LP232 or cerebellin encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-

-83-

purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature* 340(6230): 245-6 (1989); Chien et al., *Proc. Natl. Acad. Sci. USA* 88(21): 9578-82 (1991); Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89(13): 5789-93 (1992)]. Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other functions as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another in which candidate activating proteins are fused to the activation domain. The expression of GAL1-lacZ reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with chromogenic substrate for β -galactosidase. A complete kit (MATCHMAKERTM) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding an LP232 or cerebellin identified herein and other intra- or extracellular components can be tested as follows.

-84-

Typically, a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture to serve as a positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

Antagonists may be detected by combining the LP232 or cerebellin and a potential antagonist with membrane-bound LP232 or cerebellin receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The LP232 or cerebellin can be labeled, such as by radioactivity, such that the number of LP232 or cerebellin molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. See Coligan et al., *Current Protocols in Immunology* 1(2): Ch. 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the LP232 polypeptide, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the LP232 or cerebellin. Transfected cells that are grown on

-85-

glass slides are exposed to labeled LP232 or cerebellin. The LP232 or cerebellin can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled LP232 or cerebellin can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled LP232 or cerebellin in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be removed.

More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of immunoglobulin with LP232 or cerebellin, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and

-86-

antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the LP232 or cerebellin that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the LP232 or cerebellin.

Another potential LP232 or cerebellin antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and prevent its translation into protein. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature LP232 or cerebellins herein, is used to design an antisense RNA oligonucleotide sequence of about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription [triple helix; see Lee et al., *Nucl. Acids Res* 6(9): 3073-91 (1979); Cooney et al., *Science* 241(4864): 456-9 (1988); Beal and Dervan, *Science* 251(4999): 1360-3 (1991)], thereby preventing transcription and the production of the LP232 polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the LP232 or cerebellin [antisense; see Okano, *J. Neurochem.* 56(2): 560-7 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression* (CRC Press: Boca Raton, FL 1988)]. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the LP232 polypeptide. When antisense DNA is used,

-87-

oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the LP232 polypeptide, thereby blocking the normal biological activity of the LP232 or cerebellin. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details, see, e.g., Rossi, *Current Biology* 4(5): 469-71 (1994) and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

I. Diagnostic Uses

Another use of the compounds of the invention (e.g., LP232 variants and anti-LP232 antibodies) described herein is

-88-

to help diagnose whether a disorder is driven to some extent by LP232 modulated signaling.

A diagnostic assay to determine whether a particular disorder is driven by LP232 signaling can be carried out using the following steps: (1) culturing test cells or tissues expressing LP232 ; (2) administering a compound which can inhibit LP232 modulated signaling; and (3) measuring the LP232 mediated phenotypic effects in the test cells. The steps can be carried out using standard techniques in light of the present disclosure. For example, standard techniques can be used to isolate cells or tissues and to culture them *in vivo*. Compounds of varying degrees of selectivity are useful for diagnosing the role of LP232. For example, compounds which can inhibit LP232 modulated signaling, in addition to another form of adaptor molecule, can be used as an initial test compound to determine if one of several adaptor molecules drive the disorder. The selective compounds can then be used to further eliminate the possible role of the other adaptor proteins in driving the disorder. Test compounds should be more potent in inhibiting intracellular signaling activity than in exerting a cytotoxic effect (e.g., an IC_{50} and LD_{50} of greater than one). The IC_{50} and LD_{50} can be measured by standard techniques, such as an MTT assay or by measuring the amount of LDH released. The degree of IC_{50} and LD_{50} of a compound should be taken into account in evaluating the diagnostic assay. Generally, the larger the ratio, the more relative the information. Appropriate controls take into account the possible cytotoxic effect of a compound, such as treating cells not associated with a cell proliferative disorder (e.g., control cells) with a test compound and can also be used as part of the diagnostic assay. The diagnostic methods of the invention involve the screening for agents that

-89-

modulate the effects of LP232 upon synaptic disorders. Exemplary detection techniques include radioactive labeling and immunoprecipitating (U. S. Patent No. 5,385,915).

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by the disease-related genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art.

In situ detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

J. Pharmaceutical Compositions

The LP232 antagonists or agonists thereof (e.g., antibodies), as well as other molecules identified by the screening assays disclosed hereinbefore, can be employed as therapeutic agents. Such therapeutic agents are formulated according to known methods to prepare pharmaceutically useful compositions, whereby the LP232 antagonist or agonist thereof is combined in a mixture with a pharmaceutically acceptable carrier.

In the case of LP232 antagonist or agonist antibodies, if the protein encoded by the amplified gene is intracellular and

-90-

whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology [see, e.g., Marasco et al., *Proc. Natl. Acad. Sci. USA* 90(16): 7889-93 (1993)].

Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers [*Remington's Pharmaceutical Sciences* 16th edition (1980)], in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include buffers such as phosphate, citrate, and other organic acids; antioxidants include ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose,

-91-

mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICTM or polyethylene glycol (PEG).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, and intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers

-92-

containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels [for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)], polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as those used in the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon, interleukin-2, and MN rpg 120. Johnson et al., *Nat. Med.* 2(7): 795-9 (1996); Yasuda et al., *Biomed. Ther.* 27: 1221-3 (1993); Hora et al., *Bio/Technology* 8(8): 755-8 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems" in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, Eds., Plenum Press, NY, 1995, pp. 439-462 WO 97/03692; WO 96/40072; WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins may be developed using polylactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. See Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer" in *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker; New York, 1990), M. Chasin and R. Langer (Eds.) pp. 1-41.

-93-

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

K. Methods of Treatment

It is contemplated that the compounds of the present invention may be used to treat various conditions including those characterized by overexpression and/or activation of the disease-associated genes identified herein. Exemplary conditions or disorders to be treated with such antibodies and other compounds, including, but not limited to, small organic and inorganic molecules, peptides, antisense molecules, etc., include Parkinson's disease, Alzheimer's disease, bipolar and unipolar affective disorders, schizophrenia, olivopontocerebellar atrophy, and Shy-Dager syndrome, especially those characterized by disruption of synapse function..

The active agents of the present invention, e.g., antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous

-94-

administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebral, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intraocular, intralesional, oral, topical, inhalation or through sustained release.

Other therapeutic regimens may be combined with the administration of the LP232 antagonists or antagonists, anti-cancer agents, e.g., antibodies of the instant invention.

For the prevention or treatment of disease, the appropriate dosage of an active agent, (e.g., an antibody) will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti and Chappell, "The Use of Interspecies Scaling in Toxicokinetics," in *Toxicokinetics and New Drug Development*, Yacobi et al., Eds., Pergamon Press, NY 1989, pp.4246.

When *in vivo* administration of an LP232 polypeptide or agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg up to 100 mg/kg of mammal

-95-

body weight or more per day, preferably about 1 pg/kg/day up to 100 mg/kg of mammal body weight or more per day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760, 5,206,344 or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. Conventional techniques and assays easily monitor the progress of therapy.

L. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is typically an

-96-

LP232 polypeptide, antagonist or agonist thereof. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

Example 1

Expression and Purification of LP232 in *E. coli*

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., Chatsworth, CA). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide can be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of

-97-

that polypeptide. However, a polypeptide coding sequence can optionally be inserted such that translation of the six His codons is prevented and, therefore, a polypeptide is produced with no 6 X His tag.

The nucleic acid sequence encoding the desired portion of the LP232 lacking the hydrophobic leader sequence is amplified from a cDNA clone using PCR oligonucleotide primers (based on the sequences presented, e.g., as presented in SEQ ID NO:2), which anneal to the amino terminal encoding DNA sequences of the desired portion of the LP232 and to sequences in the construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning a LP232, the 5' and 3' primers have nucleotides corresponding or complementary to a portion of the coding sequence of LP232, e.g., as presented in SEQ ID NO:2, according to known method steps. One of ordinary skill in the art would appreciate, of course, that the point in a polypeptide coding sequence where the 5' primer begins can be varied to amplify a desired portion of the complete polypeptide shorter or longer than the mature form.

The amplified LP232 nucleic acid fragments and the vector pQE60 are digested with appropriate restriction enzymes and the digested DNAs are then ligated together. Insertion of the LP232 DNA into the restricted pQE60 vector places the LP232 polypeptide coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG codon. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

-98-

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook, et al., 1989; Ausubel, 1987-1998. *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing LP232 polypeptide, is available commercially from QIAGEN, Inc. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the LP232 is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, a polypeptide can be successfully refolded by dialyzing it against 500 mM NaCl,

-99-

20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors.

If insoluble protein is generated, the protein is made soluble according to known method steps. After renaturation, the polypeptide is purified by ion exchange, hydrophobic interaction, and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column is used to obtain pure LP232. The purified polypeptide is stored at 4°C or frozen at -40°C to -120°C.

Example 2

Cloning and Expression of LP232 in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding the mature polypeptide into a baculovirus to express LP232, using a baculovirus leader and standard methods as described in Summers, et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 polypeptide and convenient restriction sites such as BamHI, Xba I, and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-

-100-

mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Other baculovirus vectors are used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow, *et al.*, **Virology** 170:31-39.

The cDNA sequence encoding the mature LP232 polypeptide in a clone, lacking the AUG initiation codon and the naturally associated nucleotide binding site, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequence of a LP232 polypeptide, e.g., as presented in SEQ ID NO:2, according to known method steps.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (e.g., "Geneclean," BIO 101 Inc., La Jolla, CA). The fragment then is then digested with the appropriate restriction enzyme and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean"

-101-

BIO 101 Inc., La Jolla, CA). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid bearing the human LP232 gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the LP232 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac LP232 .

Five µg of the plasmid pBacLP232 is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner, et al., *Proc. Natl. Acad. Sci. USA* **84**:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac LP232 are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies, Inc., Rockville, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium

-102-

supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed. An agarose gel with "Blue Gal" (Life Technologies, Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies, Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with a micropipettor tip (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-LP232.

To verify the expression of the LP232 gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-LP232 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available, e.g., from Life Technologies, Inc., Rockville, MD). If radiolabeled polypeptides are desired, 42 hours later, 5 mCi of ³⁵S-methionine and 5 mCi ³⁵S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The polypeptides

-103-

in the supernatant as well as the intracellular polypeptides are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified polypeptide can be used to determine the amino terminal sequence of the mature polypeptide and thus the cleavage point and length of the secretory signal peptide.

Example 3

Cloning and Expression of LP232 in Mammalian Cells

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the polypeptide coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Other suitable mammalian host cells include human Hela 293, H9, Jurkat cells, mouse NIH3T3, C127 cells, Cos 1, Cos 7 and CV

-104-

1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded polypeptide. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., *Biochem. J.* **227**:277-279 (1991); Bebbington, et al., *Bio/Technology* **10**:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of polypeptides.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., *Molec. Cell. Biol.* **5**:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., *Cell* **41**:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

-105-

Example 3(a)Cloning and Expression in COS Cells

The expression plasmid, pLP232 HA, is made by cloning a cDNA encoding LP232 into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) or HIS tag (see, e.g, Ausubel, *supra*) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin polypeptide described by Wilson, *et al.*, *Cell* **37**:767-778 (1984). The fusion of the HA tag to the target polypeptide allows easy detection and recovery of the recombinant polypeptide with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the LP232 is cloned into the polylinker region of the vector so that recombinant polypeptide expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The LP232 cDNA of a clone is amplified using primers that contain

-106-

convenient restriction sites, much as described above for construction of vectors for expression of LP232 in *E. coli*. Non-limiting examples of suitable primers include those based on the coding sequence presented in SEQ ID NO:2, as they encode LP232 as described herein.

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with suitable restriction enzyme(s) and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the LP232-encoding fragment.

For expression of recombinant LP232, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook, *et al.*, *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of LP232 by the vector.

Expression of the LP232-HA fusion polypeptide is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow, *et al.*, *Antibodies: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ³⁵S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5,

-107-

as described by Wilson, et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated polypeptides then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b)

Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of LP232 polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary cells or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., *J. Biol. Chem.* **253**:1357-1370 (1978); J. L. Hamlin and C. Ma, *Biochem. et Biophys. Acta* **1097**:107-143 (1990); and M. J. Page and M. A. Sydenham, *Biotechnology* **9**:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which

-108-

contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al.*, *Molec. Cell. Biol.* **5**:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, *et al.*, *Cell* **41**:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the LP232 in a regulated way in mammalian cells (M. Gossen, and H. Bujard, *Proc. Natl. Acad. Sci. USA* **89**: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

-109-

The DNA sequence encoding the complete LP232 polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequence of LP232, e.g., as presented in SEQ ID NO:2, according to known method steps.

The amplified fragment is digested with suitable endonucleases and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 µg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 µg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are

-110-

obtained which grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 4

Tissue Distribution of LP232 mRNA Expression

Northern blot analysis is carried out to examine LP232 gene expression in human tissues, using methods described by, among others, Sambrook, *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of LP232 polypeptide (SEQ ID NO:1) is labeled with ^{32}P using the Rediprime™ DNA labeling system (Amersham Life Science), according to the manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to the manufacturer's protocol number PT1200-1. The purified and labeled probe is used to examine various human tissues for LP232 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and developed according to standard procedures. The results show LP232 to be selectively expressed in certain neural tissues.

-111-

WHAT IS CLAIMED IS:

1. Isolated nucleic acid comprising DNA having at least an 91% sequence identity to (a) a DNA molecule encoding an LP232 polypeptide comprising the sequence of amino acid residues 1 or 27 through 287, inclusive, of SEQ ID NO: 1, and (b) the complement of the DNA molecule of (a).

2. The nucleic acid of Claim 1, wherein said DNA comprises the sequence of corresponding nucleotide positions 178 or 259 through 1039, inclusive, of SEQ ID NO:2 .

3. The nucleic acid of Claim 1, wherein said DNA comprises the nucleotide sequence of sequence of SEQ ID NO:2.

4. The isolated nucleic acid molecule of Claim 1 comprising a nucleotide sequence that encodes the sequence of amino acid residues from 1 or about 27 to about 287 of SEQ ID NO:1.

5. An isolated nucleic acid molecule encoding an LP232 polypeptide comprising DNA that hybridizes to the complement of the nucleic acid sequence that encodes amino acids 1 or about 27 to about 287 of SEQ ID NO: 1.

6. The isolated nucleic acid molecule of claim 5, wherein the nucleic acid sequence that encodes amino acids 1 or about 27 to about 287, inclusive, of SEQ ID NO:1 or comprises nucleotides 178 or about 259 to about 1039, inclusive, of (SEQ ID NO:2.

-112-

7. The isolated nucleic acid molecule of claim 5, wherein hybridization occurs under stringent hybridization and wash conditions.

8. An isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide scoring at least 91% positives when compared to the sequence of amino acid residues selected from the group consisting of: (a) from 1 or about 27 to about 287, inclusive, of SEQ ID NO: 1; or (b) the complement of the DNA of (a).

9. An isolated nucleic acid molecule comprising at least about 250 nucleotides in length and which is produced by hybridizing a test DNA under stringent hybridization conditions with (a) a DNA molecule which encodes an LP232 polypeptide comprising a sequence of amino acid residues from 1 or about 27 to about 287, inclusive, of SEQ ID NO: 1, or (b) the complement of the DNA molecule of (a).

10. The isolated nucleic acid molecule of claim 9, which has at least about 91% sequence identity to (a) or (b).

11. A vector comprising the nucleic acid molecule of any of Claims 1 to 10.

12. The vector of Claim 11, wherein said nucleic acid molecule is operably linked to control sequences recognized by a host cell transformed with a the vector.

13. A host cell comprising the vector of Claim 12.

-113-

14. The host cell of Claim 13, wherein said cell is a CHO cell.

15. The host cell of Claim 13, wherein said cell is an E. coli cell.

16. The host cell of Claim 13, wherein said cell is a yeast cell.

17. A process for procuring an LP232 polypeptide comprising culturing the host cell of Claim 13 under conditions suitable for expression of said LP232 polypeptide and recovering said LP232 polypeptide from the cell culture.

18. An isolated polypeptide comprising an amino acid sequence comprising at least about 91% sequence identity to the sequence comprising residues 1 or about 27 to about 287 of SEQ ID NO: 1.

19. The isolated LP232 polypeptide of claim 18 comprising amino acid residues 1 or about 27 to about 287 of SEQ IDNO: 1.

20. An isolated LP232 polypeptide scoring at least 91% positives when compared to the sequence of amino acids from about 1 or about 27 to about 287 of SEQ ID NO: 1.

21. An isolated LP232 polypeptide comprising the sequence of amino acid residues from 1 or about 27 to about 287 of SEQ ID NO:1, or a fragment thereof sufficient to provide a binding site for an anti-LP232 antibody, respectively.

-114-

22. An isolated polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding an LP232 polypeptide comprising the sequence of amino acid residues from 1 or about 27 to about 287 of SEQ ID NO:1; or (b) the complement of the DNA molecule of (a); (ii) culturing a host cell comprising the said test DNA molecule under conditions suitable for the expression of said polypeptide, and (iii) recovering said polypeptide from the cell culture.

23. The isolated polypeptide of Claim 22, wherein said test DNA has at least about 91% sequence identity to (a) or (b).

24. A chimeric molecule comprising an LP232 polypeptide fused to a heterologous amino acid sequence.

25. The chimeric molecule of Claim 24, wherein said heterologous amino acid sequence is an epitope tag sequence.

26. The chimeric molecule of Claim 24, wherein said heterologous amino acid sequence is an Fc region of an immunoglobulin.

27. An antibody which specifically binds to an LP232 polypeptide.

28. The antibody of Claim 27, where said antibody is a monoclonal antibody.

-115-

29. The antibody of Claim 27, wherein said antibody is selected from the group consisting of a humanized antibody and a human antibody.

30. An agonist to LP232.

31. An antagonist to LP232.

32. A composition comprising a therapeutically effective amount of an active agent selected from the group consisting of: (a) an LP232 polypeptide, (b) an agonist to an LP232 polypeptide, (c) an antagonist to an LP232 polypeptide, and (d) an anti-LP232 antibody; in combination with a pharmaceutically acceptable carrier.

33. A method of treating a neurological disorders comprising administering a therapeutically effective amount of an LP232 agonist, or antagonist to a mammal suffering from said disorder.

34. A method of diagnosing a neurological disorder by: (1) culturing test cells or tissues expressing LP232 ; (2) administering a compound which can inhibit LP232 modulated signaling; and (3) measuring the LP232 mediated phenotypic effects in the test cells or tissues.

35. An article of manufacture comprising a container, label and therapeutically effective amount of LP232 agonist or antagonist thereof in combination with a pharmaceutically effective carrier.