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(54) Title: DNA ENCODING T–CELL LYMPHOMA INVASION AND METASTASIS 2 AND A PROCESS FOR ITS USE

(57) Abstract

The present invention provides nucleic acid sequences encoding TIAM2 proteins. The invention also includes diagnostic assays, expression vectors, antisense molecules, ribozymes, and host cells to express the polypeptide encoded by the nucleic acid sequence. The present invention also includes claims to the polypeptide sequence encoded by the nucleic acid sequences.

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DNA ENCODING T-CELL LYMPHOMA INVASION AND METASTASIS 2 AND A PROCESS FOR ITS USE

FIELD OF THE INVENTION

The present invention is in the field of molecular biology. More specifically, the present invention relates to a polynucleotide sequence and corresponding TIAM2 protein.

BACKGROUND OF THE INVENTION

The TIAM1 gene and characteristics of the encoded protein are discussed in Habets et al., *Cell 77*:537-549 (1994); Hart, M.J. et al., *J. Biol. Chem. 269*:62-65 (1994); Haslam et al., *Nature 363*:309-310 (1993); and Mayer, B.J. et al., *Cell 73*:629-630 (1993).

SUMMARY

The invention relates to a polynucleotide comprising a polynucleotide of SEQ ID NO:8.

The invention also relates to a polypeptide encoded by SEQ ID NO:8.

The invention further relates to a polynucleotide having at least 80% sequence identity to SEQ ID NO:8.

The invention still further relates to a polynucleotide having at least 85% sequence identity to SEQ ID NO:8.

The invention also relates to a polynucleotide having at least 90% sequence identity to SEQ ID NO:8.

The invention further relates to a polynucleotide having at least 95% sequence identity to SEQ ID NO:8.

The invention relates to a polynucleotide comprising a polynucleotide of SEQ ID NO:9.

The invention also relates to a polypeptide encoded by SEQ ID NO:9.

The invention further relates to a polynucleotide having at least 80% sequence identity to SEQ ID NO:9.

The invention still further relates to a polynucleotide having at least 85% sequence identity to SEQ ID NO:9.

The invention also relates to a polynucleotide having at least 90% sequence identity to SEQ ID NO:9.

The invention further relates to a polynucleotide having at least 95% sequence identity to SEQ ID NO:9.

The invention relates to a polynucleotide comprising a polynucleotide of SEQ ID NO:10.

The invention also relates to a polypeptide encoded by SEQ ID NO:10.

The invention further relates to a polynucleotide having at least 80% sequence identity to SEQ ID NO:10.

The invention still further relates to a polynucleotide having at least 85% sequence identity to SEQ ID NO:10.

The invention also relates to a polynucleotide having at least 90% sequence identity to SEQ ID NO:10.

The invention further relates to a polynucleotide having at least 95% sequence identity to SEQ ID NO:10.

The invention relates to a polypeptide having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

The invention also relates to a polypeptide having at least 85% sequence identity to the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

The invention further relates to a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

The invention still further relates to a polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

The invention further relates to an antibody capable of specifically binding to a polypeptide encoded by SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, or by a polynucleotide having at least 80% sequence identity to SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

The invention further relates to an antibody capable of specifically binding to a polypeptide having the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

The invention also relates to a method of detecting expression of a TIAM2 expression product in a biological sample, comprising obtaining polypeptides from the biological sample, contacting the polypeptides with an antibody capable of specifically binding to TIAM2, and detecting the presence or absence of antibody-polypeptide complexes.

The invention also relates to a method of obtaining a TIAM2 polypeptide, comprising expressing a polynucleotide having at least 80% sequence identity to SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

The invention further relates to a vector comprising a polynucleotide having at least 80% sequence identity to SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

The invention still further relates to a vector comprising a polynucleotide having at least 80% sequence identity to SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, and at least one regulatory region.

The invention also relates to a host cell comprising a polynucleotide having at least 80% sequence identity to SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

The invention further relates to a method of detecting a deletion of a region of chromosome 6q25 in ovarian cancer, comprising comparing the polynucleotide sequence of region of chromosome 6q25 of an ovarian tissue sample

suspected of being cancerous with the polynucleotide sequence of SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10 and determining the presence or absence of the sequence in the polynucleotides of the tissue sample.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Human brain-(A) and tissue-specific (B) blots containing 2 μg/lane of poly(A)$^+$ mRNA were hybridized with TIAM-specific probes that recognize both the long ($TIAM_L$) and the short ($TIAM_S$) forms of TIAM2. A probe derived from the 5' end of the 4.4-Kb message hybridized to only the long form (C), while a 3'-specific probe hybridized to both the long and the short forms (D), on the brain-specific Northerns.

Figure 2 shows expression of TIAM2 and mRNA in mouse brain. (A) Whole mount of a E13.5 embryo brain. TIAM2 transcripts are expressed throughout the developing telencephalon (T). (B) Parasagittal section through an E13.5 telencephalon demonstrating that TIAM2 mRNA is localized to cells near the pial (P) surface; labeling is not seen near the ventricular surface (V). (C) Coronal section through the adult mouse forebrain. TIAM2 transcripts are localized to the cerebral cortex (Ctx) and caudate putamen (CP), with intense labeling in the ependyma (small arrow) and in the indusium griseum (arrow). (D) Higher magnification view of labeling in the emendyma (arrowhead) lining the lateral ventricle (LV) below the corpus callosum. (CC). (E) TIAM2 transcripts in the olfactory bulb, glomerular layer (arrowhead), mitral cell layer (arrow). (F) Coronal section demonstrating labeling in the thalamus (arrowhead) and fasciola cinerea (arrow). (G) Hippocampal expression of TIAM2 shows expression in the granule cells of the dentate gyrus (DG) and the pyramidal cells of CA2.

Figure 3 shows a comparison of the carboxy-terminus amino acid sequence of TIAM1 protein (amino acids 576-1591) with the amino acid sequence of $TIAM2_L$. The EX domain, alternatively splice region, DHR domain, DH domain, and PH domain are underlined, and the initiator methionine for $TIAM2_S$ (amino acid 452) is in boldface and designated with an arrow.

Figure 4 shows the results of a GDP-GTP exchange assay. Partially purified His-TIAM2$_S$ protein was assayed for the ability to stimulate GDP-GTP exchange activity with immunoprecipitated Rac (A) or Ras (B) proteins. (A) The average of three separate reactions in which Rac was incubated with [^{32}P]GTP in the presence of His-TIAM2$_S$ (◇), SOS (○), or buffer alone (□). (B) A representative experiment in which Ras was incubated with [^{32}P]GTP in the presence of TIAM2$_S$ (●), SOS (▲), or buffer alone (X).

Figure 5 shows the expression of TIAM2 mRNA in tumor tissue and normal tissues.

DETAILED DESCRIPTION OF THE INVENTION

TIAM2 (DP-75), for T-cell lymphoma invasion and metastasis 2, relates to a novel DNA and amino acid sequence that has some sequence homology with TIAM1. (Habets *et al.*, *Cell 77*:537-749 (1994), and Habets *et al.*, *Oncogene 10*:1371-1376 (1995)). Overexpression of full length or truncated forms of TIAM1 increases the metastatic potential of lymphoma cells in mice. TIAM1 is a member of a family GDP dissociation stimulators (GDSs) which are proteins that activate Rho-like and Rac-like GTPases. GDSs as well as Rho and Rac have oncogenic potential.

According to the invention, a novel protein, TIAM2, with high homology to TIAM1, has been identified. TIAM2 is expressed as ~4.4- and ~3.3-kb messages that encode long (TIAM2$_L$) and short forms (TIAM2$_S$) of the TIAM2 protein. TIAM2$_L$ begins in a region with identity to the coiled-coil region of TIAM1 and includes both the EX and the PDZ domains found within TIAM1. Both TIAM2$_L$ and TIAM2$_S$ contain regions of identity to the DH and carboxyl-terminal PH domains of TIAM1. An additional level of complexity is added by alternative splicing of the ~4.4-kb message, which leads to the insertion of 24 amino acids between the EX and the DHR domains.

While analysis of the ~4.4-kb message identified an ATG at nucleotide 51 that is in good context for translation, further analysis revealed that the ORF can be extended 5' of the ATG through the first 51 nucleotides. This extended ORF could

indicate that there are additional 5' sequences that would extend $TIAM2_L$. Initiation likely begins at nucleotide 51 since the size of the clone cDNA (4586 nt) equals or exceeds the estimated size of the cerebellum message (~4.4-kb) and because, despite numerous attempts, additional 5' sequences were not identified.

A His-tagged version of $TIAM2_S$ was expressed in a baculovirus system, which is the preferred system for TIAM2 expression, and purified on a nickel column. His-$TIAM2_S$ was compared to SOS and was shown to have GEF activity that prefers RAC over RAS.

The high similarity between TIAM1 and TIAM2 protein coding sequences, expression patterns, and GEF activity suggest that, like TIAM1 (Habets et al., *Cell 77*:537-549, 1994; Hordijk et al., *Science 278*:1464-1466, 1997; Ehler et al., *Mol. Cell. Neurosci. 9*:1-12, 1997; van Leeuwen et al., *J. Cell Biol., 139*:797-807, 1997), TIAM2 may play a role in neural development. The high level of TIAM2 expression throughout the E13.5 telencephalon suggests that TIAM2 may play an important role in development of the forebrain. At E13.5 postmitotic neurons are being produced; these cells must migrate to their correct positions within the developing brain and send out growth cones to establish contacts with their correct targets. These processes all require coordinating actin-based cytoskeletal rearrangements with environmental cues. There is strong evidence that the Rac/cdc42 GTPases play important roles in neuritogenesis and target recognition within the nervous system (Albertinazzi et al., *J. Cell biol. 142*:815-825, 1998; Hing et al., *Cell 97*:853-863, 1999; van Leewen et al., *supra*, 1997). Further analysis of TIAM2 expression during mouse embryogenesis may reveal a correlation with specific morphogenetic events or with specific GTP-ase family members, regulators, or effectors. It is likely that some of the specificity established during neural development is due to cell-type-specific expression of GTPase signaling pathway components such as TIAM1, RAC3, and TIAM2.

Unlike TIAM1, TIAM2 continues to be expressed at high levels in the adult forebrain. The areas of high expression in the adult are regions of synaptic plasticity (cortex, hippocampus) or neurogenesis (dentate gyrus, ependyma/subependyma) in the adult (Gould et al., *Trends Cogn. Sci. 3*:186-192, 1999;

Temple, *Curr. Biol. 9*:R397-399, 1999; Lowenstein and Parent, *Science 283*:1126-1127, 1999).

TIAM2 may play a role in metastasis or tumor suppression. Radiation hybrid mapping of TIAM2 places it in a 4-cM region of chromosome 6q25 that is frequently deleted in ovarian cancer (Colitti et al., *Oncogene 16*:555-559, 1998). Since the majority of ovarian cancer is epithelial in nature, and since TIAM1 (21q22.1; Chen and Antonarakis, *Genomics 30*:123-127, 1995) has been suggested to act as a tumor suppressor in epithelial cells, this might suggest a role for TIAM2 in ovarian cancer.

The expression of alternative forms of TIAM2 suggests distinct roles for $TIAM2_L$ and $TIAM2_S$. Both the EX and the PDZ domains found in $TIAM2_L$ have been suggested to play roles in protein-protein interactions and intracellular localization of proteins. Since $TIAM2_S$ lacks both the EX and the PDZ domains of $TIAM2_L$, the two forms may be localized to different regions of the cell. Understanding the alternate forms of TIAM2 extends knowledge of the complexity of this emerging family of GEFs and may ultimately yield insight into the divergent roles of TIAM1 as both a promoter of invasiveness and a tumor suppressor.

GDSs are implicated in various cancers and, based on the discussion herein, TIAM2 may be useful to diagnose cancerous cells. Many techniques may be used to diagnose whether tissue samples possess TIAM2-containing tumor tissue. For example, reverse transcription and PCR amplification of the RNA of a tumor sample to identify the presence of TIAM2 mRNA sequences (see Sambrook, *et al.*, Molecular Cloning; A Laboratory Manual, Second Edition (1989), chapter 14 or Gaugler *et al.*, *J. Exp. Med 179*:921-930 (1994)). Also, immunohistochemical techniques or ELISA assays may be used to identify TIAM2-expressing tumors. For example, the TIAM2 protein can be recombinantly expressed and monoclonal antibodies can be prepared according to methods that are known in the art. For example, the methods shown in EP 174,204, Kohler and Milstein, *Nature 256*:495-497 (1975), Fong *et al.*, *J. Immun. Meth. 70*:83-90 (1984), GB 2,086,937, 2,113,715, EP 57,107, 62,409, EP 118,893, EP 124,301, and EP 131,878 are suited to the present invention. The anti-TIAM2

monoclonal antibodies can then be used in the standard assays recited above or those assays that are otherwise known in the art.

Monoclonal antibodies may also be used therapeutically. Anti-TIAM2 monoclonal antibodies can be administered by means known in the art. Preferably, the antibodies are administered parenterally or subcutaneously, more preferably, they are administered intravenously. The monoclonal antibodies can be administered in combination with other agents designed to promote the activity of the antibodies or to treat the underlying condition involving the TIAM2 expressing cell.

Additionally, branched DNA testing may be performed to assay for TIAM2 DNA as shown in U.S. Patent Nos. 5,124,246, and 4,868,105 (hereby incorporated by reference in their entireties). TIAM2 nucleic acid probe molecules for the branched DNA testing are preferably from 10 to 50 bases in length, more preferably, between 15 and 40 bases in length, most preferably, between 20 and 30 bases in length

Ribozymes may be designed to act on the TIAM2 sequence identified in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof. (Kashani-Cabet and Scanlon, *Cancer Gene Therapy*, *2*:213-223 (1995), hereby incorporated by reference in its entirety.) To obtain cellular expression, the ribozyme gene is cloned into an available vector and transfected into the cells of choice. Different vectors may be chosen based on the target cell to be infected. For example, respiratory cells may be targeted by an adeno or adeno associated virus (AAV) vector. Appropriate promoters may be inserted into these vectors to ensure regulatable expression. (Kashani-Cabet at p. 216).

Antisense molecules can be developed based on the TIAM2 sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10. For example, see U. S. Patent Nos. 5,491,133 and 5,271,941 which are hereby incorporated by reference in their entireties.

Antisense RNA sequences have been described as naturally occurring biological inhibitors of gene expression in both prokaryotes (Mizuno, T., Chou, M-Y, and Inouye, M. (1984), *Proc. Natl. Acad. Sci. USA 81*, (1966-1970)) and eukaryotes (Heywood, S. M. *Nucleic Acids Res., 14*, 6771-6772 (1986) and these sequences

function by hybridizing to complementary mRNA sequences, resulting in hybridization arrest of translation (Paterson, B. M., Roberts, B. E., and Kuff, E. L., (1977) *Proc. Natl. Acad. Sci. USA, 74*, 4370-4374.

Antisense oligodeoxynucleotides are short synthetic nucleotide sequences formulated to be complementary to a specific gene or RNA message. Through the binding of these oligomers to a target DNA or mRNA sequence, transcription or translation of the gene can be selectively blocked and the disease process generated by that gene can be halted. The cytoplasmic location of mRNA provides a target considered to be readily accessible to antisense oligodeoxynucleotides entering the cell; hence much of the work in the field has focused on RNA as a target.

Antisense therapy is the administration of oligonucleotides which bind to a target polynucleotide located within the cells. These oligonucleotides are usually exogenous, but they can be endogenously expressed. The term "antisense" refers to the fact that such oligonucleotides are complementary to their intracellular targets, *e.g.*, TIAM2. See for example, Jack Cohen, Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression, CRC Press, 1989; and *Synthesis 1*:1-5 (1988).

The TIAM2 antisense oligonucleotides of the present invention include derivatives such as S-oligonucleotides (phosphorothioate derivatives or S-oligos, see, Jack Cohen, supra) which exhibit enhanced cancer cell growth inhibitory action. The TIAM2 antisense oligonucleotides of the present invention may be RNA or DNA which are complementary to and stably hybridize with the TIAM2 genome or the corresponding mRNA. Use of an oligonucleotide complementary to this region allows for the selective hybridization to TIAM2 mRNA and not to other mRNAs.

Preferably, the TIAM2 antisense oligonucleotides of the present invention are 15 to 40-mer fragments of the antisense DNA molecules which hybridize to TIAM2 mRNA. Alternatively, the preferred TIAM2 antisense oligonucleotide is a 20- to 30-mer oligonucleotide which is complementary to a region in TIAM2. Included in the present invention are pharmaceutical compositions comprising an effective amount of at least one of the TIAM2 antisense oligonucleotides of the invention in combination with a pharmaceutically acceptable carrier. In one embodiment, a single

TIAM2 antisense oligonucleotide is utilized. In another embodiment, two TIAM2 antisense oligonucleotides are utilized which are complementary to adjacent regions of the TIAM2 genome.

Administration of two TIAM2 antisense oligonucleotides which are complementary to adjacent regions of the TIAM2 genome or corresponding mRNA may allow for more efficient inhibition of TIAM2 genomic transcription or mRNA translation, resulting in more effective inhibition of cancer cell growth. Preferably, the TIAM2 antisense oligonucleotide is coadministered with an agent which enhances the uptake of the antisense molecule by the cells. For example, the TIAM2 antisense oligonucleotide may be combined with a lipophilic cationic compound which may be in the form of liposomes.

The use of liposomes to introduce nucleotides into cells is taught, for example, in U.S. Pat. Nos. 4,897,355 and 4,394,448, the disclosures of which are incorporated by reference in their entireties. See also U.S. Pat. Nos. 4,235,871, 4,231,877, 4,224,179, 4,753,788, 4,673,567, 4,247,411, 4,814,270 for general methods of preparing liposomes comprising biological materials.

Alternatively, the TIAM2 antisense oligonucleotide may be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol. In addition, the TIAM2 antisense oligonucleotide may be conjugated to a peptide that is ingested by cells. Examples of useful peptides include peptide hormones, antigens or antibodies, and peptide toxins. By choosing a peptide that is selectively taken up by the neoplastic cells, specific delivery of the antisense agent may be effected.

The TIAM2 antisense oligonucleotide may be covalently bound via the 5' H group by formation of an activated aminoalkyl derivative. The peptide of choice may then be covalently attached to the activated TIAM2 antisense oligonucleotide via an amino and sulfhydryl reactive hetero bifunctional reagent. The latter is bound to a cysteine residue present in the peptide. Upon exposure of cells to the TIAM2 antisense oligonucleotide bound to the peptide, the peptidyl antisense agent is endocytosed and

the TIAM2 antisense oligonucleotide binds to the target TIAM2 mRNA to inhibit translation. See PCT Application Publication No. PCT/US89/02363.1.

The TIAM2 antisense oligonucleotides and the pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration of the antisense compounds or other compounds of the present invention may be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes.

The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. Compositions within the scope of this invention include all compositions wherein the TIAM2 antisense oligonucleotide is contained in an amount which is effective to achieve inhibition of proliferation and/or stimulate differentiation of the subject cancer cells.

While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typically, the TIAM2 antisense oligonucleotide may be administered to mammals, *e.g.*, humans, at a dose of 0.005 to 1 mg/kg/day, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated.

In addition to administering the TIAM2 antisense oligonucleotides as a raw chemical in solution, the TIAM2 antisense oligonucleotides may be administered as part of a pharmaceutical preparation containing suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the TIAM2 antisense oligonucleotide into preparations which can be used pharmaceutically. The term pharmaceutically acceptable refers to compounds and compositions which may be administered to mammals without undue toxicity. Exemplary pharmaceutically acceptable salts include mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like.

Suitable formulations for parenteral administration include aqueous solutions of the TIAM2 antisense oligonucleotides in water-soluble form, for example,

water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The antisense oligonucleotides of the present invention may be prepared according to any of the methods that are well known to those of ordinary skill in the art. Preferably, the antisense oligonucleotides are prepared by solid phase synthesis. See, Goodchild, J., *Bioconjugate Chemistry*, *1*:165-167 (1990), for a review of the chemical synthesis of oligonucleotides. Alternatively, the antisense oligonucleotides can be obtained from a number of companies which specialize in the custom synthesis of oligonucleotides.

TIAM2 polypeptides, polynucleotides, or antibodies can be administered orally, topically, or by parenteral means. including subcutaneous and intramuscular injection, implantation of sustained release depots, intravenous injection, intranasal administration, and the like. When used to treat tumors, it may be advantageous to apply the TIAM2 polynucleotides or antibodies, for example, directly to the site, *e.g.*, during surgery to remove the bulk of the tumor. Accordingly, TIAM2 polypeptides, polynucleotides, or antibodies may be administered as a pharmaceutical composition comprising a pharmaceutically acceptable excipient. Such compositions may be aqueous solutions, emulsions, creams, ointments, suspensions, gels, liposomal suspensions, and the like. Suitable excipients include water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol (PEG), phosphate, acetate, gelatin, collagen, Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like. Cream or ointment bases useful in formulation include lanolin, Silvadene® (Marion), Aquaphor®

(Duke Laboratories), and the like. Other topical formulations include aerosols, bandages, and other wound dressings.

Alternatively, one may incorporate or encapsulate the TIAM2 polypeptides, polynucleotides, or antibodies in a suitable polymer matrix or membrane, thus providing a sustained-release delivery device suitable for implantation near the site to be treated locally. Other devices include indwelling catheters and devices such as the Alzet® minipump. Ophthalmic preparations may be formulated using commercially available vehicles such as Sorbi-care® (Allergan), Neodecadron® (Merck, Sharp & Dohme), Lacrilube®, and the like, or may employ topical preparations such as that described in US 5,124,155, incorporated herein by reference. Further, one may provide a TIAM2 polypeptide, polynucleotide, or antibody in solid form, especially as a lyophilized powder. Lyophilized formulations typically contain stabilizing and bulking agents, for example human serum albumin, sucrose, mannitol, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co.).

The amount of TIAM2 polypeptide, polynucleotide, or antibody required to treat any particular disorder will of course vary depending upon the nature and severity of the disorder, the age and condition of the subject, and other factors readily determined by one of ordinary skill in the art. The appropriate dosage may be determined by one of ordinary skill in the art.

It may be useful to administer the nucleic acid molecules described above, *i.e.*, the ribozyme or antisense molecules, in a gene therapy method. Accordingly, the vectors and techniques described below will be useful. The following expression systems describe vectors, promoters and regulatory elements that are useful for gene therapy applications for the delivery of the above polynucleotides.

Vectors and expression systems useful for the present invention include viral and non-viral systems. Examples of viral delivery systems include retroviruses, adenoviruses, adeno-associated viruses (AAV), sindbis and herpes viruses. In one aspect of the present invention, the viral vector is capable of integrating the above nucleic acid sequence into the host cell genome for long term expression.

One preferred retrovirus is a murine leukemia virus. However, it may be preferred to avoid integration into the host cell genome. Non-viral vectors include naked DNA and DNA formulated with cationic lipids or liposomes. A non-viral system that can be used is the T7/T7 system.

Additionally, it will be useful to produce TIAM2 protein from the presently disclosed nucleic acid sequence to be used in an assay to test for inhibitors or for the preparation of monoclonal antibodies, for example. TIAM2 can be expressed by a baculovirus that has been transformed with a native or modified TIAM2 nucleic acid sequence. The TIAM2 nucleic acid sequence useful in the present invention encodes a protein having an amino acid sequence that is substantially identical to the amino acid sequence of native TIAM2.

Preferably, the TIAM2 nucleic acid or protein sequence will be homologous to the partial sequences listed below. Preferably, the above sequence will be greater than 80% homologous to SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof, more preferably it will be greater than 85% homologous, more preferably greater than 90% homologous, even more preferably greater than 91%, 92%, 93% , 94% or 95% homologous. Most preferably, it will be greater than 96%, 97%, 98% or 99% homologous. Substantial identity means the sequences are identical or differ by one or more alterations (deletion, additions, substitutions) that do not adversely affect the activity of the protein. It is preferable that the protein sequences are homologous in the same percentages noted above. Percent identity or homology can be calculated using methods and algorithms known in the art. A suitable but non-limiting method is the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Biomolecular) using an affine gap search with the following search parameters: gap open penalty of 12, and gap extension penalty of 1.

The precise chemical structure of the TIAM2 sequence can depend on a number of factors. As ionizable amino and carboxyl groups are present in the molecule, a particular protein may be obtained as a acidic or basic salt, or in neutral form. All such preparations which retain their activity when placed in suitable environmental

conditions are included in the definition of proteins herein. Further, the primary amino acid sequence of the protein may be augmented by derivitization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like. Certain aspects of such augmentation are accomplished through post-translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In any event, such modifications are included in the definition of protein herein so long as the activity of the protein is not destroyed. It is expected that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the protein, in the various assays. Further, individual amino acid residues in the chain may be modified by oxidation, reduction, or derivatization, and the protein may be cleaved to obtain fragments which retain activity. Such alterations which do not destroy activity do not remove the protein sequence from the definition of TIAM2 herein.

Modifications to the primary structure itself, by deletion, addition, or alteration of the amino acids incorporated into the sequence during translation, can be made without destroying the activity of the protein. For example, site specific mutagenesis can enable specific changes in the DNA structure to effect a change in the polypeptide structure. See Mark *et al.* U. S. Pat. No. 4,959,314, and Sambrook, *et al.*, *supra*, Volume 2, chapter 15 which is hereby incorporated by reference in its entirety.

The TIAM2 proteins include mutants, fragments, fusions, and the protein encoded by the sequence listed in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof. The native TIAM2 proteins are those that occur in nature. The amino acid sequence of native polypeptides will comprise a sequence that varies slightly; typically, less than by 10-20 amino acids encoded by SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

The term "polynucleotide" or "nucleic acid sequence" as used herein refers to a polymer of nucleotides of any length, preferably deoxyribonucleotides, and is used interchangeably herein with the terms "oligonucleotide" and "oligomer." The term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, as well as antisense polynucleotides. It also includes known

types of modifications, for example, the presence of labels which are known in the art, methylation, end "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, replacement with certain types of uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) or charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), introduction of pendant moieties, such as, for example, proteins (including nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), intercalators (*e.g.*, acridine, psoralen, etc.), chelators (*e.g.*, metals, radioactive species, boron, oxidative moieties, etc.), alkylators (*e.g.*, alpha anomeric nucleic acids, etc.).

A polynucleotide sequence encoding a native TIAM2 protein can be easily modified to encode other classes of TIAM2 proteins. It will be recognized in the art that some amino acid sequence of the TIAM2 polypeptide can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there are critical areas on the protein which determine activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein. The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade et al., *Nature* *361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

Thus, the invention further includes variations of the TIAM2 polypeptide which show substantial TIAM2 polypeptide activity or which include regions of TIAM2 protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., et al., "Deciphering the Message in Protein Sequences:

Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990).

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TIAM2 proteins. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., *Clin. Exp. Immunol. 2*:331-340 (1967); Robbins et al., *Diabetes 36*:838-845 (1987); Cleland et al. *Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

Amino acids in the polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or in vitro, or in vitro proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J. Mol. Biol. 224*:899-904 (1992) and de Vos et al. *Science 255*:306-312 (1992)).

For example, mutants can be constructed by making conservative amino acid substitutions. The following are examples of conservative substitutions: Gly √ Ala; Val √ Ile √ Leu; Asp √ Glu; Lys √ Arg; Asn √ Gln; and Phe √ Trp √ Tyr. A subset of mutants, called muteins, is a group of polypeptides with the non-disulfide bond participating cysteines substituted with a neutral amino acid, generally, with serines. These mutants may be stable over a broader temperature range than native TIAM2 proteins. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of substitutions for any given TIAM2 polypeptide will not be more than 50, 40,

30, 25, 20, 15, 10, 5 or 3. The coding sequence of mutants can be constructed by *in vitro* mutagenesis of the native TIAM2 polypeptide coding sequences.

As used herein, the term "protein" or "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, polypeptides, proteins, and polyproteins, as well as fragments of these, are included within this definition. This term also does not refer to, or exclude, post expression modifications of the protein, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, proteins containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), proteins with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

A polypeptide or protein or amino acid sequence "derived from" or "coded by" or "encoded by" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 consecutive amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence.

"Purified" and "isolated" mean, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000, can be present).

Also included within the scope of the invention are fragments of polynucleotides that encode the TIAM2 polypeptides disclosed herein. Such fragments may encode antigenic regions of the proteins or biologically functional regions of the

proteins as described herein. Such fragments also encode polypeptide components of the fusion proteins disclosed herein. Fragments are also suitable for use as probes for identifying polynucleotides capable of hybridizing with SEQ ID NO:8, 9, and/or 10. Fragments can range in length from about 10 basepairs to the full length polynucleotide, which is 3344 basepairs for SEQ ID NO:8, 4590 basepairs for SEQ ID NO:9, and 4514 basepairs for SEQ ID NO:10. Preferred fragments are 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, or 3300 basepairs for SEQ ID NO: 8, 9, or 10; 3400, 3500, 3600, 3700, 3800, 4000, 4100, 4200, 4300, 4400, 4500, or 4510 basepairs for SEQ ID NO:9 or 10; and 4520, 4550, 4575, or 4580 basepairs for SEQ ID NO:10. A particularly preferred fragment comprises nucleotides 1-104 of SEQ ID NO:8.

Polypeptide fragments are amino and/or carboxyl terminal amino acid deletions of mutant or native TIAM2 proteins. The number of amino acids that are truncated is not critical as long as the polypeptide fragment exhibits the desired sequence homology, immunological or biological activity. Preferred fragments are 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600 or 625 amino acids of SEQ ID NO:7, 11, 12 or 13; 650, 675, 700, 750, 800, 825, 900, 950, 1000, 1025, or 1050 amino acids of SEQ ID NO:12 or 13; and 1060, 1065, 1070 or 1075 amino acids of SEQ ID NO:13. Polypeptide fragments of immunological significance comprise, for example, at least one epitope shared by a native TIAM2 protein. Such TIAM2 proteins may be only 5-15 amino acids in length. Examples of amino acid sequence of fragments comprise amino acid number 1-8 (aa1 to aa8) of SEQ ID NO:7, 11, 12, or 13; aa2 to aa9 of SEQ ID NO:7, 11, 12, or 13; aa3 to aa10 of SEQ ID NO:7, 11, 12, or 13; aa4 to aa11 of SEQ ID NO:7, 11, 12, or 13; aa5 to aa12 of SEQ ID NO:7, 11, 12, or 13; aa6 to aa13 of SEQ ID NO:7, 11, 12, or 13; aa7 to aa14 of SEQ ID NO:7, 11, 12, or 13; aa8 to aa15 of SEQ ID NO:7, 11, 12, or 13; aa9 to aa16 of SEQ ID NO:7, 11, 12, or 13; aa10 to aa17 of SEQ ID NO:7, 11, 12, or 13; aa11 to aa18 of SEQ ID NO:7, 11, 12, or 13; aa12 to aa19 of

SEQ ID NO:7, 11, 12, or 13; aa13 to aa20 of SEQ ID NO:7, 11, 12, or 13: aa14 to aa21 of SEQ ID NO:7, 11, 12, or 13; aa15 to aa22 of SEQ ID NO:7, 11, 12, or 13; aa16 to aa23 of SEQ ID NO:7, 11, 12, or 13; aa17 to aa24 of SEQ ID NO:7, 11, 12, or 13; aa18 to aa25 of SEQ ID NO:7, 11, 12, or 13; aa19 to aa26 of SEQ ID NO:7, 11, 12, or 13; aa20 to aa27 of SEQ ID NO:7, 11, 12, or 13; aa21 to aa28 of SEQ ID NO:7, 11, 12, or 13; aa22 to aa29 of SEQ ID NO:7, 11, 12, or 13; aa23 to aa30 of SEQ ID NO:7, 11, 12, or 13; aa24 to aa31 of SEQ ID NO:7, 11, 12, or 13; aa25 to aa32 of SEQ ID NO:7, 11, 12, or 13; aa26 to aa33 of SEQ ID NO:7, 11, 12, or 13; aa27 to aa34 of SEQ ID NO:7, 11, 12, or 13; aa28 to aa35 of SEQ ID NO:7, 11, 12, or 13; aa29 to aa36 of SEQ ID NO:7, 11, 12, or 13; aa30 to aa37 of SEQ ID NO:7, 11, 12, or 13; aa31 to aa38 of SEQ ID NO:7, 11, 12, or 13; aa32 to aa39 of SEQ ID NO:7, 11, 12, or 13; aa33 to aa40 of SEQ ID NO:7, 11, 12, or 13; aa34 to aa41 of SEQ ID NO:7, 11, 12, or 13; aa35 to aa42 of SEQ ID NO:7, 11, 12, or 13; aa36 to aa43 of SEQ ID NO:7, 11, 12, or 13; aa37 to aa44 of SEQ ID NO:7, 11, 12, or 13, aa38 to aa45 of SEQ ID NO:7, 11, 12, or 13; aa39 to aa46 of SEQ ID NO:7, 11, 12, or 13; aa40 to aa47 of SEQ ID NO:7, 11, 12, or 13; aa41 to aa48 of SEQ ID NO:7, 11, 12, or 13; aa42 to aa49 of SEQ ID NO:7, 11, 12, or 13; aa43 to aa50 of SEQ ID NO:7, 11, 12, or 13; aa44 to aa51 of SEQ ID NO:7, 11, 12, or 13; aa45 to aa52 of SEQ ID NO:7, 11, 12, or 13; aa46 to aa53 of SEQ ID NO:7, 11, 12, or 13; aa47 to aa54 of SEQ ID NO:7, 11, 12, or 13; aa48 to aa55 of SEQ ID NO:7, 11, 12, or 13; aa49 to aa56 of SEQ ID NO:7, 11, 12, or 13; aa50 to aa57 of SEQ ID NO:7, 11, 12, or 13; aa51 to aa58 of SEQ ID NO:7, 11, 12, or 13; aa52 to aa59 of SEQ ID NO:7, 11, 12, or 13; aa53 to aa60 of SEQ ID NO:7, 11, 12, or 13; aa54 to aa61 of SEQ ID NO:7, 11, 12, or 13; aa55 to aa62 of SEQ ID NO:7, 11, 12, or 13; aa56 to aa63 of SEQ ID NO:7, 11, 12, or 13; aa57 to aa64 of SEQ ID NO:7, 11, 12, or 13; aa58 to aa65 of SEQ ID NO:7, 11, 12, or 13; aa59 to aa66 of SEQ ID NO:7, 11, 12, or 13; aa60 to aa67 of SEQ ID NO:7, 11, 12, or 13; aa61 to aa68 of SEQ ID NO:7, 11, 12, or 13; aa62 to aa69 of SEQ ID NO:7, 11, 12, or 13; aa63 to aa70 of SEQ ID NO:7, 11, 12, or 13; aa64 to aa71 of SEQ ID NO:7, 11, 12, or 13; aa65 to aa72 of SEQ ID NO:7, 11, 12, or 13; aa66 to aa73 of SEQ ID NO:7, 11, 12, or 13; aa67 to aa74 of SEQ ID NO:7, 11, 12, or 13; aa68 to aa75 of SEQ ID NO:7, 11, 12, or 13; aa69 to aa76 of SEQ ID NO:7,

11, 12, or 13; aa70 to aa77 of SEQ ID NO:7, 11, 12, or 13; aa71 to aa78 of SEQ ID NO:7, 11, 12, or 13; aa72 to aa79 of SEQ ID NO:7, 11, 12, or 13; aa73 to aa80 of SEQ ID NO:7, 11, 12, or 13; aa74 to aa81 of SEQ ID NO:7, 11, 12, or 13; aa75 to aa82 of SEQ ID NO:7, 11, 12, or 13; aa76 to aa83 of SEQ ID NO:7, 11, 12, or 13; aa77 to aa84 of SEQ ID NO:7, 11, 12, or 13; aa78 to aa84 of SEQ ID NO:7, 11, 12, or 13; aa79 to aa86 of SEQ ID NO:7, 11, 12, or 13; aa80 to aa87 of SEQ ID NO:7, 11, 12, or 13; aa81 to aa88 of SEQ ID NO:7, 11, 12, or 13; aa82 to aa89 of SEQ ID NO:7, 11, 12, or 13; aa83 to aa90 of SEQ ID NO:7, 11, 12, or 13; aa84 to aa91 of SEQ ID NO:7, 11, 12, or 13; aa85 to aa92 of SEQ ID NO:7, 11, 12, or 13; aa86 to aa93 of SEQ ID NO:7, 11, 12, or 13; aa87 to aa94 of SEQ ID NO:7, 11, 12, or 13; aa88 to aa95 of SEQ ID NO:7, 11, 12, or 13; aa89 to aa96 of SEQ ID NO:7, 11, 12, or 13; aa90 to aa97 of SEQ ID NO:7, 11, 12, or 13; aa91 to aa98 of SEQ ID NO:7, 11, 12, or 13; aa92 to aa99 of SEQ ID NO:7, 11, 12, or 13; aa93 to aa100 of SEQ ID NO:7, 11, 12, or 13; aa94 to aa101 of SEQ ID NO:7, 11, 12, or 13; aa95 to aa102 of SEQ ID NO:7, 11, 12, or 13; aa96 to aa103 of SEQ ID NO:7, 11, 12, or 13; aa97 to aa104 of SEQ ID NO:7, 11, 12, or 13; aa98 to aa105 of SEQ ID NO:7, 11, 12, or 13; aa99 to aa106 of SEQ ID NO:7, 11, 12, or 13; aa100 to aa107 of SEQ ID NO:7, 11, 12, or 13 aa101 to aa108 of SEQ ID NO:7, 11, 12, or 13 aa102 to aa109 of SEQ ID NO:7, 11, 12, or 13; aa103 to aa110 of SEQ ID NO:7, 11, 12, or 13; aa104 to aa111 of SEQ ID NO:7, 11, 12, or 13; aa105 to aa112 of SEQ ID NO:7, 11, 12, or 13; aa106 to aa113 of SEQ ID NO:7, 11, 12, or 13; aa107 to aa114 of SEQ ID NO:7, 11, 12, or 13; aa108 to aa115 of SEQ ID NO:7, 11, 12, or 13; aa109 to aa116 of SEQ ID NO:7, 11, 12, or 13; aa110 to aa117 of SEQ ID NO:7, 11, 12, or 13; aa111 to aa118 of SEQ ID NO:7, 11, 12, or 13; aa112 to aa119 of SEQ ID NO:7, 11, 12, or 13; aa113 to aa120 of SEQ ID NO:7, 11, 12, or 13; aa114 to aa121 of SEQ ID NO:7, 11, 12, or 13; aa115 to aa122 of SEQ ID NO:7, 11, 12, or 13; aa116 to aa123 of SEQ ID NO:7, 11, 12, or 13; aa117 to aa124 of SEQ ID NO:7, 11, 12, or 13; aa118 to aa125 of SEQ ID NO:7, 11, 12, or 13; aa119 to aa126 of SEQ ID NO:7, 11, 12, or 13; aa120 to aa127 of SEQ ID NO:7, 11, 12, or 13; aa121 to aa128 of SEQ ID NO:7, 11, 12, or 13; aa122 to aa129 of SEQ ID NO:7, 11, 12, or 13; aa123 to aa130 of SEQ ID NO:7, 11, 12, or 13; aa124 to aa131 of SEQ ID NO:7, 11, 12, or 13; aa125 to

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NO:12 or 13; aa853 to aa860 of SEQ ID NO:12 or 13; aa854 to aa861 of SEQ ID NO:12 or 13; aa855 to aa862 of SEQ ID NO:12 or 13; aa856 to aa863 of SEQ ID NO:12 or 13; aa857 to aa864 of SEQ ID NO:12 or 13; aa858 to aa865 of SEQ ID NO:12 or 13; aa859 to aa866 of SEQ ID NO:12 or 13; aa860 to aa867 of SEQ ID NO:12 or 13; aa861 to aa868 of SEQ ID NO:12 or 13; aa862 to aa869 of SEQ ID NO:12 or 13; aa863 to aa870 of SEQ ID NO:12 or 13; aa864 to aa871 of SEQ ID NO:12 or 13; aa865 to aa872 of SEQ ID NO:12 or 13; aa866 to aa873 of SEQ ID NO:12 or 13; aa867 to aa874 of SEQ ID NO:12 or 13; aa868 to aa875 of SEQ ID NO:12 or 13; aa869 to aa876 of SEQ ID NO:12 or 13; aa870 to aa877 of SEQ ID NO:12 or 13; aa871 to aa878 of SEQ ID NO:12 or 13; aa872 to aa879 of SEQ ID NO:12 or 13; aa873 to aa880 of SEQ ID NO:12 or 13; aa874 to aa881 of SEQ ID NO:12 or 13; aa875 to aa882 of SEQ ID NO:12 or 13; aa876 to aa883 of SEQ ID NO:12 or 13; aa877 to aa884 of SEQ ID NO:12 or 13; aa878 to aa884 of SEQ ID NO:12 or 13; aa879 to aa886 of SEQ ID NO:12 or 13; aa880 to aa887 of SEQ ID NO:12 or 13; aa881 to aa888 of SEQ ID NO:12 or 13; aa882 to aa889 of SEQ ID NO:12 or 13; aa883 to aa890 of SEQ ID NO:12 or 13; aa884 to aa891 of SEQ ID NO:12 or 13; aa885 to aa892 of SEQ ID NO:12 or 13; aa886 to aa893 of SEQ ID NO:12 or 13; aa887 to aa894 of SEQ ID NO:12 or 13; aa888 to aa895 of SEQ ID NO:12 or 13; aa889 to aa896 of SEQ ID NO:12 or 13; aa890 to aa897 of SEQ ID NO:12 or 13; aa891 to aa898 of SEQ ID NO:12 or 13; aa892 to aa899 of SEQ ID NO:12 or 13; aa893 to aa900 of SEQ ID NO:12 or 13; aa894 to aa901 of SEQ ID NO:12 or 13; aa895 to aa902 of SEQ ID NO:12 or 13; aa896 to aa903 of SEQ ID NO:12 or 13; aa897 to aa904 of SEQ ID NO:12 or 13; aa898 to aa905 of SEQ ID NO:12 or 13; aa899 to aa906 of SEQ ID NO:12 or 13; aa900 to aa907 of SEQ ID NO:12 or 13; aa901 to aa908 of SEQ ID NO:12 or 13; aa902 to aa909 of SEQ ID NO:12 or 13; aa903 to aa910 of SEQ ID NO:12 or 13; aa904 to aa911 of SEQ ID NO:12 or 13; aa905 to aa912 of SEQ ID NO:12 or 13; aa906 to aa913 of SEQ ID NO:12 or 13; aa907 to aa914 of SEQ ID NO:12 or 13; aa908 to aa915 of SEQ ID NO:12 or 13; aa909 to aa916 of SEQ ID NO:12 or 13; aa910 to aa917 of SEQ ID NO:12 or 13; aa911 to aa918 of SEQ ID NO:12 or 13; aa912 to aa919 of SEQ ID

NO:12 or 13; aa913 to aa920 of SEQ ID NO:12 or 13; aa914 to aa921 of SEQ ID NO:12 or 13; aa915 to aa922 of SEQ ID NO:12 or 13; aa916 to aa923 of SEQ ID NO:12 or 13; aa917 to aa924 of SEQ ID NO:12 or 13; aa918 to aa925 of SEQ ID NO:12 or 13; aa919 to aa926 of SEQ ID NO:12 or 13; aa920 to aa927 of SEQ ID NO:12 or 13; aa921 to aa928 of SEQ ID NO:12 or 13; aa922 to aa929 of SEQ ID NO:12 or 13; aa923 to aa930 of SEQ ID NO:12 or 13; aa924 to aa931 of SEQ ID NO:12 or 13; aa925 to aa932 of SEQ ID NO:12 or 13; aa926 to aa933 of SEQ ID NO:12 or 13; aa927 to aa934 of SEQ ID NO:12 or 13; aa928 to aa935 of SEQ ID NO:12 or 13; aa929 to aa936 of SEQ ID NO:12 or 13; aa930 to aa937 of SEQ ID NO:12 or 13; aa931 to aa938 of SEQ ID NO:12 or 13; aa932 to aa939 of SEQ ID NO:12 or 13; aa933 to aa940 of SEQ ID NO:12 or 13; aa934 to aa941 of SEQ ID NO:12 or 13; aa935 to aa942 of SEQ ID NO:12 or 13; aa936 to aa943 of SEQ ID NO:12 or 13; aa937 to aa944 of SEQ ID NO:12 or 13; aa938 to aa945 of SEQ ID NO:12 or 13; aa939 to aa946 of SEQ ID NO:12 or 13; aa940 to aa947 of SEQ ID NO:12 or 13; aa941 to aa948 of SEQ ID NO:12 or 13; aa942 to aa949 of SEQ ID NO:12 or 13; aa943 to aa950 of SEQ ID NO:12 or 13; aa944 to aa951 of SEQ ID NO:12 or 13; aa945 to aa952 of SEQ ID NO:12 or 13; aa946 to aa953 of SEQ ID NO:12 or 13; aa947 to aa954 of SEQ ID NO:12 or 13; aa948 to aa955 of SEQ ID NO:12 or 13; aa949 to aa956 of SEQ ID NO:12 or 13; aa950 to aa957 of SEQ ID NO:12 or 13; aa951 to aa958 of SEQ ID NO:12 or 13; aa952 to aa959 of SEQ ID NO:12 or 13; aa953 to aa960 of SEQ ID NO:12 or 13; aa954 to aa961 of SEQ ID NO:12 or 13; aa955 to aa962 of SEQ ID NO:12 or 13; aa956 to aa963 of SEQ ID NO:12 or 13; aa957 to aa964 of SEQ ID NO:12 or 13; aa958 to aa965 of SEQ ID NO:12 or 13; aa959 to aa966 of SEQ ID NO:12 or 13; aa960 to aa967 of SEQ ID NO:12 or 13; aa961 to aa968 of SEQ ID NO:12 or 13; aa962 to aa969 of SEQ ID NO:12 or 13; aa963 to aa970 of SEQ ID NO:12 or 13; aa964 to aa971 of SEQ ID NO:12 or 13; aa965 to aa972 of SEQ ID NO:12 or 13; aa966 to aa973 of SEQ ID NO:12 or 13; aa967 to aa974 of SEQ ID NO:12 or 13; aa968 to aa975 of SEQ ID NO:12 or 13; aa969 to aa976 of SEQ ID NO:12 or 13; aa970 to aa977 of SEQ ID NO:12 or 13; aa971 to aa978 of SEQ ID NO:12 or 13; aa972 to aa979 of SEQ ID

NO:12 or 13; aa973 to aa980 of SEQ ID NO:12 or 13; aa974 to aa981 of SEQ ID NO:12 or 13; aa975 to aa982 of SEQ ID NO:12 or 13; aa976 to aa983 of SEQ ID NO:12 or 13; aa977 to aa984 of SEQ ID NO:12 or 13; aa978 to aa984 of SEQ ID NO:12 or 13; aa979 to aa986 of SEQ ID NO:12 or 13; aa980 to aa987 of SEQ ID NO:12 or 13; aa981 to aa988 of SEQ ID NO:12 or 13; aa982 to aa989 of SEQ ID NO:12 or 13; aa983 to aa990 of SEQ ID NO:12 or 13; aa984 to aa991 of SEQ ID NO:12 or 13; aa985 to aa992 of SEQ ID NO:12 or 13; aa986 to aa993 of SEQ ID NO:12 or 13; aa987 to aa994 of SEQ ID NO:12 or 13; aa988 to aa995 of SEQ ID NO:12 or 13; aa989 to aa996 of SEQ ID NO:12 or 13; aa990 to aa997 of SEQ ID NO:12 or 13; aa991 to aa998 of SEQ ID NO:12 or 13; aa992 to aa999 of SEQ ID NO:12 or 13; aa993 to aa1000 of SEQ ID NO:12 or 13; aa994 to aa1001 of SEQ ID NO:12 or 13; aa995 to aa1002 of SEQ ID NO:12 or 13; aa996 to aa1003 of SEQ ID NO:12 or 13; aa997 to aa1004 of SEQ ID NO:12 or 13; aa998 to aa1005 of SEQ ID NO:12 or 13; aa999 to aa1006 of SEQ ID NO:12 or 13; aa1000 to aa1007 of SEQ ID NO:12 or 13; aa1001 to aa1008 of SEQ ID NO:12 or 13; aa1002 to aa1009 of SEQ ID NO:12 or 13; aa1003 to aa1010 of SEQ ID NO:12 or 13; aa1004 to aa1011 of SEQ ID NO:12 or 13; aa1005 to aa1012 of SEQ ID NO:12 or 13; aa1006 to aa1013 of SEQ ID NO:12 or 13; aa1007 to aa1014 of SEQ ID NO:12 or 13; aa1008 to aa1015 of SEQ ID NO:12 or 13; aa1009 to aa1016 of SEQ ID NO:12 or 13; aa1010 to aa1017 of SEQ ID NO:12 or 13; aa1011 to aa1018 of SEQ ID NO:12 or 13; aa1012 to aa1019 of SEQ ID NO:12 or 13; aa1013 to aa1020 of SEQ ID NO:12 or 13; aa1014 to aa1021 of SEQ ID NO:12 or 13; aa1015 to aa1022 of SEQ ID NO:12 or 13; aa1016 to aa1023 of SEQ ID NO:12 or 13; aa1017 to aa1024 of SEQ ID NO:12 or 13; aa1018 to aa1025 of SEQ ID NO:12 or 13; aa1019 to aa1026 of SEQ ID NO:12 or 13; aa1020 to aa1027 of SEQ ID NO:12 or 13; aa1021 to aa1028 of SEQ ID NO:12 or 13; aa1022 to aa1029 of SEQ ID NO:12 or 13; aa1023 to aa1030 of SEQ ID NO:12 or 13; aa1024 to aa1031 of SEQ ID NO:12 or 13; aa1025 to aa1032 of SEQ ID NO:12 or 13; aa1026 to aa1033 of SEQ ID NO:12 or 13; aa1027 to aa1034 of SEQ ID NO:12 or 13; aa1028 to aa1035 of SEQ ID NO:12 or 13; aa1029 to aa1036 of SEQ ID NO:12 or 13; aa1030 to aa1037 of SEQ ID NO:12 or 13; aa1031 to aa1038 of SEQ ID NO:12 or 13; aa1032 to aa1039 of SEQ ID

NO:12 or 13; aa1033 to aa1040 of SEQ ID NO:12 or 13; aa1034 to aa1041 of SEQ ID NO:12 or 13; aa1035 to aa1042 of SEQ ID NO:12 or 13; aa1036 to aa1043 of SEQ ID NO:12 or 13; aa1037 to aa1044 of SEQ ID NO:12 or 13; aa1038 to aa1045 of SEQ ID NO:12 or 13; aa1039 to aa1046 of SEQ ID NO:12 or 13; aa1040 to aa1047 of SEQ ID NO:12 or 13; aa1041 to aa1048 of SEQ ID NO:12 or 13; aa1042 to aa1049 of SEQ ID NO:12 or 13; aa1043 to aa1050 of SEQ ID NO:12 or 13; aa1044 to aa1051 of SEQ ID NO:12 or 13; aa1045 to aa1052 of SEQ ID NO:12 or 13; aa1046 to aa1053 of SEQ ID NO:12 or 13; aa1047 to aa1054 of SEQ ID NO:12 or 13; aa1048 to aa1055 of SEQ ID NO:13; aa1049 to aa1056 of SEQ ID NO: 13; aa1050 to aa1057 of SEQ ID NO:13; aa1051 to aa1058 of SEQ ID NO: 13; aa1052 to aa1059 of SEQ ID NO:13; aa1053 to aa1060 of SEQ ID NO:13; aa1054 to aa1061 of SEQ ID NO:13; aa1055 to aa1062 of SEQ ID NO:13; aa1056 to aa1063 of SEQ ID NO:13; aa1057 to aa1064 of SEQ ID NO:13; aa1058 to aa1065 of SEQ ID NO:13; aa1059 to aa1066 of SEQ ID NO:13; aa1060 to aa1067 of SEQ ID NO:13; aa1061 to aa1068 of SEQ ID NO:13; aa1062 to aa1069 of SEQ ID NO:13; aa1063 to aa1070 of SEQ ID NO:13; aa1064 to aa1071 of SEQ ID NO:13; aa1065 to aa1072 of SEQ ID NO:13; aa1066 to aa1073 of SEQ ID NO:13; aa1067 to aa1074 of SEQ ID NO:13; aa1068 to aa1075 of SEQ ID NO:13; aa1069 to aa1076 of SEQ ID NO:13; aa1070 to aa1077 of SEQ ID NO:13; and aa1071 to aa1078 of SEQ ID NO:13. The coding sequence of fragments can be constructed by cleaving the unwanted nucleotides from the mutant or native TIAM2 protein coding sequences.

Fusions are fragment, mutant, or native TIAM2 proteins with additional amino acids at either or both of the termini. The additional amino acid sequence generally is not homologous to sequence found in native TIAM2 polypeptides. The additional amino acid residues can facilitate expression, detection, or activity of the polypeptide, for example. The additional amino acid sequence can also be used as linker to construct multimers of TIAM2 proteins. All fusion polypeptides exhibit the desired sequence homology, immunological or biological activity. Recombinant TIAM2 fusion proteins can be produced using the preferred cell system, baculovirus.

After inserting the TIAM2 DNA sequence into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987).

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above-described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate construct (transfer vector).

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 base pairs downstream from the ATT; *see* Luckow and Summers, *Virology 17*:31 (1989).

The plasmid usually also contains the polyhedron polyadenylation signal (Miller *et al. Ann. Rev. Microbiol.*, *42*:177 (1988)) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen *et al.*, (1986) "The

Regulation of Baculovirus Gene Expression," in: The Molecular Biology of Baculoviruses (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak *et al.*, *J. Gen. Virol. 69*:765 (1988).

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell *et al. Gene*, *73*:409 (1988)). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α-interferon, Maeda *et al.*, *Nature 315*:592 (1985); human gastrin-releasing peptide, Lebacq-Verheyden *et al.*, *Molec. Cell. Biol. 8*:3129 (1988); human IL-2, Smith *et al.*, *Proc. Nat'l Acad. Sci. USA, 82*:8404 (1985); mouse IL-3, (Miyajima *et al.*, *Gene 58*:273 (1987); and human glucocerebrosidase, Martin *et al. DNA 7*:99 (1988), can also be used to provide for secretion in insects.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus, usually by co-transfection. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith; Ju *et al.* (1987); Smith *et al.*, *Mol. Cell. Biol. 3*:2156 (1983); and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller *et al.*, *Bioessays 4*:91 (1989).

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Methods to identify recombinant viruses are described in "Current Protocols in Microbiology" Vol. 2 (Ausubel *et al.* eds) at 16.8 (Supp. 10, 1990); Summers and Smith; Miller *et al.* (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, inter alia: *Aedes aegypti* , *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell *et al.*, *J. Virol. 56*:153 (1985); Wright *Nature 321*:718 (1986); Smith *et al.*, *Mol. Cell. Biol. 3*:2156 (1983); and see generally, Fraser, *et al.* In *Vitro Cell. Dev. Biol. 25*:225 (1989)).

The TIAM2 protein can be used in an assay for inhibitors and for preparing antibodies directed to TIAM2. TIAM2 protein may also be useful as a factor that promotes the growth of cancer cells in culture. The TIAM2 protein may be combined with the pharmaceutically acceptable carrier noted above for use with the TIAM2 antisense molecule.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

EXAMPLE 1:

IDENTIFICATION OF THE TIAM2 GENE

In a degenerative PCR-based screen for new genes, a single 377-nucleotide clone (DP-75) was identified, with high homology to the mouse and human TIAM1 genes. Due to its similarity to TIAM1, this new gene and its predicted protein product were designated T-cell lymphoma invasion and metastasis 2 (TIAM2).

Northern analysis with the DP-75 probe revealed a ~3.3-kb transcript in cerebral cortex, occipital pole, frontal lobe, and temporal lobe, and a ~4.4-kb transcript in testis and cerebellum (Figures 1A and 1B). Through cDNA library screens and RACE cloning, overlapping clones were isolated and compiled into full-length

sequences representing the ~4.4- and ~3.3-kb messages. To perform Northern analyses, Human Multiple Tissue Northern Blots (Clontech) were hybridized with DP-75 probe in SpeedHyb solution (BIOS lab) at 65° C. Blots were washed two times with 0.5 x SSC, 0.5% SDS at room temperature for 10 min. and one time with 0.1 x SSC, 0.5% SDS at 65° C for I hr.

The compiled cDNA for the ~4.4-kb message is 4586 nucleotides long, without polyadenylation, and is predicted to encode the 1077-amino-acid-long form of the TIAM2 protein ($TIAM2_L$). The first ATG is at nucleotide 51 and is in good context for translation. An alignment of the predicted protein to TIAM1 (Figure 3) shows significant identity beginning with the first methionine of TIAM2 and a methionine at position 576 of TIAM1, a region that corresponds to the last 14 amino acids of the coiled coil domain of TIAM1 (aa 548-590). The identity continues into the EX domain, which extends from amino acid 599 to amino acid 691 of human TIAM1. A comparison of the TIAM1/$TIAM2_L$ EX domains shows 62% overall identity, but with 85% identity in a 60-aa subdomain. Following the EX domain, alternative splicing of a 72-nucleotide miniexon leads to the presence or absence of a 24-amino acid sequence with no significant identity to TIAM1 or *SIF* sequences. Following the alternatively spliced region is a sequence with weak identity to TIAM1 (18%), but which contains the conserved residues of PDZ or DHR domains (Michiels et al., Nature 375:338-340, 1995). The remaining sequence contains identity to the DH catalytic region (70%) and the carboxyl-terminal PH domain (54%).

The compiled sequence for the ~3.3-kb message is 3344 nucleotides long and is predicted to encode a short form (626 aa) of TIAM2 ($TIAM2_S$). The ~3.3-kb message differs from the ~4.4-kb message in that the first 104 nucleotides are unique, suggesting that the ~3.3-kb message results from initiation at a promoter distinct from that of the ~4.4-kb message. The initiator methionine at position 162 coincides with a methionine at amino acid 428 of the 4.4-kb TIAM2 predicted protein and for the remainder of the protein shows complete identity with $TIAM2_L$. Similar to what is seen with the TIAM1 gene (Habets et al., Cell 77:537-549, 1994), there are two upstream ATGs in the ~3.3-kb message that precede the long open reading frame (nucleotide

162), but only the start codon at nucleotide 162 is in good context for translation. A comparison with TIAM1 shows that $TIAM2_S$, begins immediately upstream (24 aa) of the DH domain.

Northern analysis with a probe isolated from the 5' end of the ~4.4-kb message identifies only the larger TIAM2 transcript (Figure 1C), while a probe isolated from the 3' end of TIAM2 identifies both transcripts (Figure 1D).

A Radiation Hybrid Mapping experiment was performed as follows. TIAM2-specific oligos AGTCCTACCTCATCAAGCCG (SEQ ID NO:14) and TTAGTGCTTCCGTCAGGTGG (SEQ ID NO:15) were synthesized and used with the G3 panel (Research Genetics) as per the manufacturer's instructions. The resulting PCR products were analyzed by gel electrophoresis, and the resulting data were analyzed according to methods of Stewart and Cox, *In* "Genome Mapping: A Practical Approach" (P. Dear et al. Ed.) pp. 73-93, Oxford Univ. Press, Oxford, 1997. A two-point maximum-likelihood analysis showed that the markers were linked to D6S1556 (6q25) with a lod score of 1000.

EXAMPLE 2

IN SITU HYBRIDIZATION

Oligos designed from nucleotides 566-586 (AGTACAGAAGGTT-CTGGAGCG) and from nucleotides 1348-1367 (TACCAGGCGATCCTTACACG) of the human 3.3 kb TIAM2 cDNA were used to perform PCR on mouse brain cDNA (Clontech). Thirty cycles of PCR performed with an annealing temperature of 40° C led to the production of a product (MDP75) of the expected size (802 nt). Cloning and sequencing confirmed that MDP75 was 90% identical to human TIAM2 throughout the length of the clone. Mouse embryos (CD-1) were processed for whole-mount RNA *in situ* hybridization following the protocol of Nieto *et al.*, *In* "Methods in Avian Embryology" (M. Bronner-Fraser, Ed.), Academic Press, San Diego (1995). Adult brain sections were processed using the protocol for frozen sections as described (Schaeren-Wiemers and Gerfin-Moser, *Histochemistry 100*:431-440 (1993)).

The expression of TIAM2 mRNA in the embryonic day 13.5 (E13.5) and adult mouse brain was examined by *in situ* hybridization with a digoxigenin-labeled ribo-probe. Discrete and reproducible labeling was observed at both ages with an antisense probe; no labeling was detected with a sense probe. In both E13.5 and adult brain, the cellular localization of TIAM-2 transcripts is consistent with TIAM2 expression in neurons rather than glial cells. At E13.5, TIAM2 transcripts are present throughout the telencephalon (Figure 2A). Parasagittal sections through the telencephalon demonstrate that expression is localized to the pial surface where early born postmitotic neurons are located (Figure 2B). TIAM2 is not expressed in the proliferating neural precursor cells that line the ventricles.

In the adult mouse brain, TIAM2 continues to be expressed primarily in regions derived from the telencephalon (Figures 2C-2G, Table 1). Areas of strong expression include the olfactory bulb, cerebral cortex, caudate putamen, and hippocampus. The hippocampus expression is highly specific in that cells of the dentate gyrus and regions derived from it, induseum griseum and fasciola cinerea; and pyramidal cells of one subregion of the CA fields (CA2) are labeled very strongly while CA1 and CA3 show very little expression. TIAM2 is also strongly expressed in the ependymal cells of the lateral surface of the lateral ventricles (Figure 2D). This region is a germinal zone where neurons are generated throughout adult life. The majority of neurons born in this region are believed to migrate to the olfactory bulb.

TABLE 1

EXPRESSION OF TIAM2 MRNA IN THE ADULT MOUSE BRAIN

Olfactory bulb	
Mitral cell layer	+++
Glomerular layer	++
Granular cell layer	+++
Olfactory nuclei	++
Cerebral cortex	+++
Hippocampus	
CA1	+
CA2	++++
CA3	+
Dentate gyrus	++++
Insidium gresium	++++
Fasciola cinerea	++++
Amygdala	+
Septum	++
Caudate putamen	+++
Ependymal and subependymal layers	+++
Thalamus	
Reticular nucleus	+++
Ventral posterolateral nucleus	+++
Pons	++

Note. Relative levels of expression based on bright-field microscopy. The highest levels and lowest levels of expression are indicated by ++++ and +, respectively.

EXAMPLE 3

GDP-GTP EXCHANGE ACTIVITY

An amino-terminal HIS tagged version of the 3.3-kb $TIAM_S$ protein (His-TIAM2) was expressed in a baculovirus system. A protein of the predicted molecular mass (75,661 Da) was partially purified on a nickel column, and its identity was confirmed by amino-terminal sequencing and mass spectrometry. To generate the His-tagged $TIAM2_S$ expression construct, a linker-adapter was produced such that it added an *Eco*RI site to the 5' end of the first 29 bp of the $TIAM2_S$ sequence (GAATTCATGGAAGGACCGCGGGA-GAATCAGGATCC). This kinased linker/adapter, terminating in a *Bam*HI site (bases 23-29 of the $TIAM2_S$ sequence), was ligated to a cDNA clone DP-75#1, which had been linearized by a *Bam*HI fragment that encompassed the entire coding region of $TIAM2_S$. A subsequent *Sal*I digest liberated an ~2000-bp *Eco*RI-*Sal*I fragment that encompassed the entire coding region of $TIAM2_S$. The fragment was isolated, purified, and ligated into *Eco*RI-*Sal*I-digested pBlueBacHis2B vector (Invitrogen). The resulting clone (pHIS- $TIAM2_S$) was confirmed by sequence analysis.

pHIS-$TIAM2_S$ was introduced into SF9 cells, and clones expressing high levels of pHIS- $TIAM2_S$ were identified by Western blot. Following clonal expansion, pHIS- $TIAM2_S$-expressing cells were concentrated, lysed, and applied to a nickel column (Invitrogen). pHIS-$TIAM2_S$ protein was purified from the column as per the manufacturer's recommendations except that following the 50 mM imidazole wash, the protein was directly eluted in 500 mM imidazole. The fractions containing pHIS-$TIAM2_S$ were concentrated on a Centricon (Amicon) 30 column spun at 5000*g* for 30 min.

His-$TIAM2_S$ was tested for its ability to stimulate the GDP-GTP exchange activity with Rac or Ras proteins and was compared to the activity of SOS, a known Ras GEF (Jefferson et al., Oncogene 16:2303-2310, 1998). In these experiments (Figure 4), immunoprecipitated Rac or Ras protein was preloaded with GDP and incubated in [^{32}P]GTP-containing buffer alone or in buffer containing either His-

$TIAM2_S$ or purified SOS. In multiple experiments, His-$TIAM_2$ showed approximately fivefold stimulation of Rac exchange activity at 30 min relative to buffer alone, while SOS showed a modest stimulation (1.6×) at best (Figure 4A). Conversely, when His-$TIAM2_S$ and SOS were incubated with RAS, SOS demonstrated a four- to fivefold stimulation of exchange activity while His-$TIAM2_S$ had little or no effect (Figure 4B).

EXAMPLE 4:

UTILIZING POLYNUCLEOTIDES TO DIAGNOSE CANCER

In a dot blot assay, TIAM2 was hybridized to RNA from both cancerous and normal tissue. The source of cancerous tissue include renal, thyroid, breast, colon, ureter, lung, nose, stomach, esophagus, liver, lymphoma, uterus, bladder, rectum, and brain.

The blots were from BioChain Institute, Inc., San Leandro, California, USA. ExpressHyb™ hybridization buffer (Clontech, Palo Alto, California, USA) was used for the blotting at 68°C with TIAM2 (SEQ ID NO:1) at $1x10^6$ cpm/ml for 2 hours.

As shown in Figure 5, in four of four thyroid samples, SEQ ID NO:1 mRNA levels were higher in the cancer than the normal samples. In two of the four colon samples, SEQ ID NO:1 mRNA levels were higher in the cancer than the normal samples. In one of two ureter samples, the SEQ ID NO:1 (TIAM2) mRNA level were higher in the cancer than the normal sample. In one of four breast samples, the SEQ ID NO:1 mRNA level were higher in the cancer than the normal sample. In one of four renal sample, the SEQ ID NO:1 mRNA level were higher in the cancer than the normal sample. In all other tissue types tested, the SEQ ID NO:1 mRNA levels were the same or higher in the normal samples than the cancer samples.

SEQ ID NO:1

AAGGCCTTTGTTGGGTGCCCGGAACCCCACCAAGCAGCATTCCTCACGCTG
GAGTCCTACCTCATCAAGCCGGTTCAGAGAGCGCTCAGGTACCCGCTGCTG
CTCAAGGAGCTGGTGTCCCTGACGGACCAGGAGAGCGAGGAGCACTACCAC
CTGACGGAAGCACTAAAGGCAATGGAGAAAGTAGCGAGCCACATCAATGA
GATGCAGAAGATCTATGAGGATTATGGGACCGTGTTTGACCAGCTAGTAGC
TGAGCAGAGCGGAACAGAGAAGGAGGTAACAGAACTTTCGATGGGAGAGC
TTCTGATGCACTCTACGGTTTCCTGGTTGAACCCAATGTTGATCCCCGGGG

EXAMPLE 5:

ISOLATION OF SEQ ID NO:6

SEQ ID NO:6 was isolated from a frontal cortex library utilizing a phage vector Stratagene, La Jolla, California, USA). The library was probed with SEQ ID NO:1, which was generated by a random primed label with a final radioactive count of approximately $1x10^6$ cpm/ml. The probe was labeled according to manufacturer's instruction with a RediPrime™ DNA labeling kit (Amersham, Arlington Heights, Illinois, USA).

The phage library was propagated and plated onto twenty plates according to the manufacturer's instructions with a 3.0-5.0 x 10^5 plaques/plate. The plaques were transferred to a nitrocellulose membranes. Each membrane was incubated with the SEQ ID NO:1 probe for 2 hours at 65°C in ExpressHyb™ hybridization solution purchased from Clontech, Palo Alto, California, USA. The filters were washed according to the Clontech instruction. Film was exposed to the membranes to identify putative positive plaques containing the desired TIAM2 polynucleotide.

A second round of plating and hybridization was performed to identify a single positive plaque. The positive plaques from the first round were propagated and

plated onto agar medium according to the instructions provided by Stratagene. The plaques were transferred to filters. These filters were incubated with the SEQ ID NO:1 probe. The probe and hybridization conditions were the same as described above. Positive plaques were identified and propagated.

According to manufacturer's instructions, a BlueScript plasmid was rescued from the phage vector. The EcoRI insert from the plasmid was sequenced. The polynucleotide sequence is shown in SEQ ID NO:6.

The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions which may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

Deposit Information:

The following materials were deposited with the American Type Culture Collection:

Name	Deposit Date	Accession No.
Escherichia coli INVαF' DP 75	25 April 1996	98030

The above materials have been deposited with the American Type Culture Collection, Manassas, Virginia, under the accession numbers indicated. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for purposes of Patent Procedure. The deposits will be maintained for a period of 30 years following issuance of this patent, or for the enforceable life of the patent, whichever is greater. Upon issuance of the patent, the deposits will be available to the public from the ATCC without restriction.

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained within the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by

reference and are controlling in the event of any conflict with the written description of sequences herein. A license may be required to make, use, or sell the deposited materials, and no such license is granted hereby.

CLAIMS

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:11;

(b) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:11;

(c) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:12;

(d) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:12;

(e) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:13;

(f) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:13;

(g) a polynucleotide at least 80 % identical to the polynucleotide of (a), (b), (c), (d), (e) or (f); and

(h) the polynucleotide complement of the polynucleotide of (a), (b), (c), (d), (e), (f), (g).

2. An isolated nucleic acid molecule comprising 10 contiguous nucleotides from the coding region of SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

3. The isolated nucleic acid molecule of claim 2, which comprises 20 contiguous nucleotides from the coding region of SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

4. The isolated nucleic acid molecule of claim 3, which comprises

50 contiguous nucleotides from the coding region of SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

5. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:11;

(b) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:11;

(c) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:12;

(d) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:12;

(e) a polynucleotide encoding amino acids from about 1 to about 626 of SEQ ID NO:13; and

(f) a polynucleotide encoding amino acids from about 2 to about 626 of SEQ ID NO:13.

6. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 1 into a vector in operable linkage to a promoter.

7. A recombinant vector produced by the method of claim 6.

8. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 7 into a host cell.

9. A recombinant host cell produced by the method of claim 8.

10. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 9 under conditions such that said polypeptide is expressed and recovering said polypeptide.

11. An isolated polypeptide comprising amino acids at least 90% identical to amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 626 of SEQ ID NO:11;

(b) amino acids from about 2 to about 626 of SEQ ID NO:11;

(c) amino acids from about 1 to about 626 of SEQ ID NO:12;

(d) amino acids from about 2 to about 626 of SEQ ID NO:12;

(e) amino acids from about 1 to about 626 of SEQ ID NO:13; and

(f) amino acids from about 2 to about 626 of SEQ ID NO:13.

12. An isolated polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 626 of SEQ ID NO:11;

(b) amino acids from about 2 to about 626 of SEQ ID NO:11;

(c) amino acids from about 1 to about 626 of SEQ ID NO:12;

(d) amino acids from about 2 to about 626 of SEQ ID NO:12;

(e) amino acids from about 1 to about 626 of SEQ ID NO:13; and

(f) amino acids from about 2 to about 626 of SEQ ID NO:13.

13. An isolated polypeptide comprising amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 626 of SEQ ID NO:11;

(b) amino acids from about 2 to about 626 of SEQ ID NO:11;

(c) amino acids from about 1 to about 626 of SEQ ID NO:12;

(d) amino acids from about 2 to about 626 of SEQ ID NO:12;

(e) amino acids from about 1 to about 626 of SEQ ID NO:13; and

(f) amino acids from about 2 to about 626 of SEQ ID NO:13.

14. An epitope-bearing portion of the polypeptide of SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

15. The epitope-bearing portion of claim 14, which comprises 10 contiguous amino acids of SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

16. The epitope-bearing portion of claim 15, which comprises 20 contiguous amino acids of SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

17. An isolated antibody that binds specifically to the polypeptide of claim 11.

18. An isolated antibody that binds specifically to a polypeptide of claim 12.

19. An isolated antibody that binds specifically to the polypeptide of claim 13.

20. An antisense vector comprising (i) an antisense polynucleotide that comprises a sequence capable of hybridizing to at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof under stringent conditions wherein said polynucleotide is capable of hybridizing to the mRNA of native human TIAM2; and (ii) and polynucleotide comprising a sequence capable of initiating transcription of said antisense polynucleotide.

21. The antisense vector of claim 20, wherein said sequences to initiate transcription are derived from a retrovirus, an adenovirus, or an adeno-associated virus.

22. The antisense vector of claim 21, further comprising an origin of replication.

23. A method of inhibiting expression of TIAM2, comprising:

(a) providing an antisense polynucleotide comprising a nucleic acid sequence capable of hybridizing to at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof under stringent conditions and is capable of hybridizing to native human TIAM2 mRNA; and

(b) contacting said antisense polynucleotide with said TIAM2.

24. A method for detecting hyperproliferative cells in a sample comprising

(a) providing a probe polynucleotide comprising a sequence capable of hybridizing to at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof under stringent conditions;

(b) contacting the probe with the polynucleotides of the sample cell under conditions permitting formation of polynucleotide hybrids; and

(c) detecting the hybrids.

25. A method to detect TIAM2 polypeptides in a sample comprising:

(a) providing an antibody that specifically binds to a TIAM2 polypeptide, wherein said TIAM2 polypeptide comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence capable of hybridizing to at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof;

(b) contacting said antibody to the sample under conditions permitting the formation of antibody/antigen complexes; and

(c) detecting the complexes.

26. A method of inhibiting the replication of a cell comprising

(a) providing an antibody that specifically binds to a TIAM2 polypeptide, wherein said TIAM2 polypeptide comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence capable of hybridizing to at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 or fragments thereof; and

(b) contacting said antibody to the sample under conditions permitting the formation of antibody/antigen complexes.

Figure 1

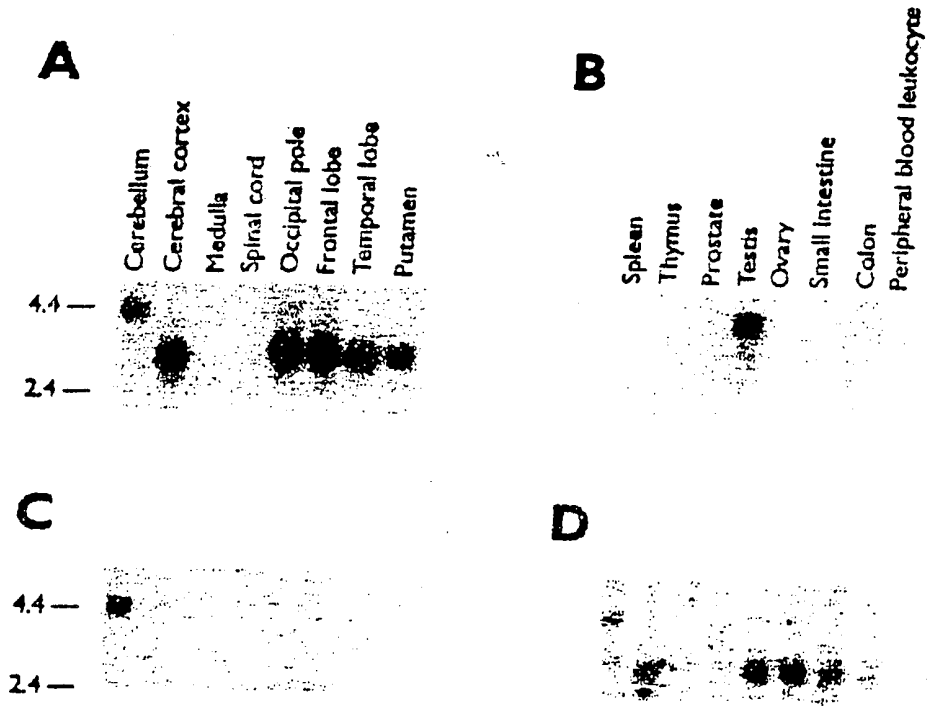

A
Cerebellum
Cerebral cortex
Medulla
Spinal cord
Occipital pole
Frontal lobe
Temporal lobe
Putamen
4.4
2.4
B
Spleen
Thymus
Prostate
Testis
Ovary
Small intestine
Colon
Peripheral blood leukocyte
C
4.4
2.4
D

Figure 2

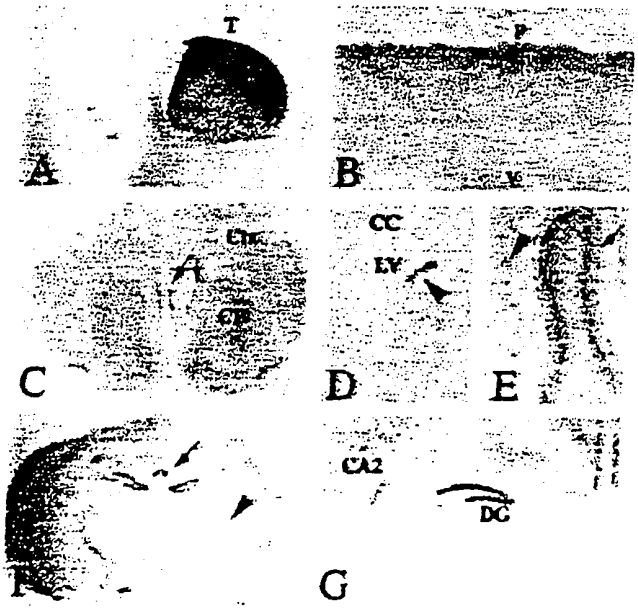


Figure 4

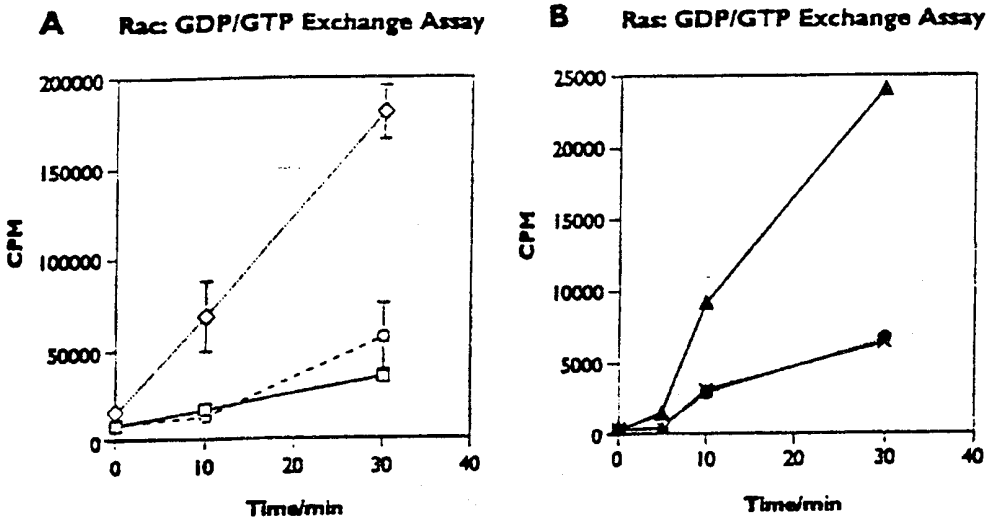
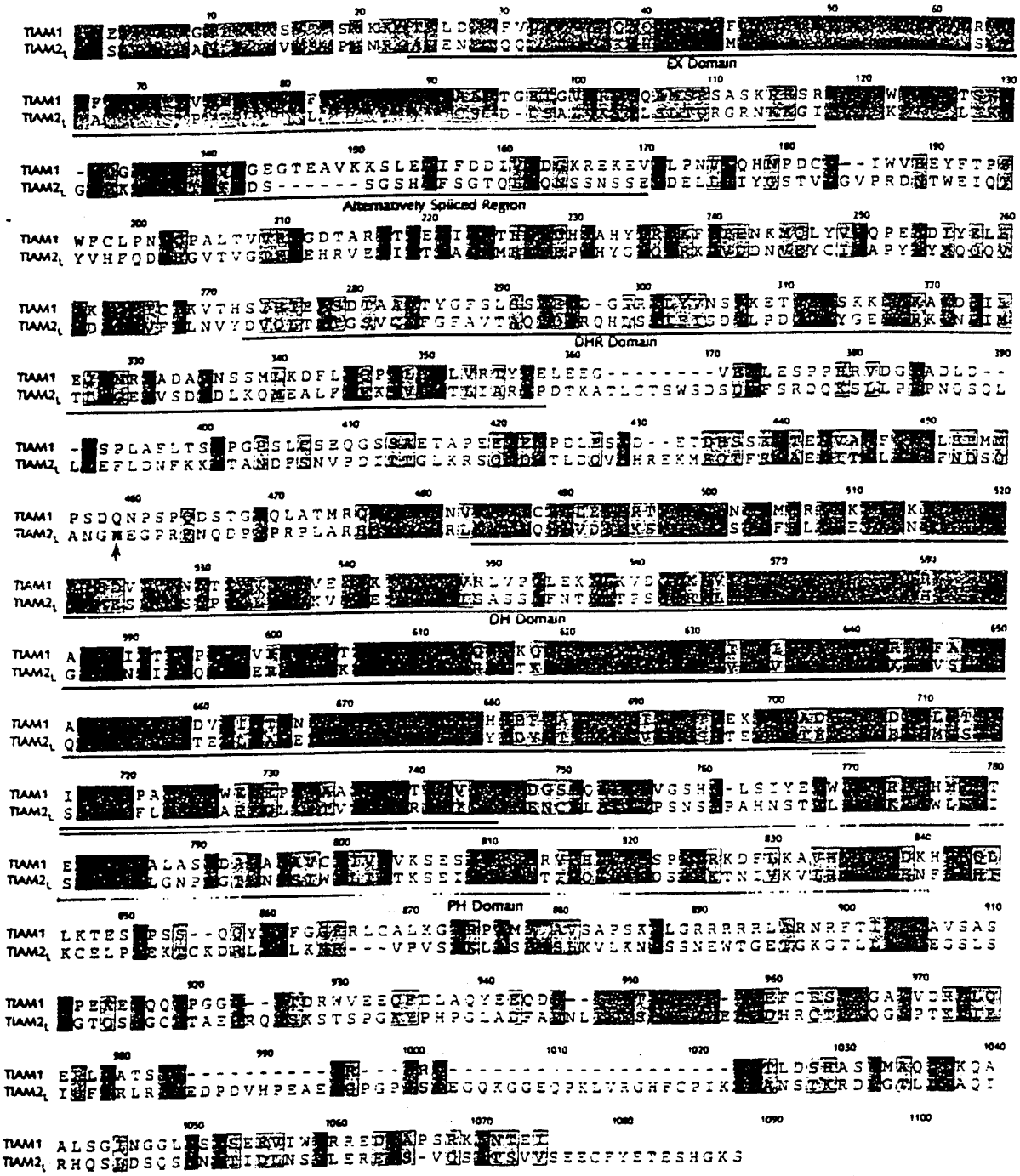


Figure 3


TIAM1
TIAM2L
EX Domain
Alternatively Spliced Region
DHR Domain
DH Domain
PH Domain

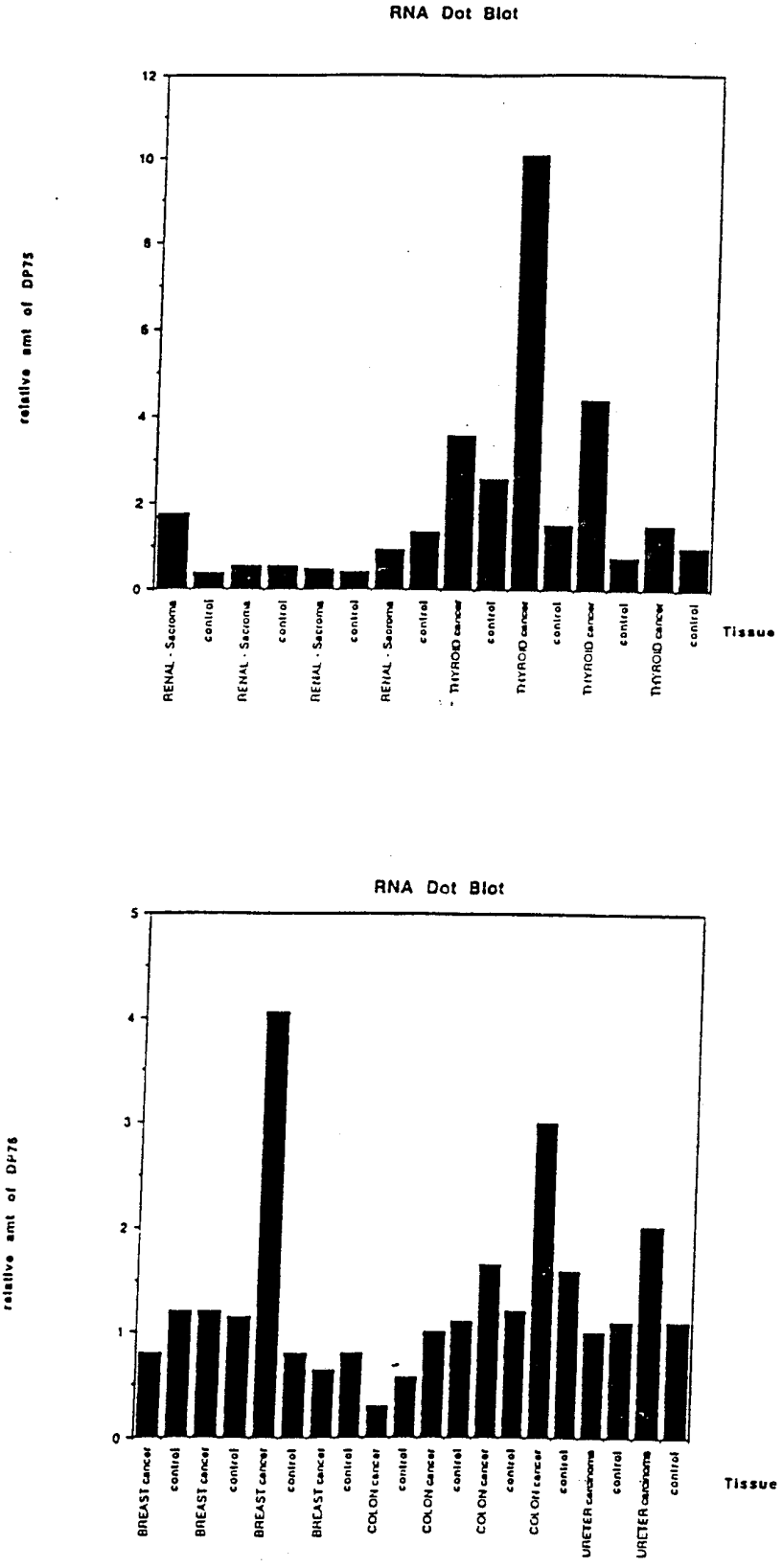

RNA Dot Blot
relative amt of DP75
12
10
8
6
4
2
0
RENAL - Sarcoma
control
RENAL - Sarcoma
control
RENAL - Sarcoma
control
RENAL - Sarcoma
control
THYROID cancer
control
THYROID cancer
control
THYROID cancer
control
THYROID cancer
control
Tissue
RNA Dot Blot
relative amt of DP76
5
4
3
2
1
0
BREAST cancer
control
BREAST cancer
control
BREAST cancer
control
BREAST cancer
control
COLON cancer
control
COLON cancer
control
COLON cancer
control
COLON cancer
control
URETER carcinoma
control
URETER carcinoma
control
Tissue

Figure 5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Duhl, David

(ii) TITLE OF INVENTION: DNA Encoding T-Cell Lymphoma Invasion and Metastasis 2 and a Process for its Use

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Chiron Corporation
(B) STREET: 4560 Horton Street
(C) CITY: Emeryville
(D) STATE: California
(E) COUNTRY: United States
(F) ZIP: 94608

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Potter, Jane E. R.
(B) REGISTRATION NUMBER: 33,332
(C) REFERENCE/DOCKET NUMBER: 1203.200PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-622-4900
(B) TELEFAX: 206-682-6031

(2) INFORMATION For SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 355 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGGCCTTTG TTGGGTGCCC GGAACCCCAC CAAGCAGCAT TCCTCACGCT GGAGTCCTAC
60

CTCATCAAGC CGGTTCAGAG AGCGCTCAGG TACCCGCTGC TGCTCAAGGA GCTGGTGTCC
120

CTGACGGACC AGGAGAGCGA GGAGCACTAC CACCTGACGG AAGCACTAAA GGCAATGGAG
180

AAAGTAGCGA GCCACATCAA TGAGATGCAG AAGATCTATG AGGATTATGG GACCGTGTTT
240

GACCAGCTAG TAGCTGAGCA GAGCGGAACA GAGAAGGAGG TAACAGAACT TTCGATGGGA
300

GAGCTTCTGA TGCACTCTAC GGTTTCCTGG TTGAACCCAA TGTTGATCCC CGGGG
355

(2) INFORMATION For SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGGCCTTTG TTGGGTGCC
19

(2) INFORMATION For SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGGCCTTTG YTGGNYNCC
19

(2) INFORMATION For SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCCGGGGAT CAACATTGGG TT
22

(2) INFORMATION For SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCCGGGGAT VADVADDGGR TT
22

(2) INFORMATION For SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2089 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCCACT TAGATGTGAT CCGTTCTCCC AGAGGGAGCA GGTTTCTTTG AACTTTTCCT
60

TTTTATGTAC AGCATAGTGC TGAGCAGATC ACTGCACTGT GCAGGAGTTT TAACGACAGT
120

CAGGCCAACG GCATGGAAGG ACCGCGGGAG AATCAGGATC CTCCTCCGAG GCCTCTGGCC
180

CGCCACCTGT CTGATGCAGA CCGCCTCCGC AAAGTCATCC AGGAGCTTGT GGACACAGAG
240

AAGTCCTACG TGAAGGATTT GAGCTGCCTC TTTGAATTAT ACTTGGAGCC ACTTCAGAAT
300

GAGACCTTTC TTACCCAAGA TGAGATGGAG TCACTTTTTG GAAGTTTGCC AGAGATGCTT
360

GAGTTTCAGA AGGTGTTTCT GGAGACCCTG GAGGATGGGA TTTCAGCATC ATCTGACTTT
420

AACACCCTAG AAACCCCCTC ACAGTTTAGA AAATTACTGT TTTCCCTTGG AGGCTCTTTC
480

CTTTATTACG CGGACCACTT TAAACTGTAC AGTGGATTCT GTGCTAACCA TATCAAAGTA
540

CAGAAGGTTC TGGAGCGAGC TAAAACTGAC AAAGCCTTCA AGGCTTTTCT GGACGCCCGG
600

AACCCCACCA AGCAGCATTC CTCCACGCTG GAGTCCTACC TCATCAAGCC GGTTCAGAGA
660

GTGCTCAAGT ACCCGCTGCT GCTCAAGGAG CTGGTGTCCC TGACGGACCA GGAGAGCGAG
720

GAGCACTACC ACCTGACGGA AGCACTAAAG GCAATGGAGA AAGTAGCGAG CCACATCAAT
780

GAGATGCAGA AGATCTATGA GGATTATGGG ACCGTGTTTG ACCAGCTAGT AGCTGAGCAG
840

AGCGGAACAG AGAAGGAGGT AACAGAACTT TCGATGGGAG AGCTTCTGAT GCACTCTACG
900

GTTTCCTGGT TGAATCCATT TCTGTCTCTA GGAAAAGCTA GAAAGGACCT TGAGCTCACA
960

GTATTTGTTT TTAAGAGAGC CGTCATACTG GTTTATAAAG AAAACTGCAA ACTGAAAAAG
1020

AAATTGCCCT CGAATTCCCG GCCTGCACAC AACTCTACTG ACTTGGACCC ATTTAAATTC
1080

CGCTGGTTGA TCCCCATCTC CGCGCTTCAA GTCAGACTGG GGAATCCAGC AGGGACAGAA
1140

AATAATTCCA TATGGGAACT GATCCATACG AAGTCAGAAA TAGAAGGACG GCCAGAAACC
1200

ATCTTTCAGT TGTGTTGCAG TGACAGTGAA AGCAAAACCA ACATTGTTAA GGTGATTCGT
1260

TCTATTCTGA GGGAGAACTT CAGGCGTCAC ATAAAGTGTG AATTACCACT GGAGAAAACG
1320

TGTAAGGATC GCCTGGTACC TCTTAAGAAC CGAGTTCCTG TTTCGGCCAA ATTAGCTTCA
1380

TCCAGGTCTT TAAAAGTCCT GAAGAATTCC TCCAGCAACG AGTGGACCGG TGAGACTGGC
1440

AAGGGAACCT TGCTGGACTC TGACGAGGGC AGCTTGAGCA GCGGCACCCA GAGCAGCGGC
1500

TGCCCCACGG CTGAGGGCAG GCAGGACTCC AAGAGCACTT CTCCCGGGAA ATACCCACAC
1560

CCCGGCTTGG CAGATTTTGC TGACAATCTC ATCAAAGAGA GTGACATCCT GAGCGATGAA
1620

GATGATGACC ACCGTCAGAC TGTGAAGCAG GGCAGCCCTA CTAAAGACAT CGAAATTCAG
1680

TTCCAGAGAC TGAGGATTTC CGAGGACCCA GACGTTCACC CCGAGGCTGA GCAGCAGCCT
1740

GGCCCGGAGT CGGGTGAGGG TCAGAAAGGA GGAGAGCAGC CCAAACTGGT CCGGGGGCAC
1800

TTCTGCCCCA TTAAACGAAA AGCCAACAGC ACCAAGAGGG ACAGAGGAAC TTTGCTCAAG
1860

GCGCAGATCC GTCACCAGTC CCTTGACAGT CAGTCTGAAA ATGCCACCAT CGACCTAAAT
1920

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2089

(2) INFORMATION For SEQ ID NO:7, 11, 12, or 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 626 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7, 11, 12, or 13:

Met Glu Gly Pro Arg Glu Asn Gln Asp Pro Pro Pro Arg Pro Leu Ala
1 5 10 15

Arg His Leu Ser Asp Ala Asp Arg Leu Arg Lys Val Ile Gln Glu Leu
20 25 30

Val Asp Thr Glu Lys Ser Tyr Val Lys Asp Leu Ser Cys Leu Phe Glu
35 40 45

Leu Tyr Leu Glu Pro Leu Gln Asn Glu Thr Phe Leu Thr Gln Asp Glu
50 55 60

Met Glu Ser Leu Phe Gly Ser Leu Pro Glu Met Leu Glu Phe Gln Lys
65 70 75 80

Val Phe Leu Glu Thr Leu Glu Asp Gly Ile Ser Ala Ser Ser Asp Phe
85 90 95

Asn Thr Leu Glu Thr Pro Ser Gln Phe Arg Lys Leu Leu Phe Ser Leu
100 105 110

Gly Gly Ser Phe Leu Tyr Tyr Ala Asp His Phe Lys Leu Tyr Ser Gly
115 120 125

Phe Cys Ala Asn His Ile Lys Val Gln Lys Val Leu Glu Arg Ala Lys
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Thr Asp Lys Ala Phe Lys Ala Phe Leu Asp Ala Arg Asn Pro Thr Lys
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Gln His Ser Ser Thr Leu Glu Ser Tyr Leu Ile Lys Pro Val Gln Arg
165 170 175

Val Leu Lys Tyr Pro Leu Leu Leu Lys Glu Leu Val Ser Leu Thr Asp
180 185 190

Gln Glu Ser Glu Glu His Tyr His Leu Thr Glu Ala Leu Lys Ala Met
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Glu Lys Val Ala Ser His Ile Asn Glu Met Gln Lys Ile Tyr Glu Asp
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Tyr Gly Thr Val Phe Asp Gln Leu Val Ala Glu Gln Ser Gly Thr Glu
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Leu Glu Leu Thr Val Phe Val Phe Lys Arg Ala Val Ile Leu Val Tyr
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Ile Glu Ile Gln Phe Gln Arg Leu Arg Ile Ser Glu Asp Pro Asp Val
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His Pro Glu Ala Glu Gln Gln Pro Gly Pro Glu Ser Gly Glu Gly Gln
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Lys Gly Gly Glu Gln Pro Lys Leu Val Arg Gly His Phe Cys Pro Ile
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Lys Arg Lys Ala Asn Ser Thr Lys Arg Asp Arg Gly Thr Leu Leu Lys
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565 570 575

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Lys Ser
625

SEQ ID NO:8
3.3 kb message

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SEQ ID NO:10

4.4 with out mini exon

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3.3 amino acid sequence = SEQ ID NO:11

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4.4 without exon amino acid sequence = SEQ ID NO:12

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ILSVSSFHALVCSRDDSALRKRTLSLTQRGRNKKGIFSSLKGLDTLARKGKEKRPSITQVDELLHIYGSTVDGVPRDN
TW
EIQTYVHFQDNHGVTVGIKPEHRVEDILTLACKMRQLEPSHYGLQLRKLVDDNVEYCIPAPYEYMQQQVYDEIEVFPL
NV
YDVQLTKTGSVCDFGFAVTAQVDERQHLSRIFISDVLPDGLAYGEGLRKGNEIMTLNGEAVSDLDLKQMEALFSEKSV
GL
TLIARPPDTKATLCTSWSDSDLFSRDQKSLLPPPNQSQLLEEFLDNFKKNTANDFSNVPDITTGLKRSQTDGTLDQVS

HR
EKMEQTFRSAEQITALCRSFNDSQANGMEGPRENQDPPPRPLARHLSDADRLRKVIQELVDTEKSYVKDLSCLFELYL
EP
LQNETFLTQDEMESLFGSLPEMLEFQKVFLETLEDGISASSDFNTLETPSQFRKLLFSLGGSFLYYADHFKLYSGFCA
NH
IKVQKVLERAKTDKAFKAFLDARNPTKQHSSTLESYLIKPVQRVLKYPLLLKELVSLTDQESEEHYHLTEALKAMEKV
AS
HINEMQKIYEDYGTVFDQLVAEQSGTEKEVTELSMGELLMHSTVSWLNPFLSLGKARKDLELTVFVFKRAVILVYKEN
CK
LKKKLPSNSRPAHNSTDLDPFKFRWLIPISALQVRLGNPAGTENNSIWELIHTKSEIEGRPETIFQLCCSDSESKTNI
VK
VIRSILRENFRRHIKCELPLEKTCKDRLVPLKNRVPVSAKLASSRSLKVLKNSSSNEWTGETGKGTLLDSDEGSLSSG
TQ
SSGCPTAEGRQDSKSTSPGKYPHPGLADFADNLIKESDILSDEDDDHRQTVKQGSPTKDIEIQFQRLRISEDPDVHPE
AE
QQPGPESGEGQKGGEQPKLVRGHFCPIKRKANSTKRDRGTLLKAQIRHQSLDSQSENATIDLNSVLEREFSVQSLTSV
VS EECFYETESHGKS

4.4 with exon amino acid sequence = SEQ ID NO:13

MDSKMKKMAELQLSVVSDPKNRKAIENQIQQWEQNLEKFHMDLFRMRCYLASLQGGELPNPKSLLAAASRPSKPALGR
LG
ILSVSSFHALVCSRDDSALRKRTLSLTQRGRNKKGIFSSLKGLDTLARKGKEKRPSITQIFDSSGSHGFSGTQLPQNS
SN
SSEVDELLHIYGSTVDGVPRDNTWEIQTYVHFQDNHGVTVGIKPEHRVEDILTLACKMRQLEPSHYGLQLRKLVDDNV
EY
CIPAPYEYMQQQVYDEIEVFPLNVYDVQLTKTGSVCDFGFAVTAQVDERQHLSRIFISDVLPDGLAYGEGLRKGNEIM
TL
NGEAVSDLDLKQMEALFSEKSVGLTLIARPPDTKATLCTSWSDSDLFSRDQKSLLPPPNQSQLLEEFLDNFKKNTAND
FS
NVPDITTGLKRSQTDGTLDQVSHREKMEQTFRSAEQITALCRSFNDSQANGMEGPRENQDPPPRPLARHLSDADRLRK
VI
QELVDTEKSYVKDLSCLFELYLEPLQNETFLTQDEMESLFGSLPEMLEFQKVFLETLEDGISASSDFNTLETPSQFRK
LL
FSLGGSFLYYADHFKLYSGFCANHIKVQKVLERAKTDKAFKAFLDARNPTKQHSSTLESYLIKPVQRVLKYPLLLKEL
VS
LTDQESEEHYHLTEALKAMEKVASHINEMQKIYEDYGTVFDQLVAEQSGTEKEVTELSMGELLMHSTVSWLNPFLSLG
KA
RKDLELTVFVFKRAVILVYKENCKLKKKLPSNSRPAHNSTDLDPFKFRWLIPISALQVRLGNPAGTENNSIWELIHTK
SE

IEGRPETIFQLCCSDSESKTNIVKVIRSILRENFRRHIKCELPLEKTCKDRLVPLKNRVPVSAKLASSRSLKVLKNSS
SN
EWTGETGKGTLLDSDEGSLSSGTQSSGCPTAEGRQDSKSTSPGKYPHPGLADFADNLIKESDILSDEDDDHRQTVKQG
SP
TKDIEIQFQRLRISEDPDVHPEAEQQPGPESGEGQKGGEQPKLVRGHFCPIKRKANSTKRDRGTLLKAQIRHQSLDSQ
SE NATIDLNSVLEREFSVQSLTSVVSEECFYETESHGKS