(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2006 (30.03.2006)

PCT

(10) International Publication Number
WO 2006/034191 A2

(51) International Patent Classification:
C07K 14/82 (2006.01)

(21) International Application Number:
PCT/US2005/033479

(22) International Filing Date:
20 September 2005 (20.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/610,954 20 September 2004 (20.09.2004) US

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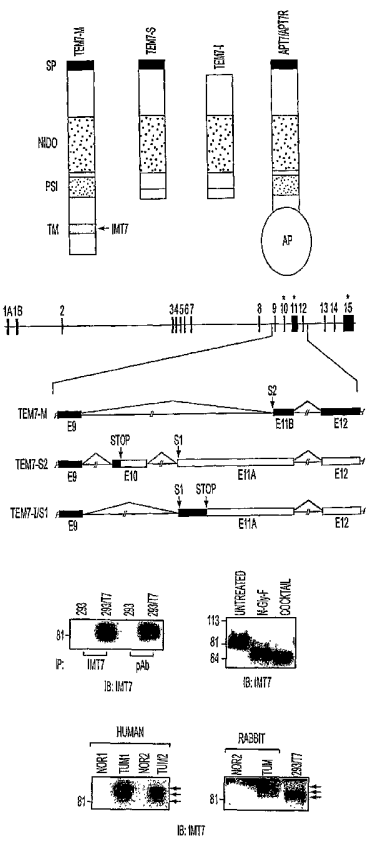
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,

[Continued on next page]

(54) Title: TEM17 BINDS TO CORTACTIN



(57) Abstract: Tumor Endothelial Marker 17 (TEM17) was recently identified as an mRNA transcript overexpressed in the endothelium of vessels in human solid tumors. We have identified several new variants of TEMI7, derived by alternative splicing, that are intracellular (TEM17-I), secreted (TEM17-S), and on the cell surface membrane (TEM17-M) of tumor endothelium. We identified cortactin as a protein capable of binding to the extracellular region of both TEM17 and its closest homologue, TEM3, which is also expressed in tumor endothelium. The binding domain of cortactin is a 9-amino acid residue region in its plexin-like domain. These provide targets for the delivery of therapeutic and imaging agents to the vessels of solid tumors.

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ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

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

TEM17 BINDS TO CORTACTIN

This application claims the benefit of provisional application 60/610,954 filed September 20, 2004, the disclosure of which is incorporated herein.

FIELD OF THE INVENTION

The invention relates to tumor endothelium and pathogenic angiogenesis. In particular it relates to diagnostic, imaging, and therapeutic targets for management and treatment of tumors.

BACKGROUND OF THE INVENTION

Targeting the endothelial cells that line tumor blood vessels is a promising new strategy for the treatment of cancer (1). However, realization of the full potential of a vascular directed approach will require the exploitation of new targets that are expressed predominantly on tumor endothelium (2). In a systematic attempt to uncover such targets, we recently employed Serial Analysis of Gene Expression (SAGE) on endothelial cells isolated from human normal or malignant colorectal tissues. These studies led to the identification of several novel Tumor Endothelial Markers (TEMs), the most abundant of which was called TEM17 (U.S. Serial No. 09/918,715; also referred to as TEM7 in ref. 3). In situ hybridization studies validated the expression of TEM17 in the endothelium of colorectal cancer and demonstrated that TEM17 mRNA was also abundantly expressed in the endothelium of a variety of other human cancer types including, breast, lung, and brain tumors. Based on the full length nucleotide sequence, TEM17 appears to be a typical type I transmembrane protein containing a signal peptide followed by a Nidogen-like domain and a single

hydrophobic domain (Figure1A). The only other protein that appears to share significant homology to TEM17 is TEM17-Related (referred to as TEM3 in USSN 09/918,715), another putative cell surface protein that also contains a nidogen-like domain. In situ hybridization revealed that TEM3, like TEM17, was abundantly expressed in the endothelium of malignant colorectal cancer but was absent or rare in normal colonic mucosa (4). Surprisingly, the mouse counterpart to human TEM17 could not be found in mouse tumor endothelium, suggesting that this protein may have evolved a different function during the course of evolution.

There is a continuing need in the art for tools and reagents for diagnosing and treating tumors and other pathogenic angiogenesis.

BRIEF SUMMARY OF THE INVENTION

In a first embodiment of the invention an isolated and purified TEM17I protein is provided. The protein comprises a nidogen G1-like domain of TEM17 protein having an amino acid sequence as shown in SEQ ID NO: 1 or variants thereof having at least 95 % identity with said sequence. TEM17I protein lacks a signal peptide, a transmembrane domain, and a plexin-like domain.

In a second embodiment of the invention an isolated and purified TEM17S protein is provided. The protein comprises a nidogen G1-like domain and a signal peptide of TEM17 protein having an amino acid sequence as shown in SEQ ID NO: 3 or SEQ ID NO: 15 or variants of either having at least 95 % identity with said sequence. TEM17S protein lacks a transmembrane domain and a plexin-like domain.

In a third embodiment of the invention a polypeptide of less than 100 amino acids is provided. The polypeptide comprises an amino acid sequence selected from the group consisting of: Thr Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 5); Xaa Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 6); Thr Xaa Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 7); Thr Ala Xaa Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 8); Thr Ala Val Xaa Leu Tyr Asp Tyr Gln (SEQ ID NO: 9); Thr Ala Val Ala Xaa Tyr Asp Tyr Gln (SEQ ID NO: 10); Thr Ala Val Ala Leu Xaa Asp Tyr Gln (SEQ ID NO: 11); Thr Ala Val Ala Leu Tyr Xaa Tyr Gln (SEQ ID NO: 12); Thr Ala Val Ala Leu

Tyr Asp Xaa Gln (SEQ ID NO: 13); and Thr Ala Val Ala Leu Tyr Asp Tyr Xaa (SEQ ID NO: 14).

In a fourth embodiment of the invention a method of delivering a therapeutic agent to tumor endothelium is provided. A therapeutic agent is administered to a patient harboring a tumor. The therapeutic agent is linked to a polypeptide which comprises an amino acid sequence selected from the group consisting of: Thr Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 5); Xaa Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 6); Thr Xaa Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 7); Thr Ala Xaa Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 8); Thr Ala Val Xaa Leu Tyr Asp Tyr Gln (SEQ ID NO: 9); Thr Ala Val Ala Xaa Tyr Asp Tyr Gln (SEQ ID NO: 10); Thr Ala Val Ala Leu Xaa Asp Tyr Gln (SEQ ID NO: 11); Thr Ala Val Ala Leu Tyr Xaa Tyr Gln (SEQ ID NO: 12); Thr Ala Val Ala Leu Tyr Asp Xaa Gln (SEQ ID NO: 13); and Thr Ala Val Ala Leu Tyr Asp Tyr Xaa (SEQ ID NO: 14).

In a fifth embodiment of the invention a method of delivering a diagnostic agent to tumor endothelium is provided. A diagnostic agent is administered to a patient harboring a tumor. The diagnostic agent is linked to a polypeptide which comprises an amino acid sequence selected from the group consisting of: Thr Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 5); Xaa Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 6); Thr Xaa Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 7); Thr Ala Xaa Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 8); Thr Ala Val Xaa Leu Tyr Asp Tyr Gln (SEQ ID NO: 9); Thr Ala Val Ala Xaa Tyr Asp Tyr Gln (SEQ ID NO: 10); Thr Ala Val Ala Leu Xaa Asp Tyr Gln (SEQ ID NO: 11); Thr Ala Val Ala Leu Tyr Xaa Tyr Gln (SEQ ID NO: 12); Thr Ala Val Ala Leu Tyr Asp Xaa Gln (SEQ ID NO: 13); and Thr Ala Val Ala Leu Tyr Asp Tyr Xaa (SEQ ID NO: 14).

In a sixth embodiment of the invention a method is provided for screening for potential therapeutic agents which inhibit binding of cortactin to TEM17. Binding of TEM17 to cortactin is compared in the presence and in the absence of a test agent. A test agent which diminishes amount of binding of TEM17 to cortactin is identified as a potential therapeutic agent for treating pathological vascularization.

In a seventh embodiment of the invention a method of screening for potential therapeutic agents which inhibit binding of cortactin to TEM3 is provided. Binding of TEM3 to cortactin is compared in the presence and in the absence of a test agent. A test agent which diminishes amount of binding of TEM3 to cortactin is identified as a potential therapeutic agent for treating pathological vascularization.

In an eighth embodiment of the invention a method is provided of screening for potential therapeutic agents which inhibit the binding of cortactin to TEM17. Binding of TEM17 to a polypeptide is compared in the presence and in the absence of the test agent. The polypeptide is less than 100 amino acid residues in length and comprises the amino acid sequence TAVALYDYQ (SEQ ID NO: 5). A test agent which diminishes the amount of binding of TEM17 to the polypeptide is identified as a potential therapeutic agent for treating pathological vascularization.

In a ninth embodiment of the invention a method is provided of screening for potential therapeutic agents which inhibit the binding of cortactin to TEM3. Binding of TEM3 to a polypeptide is compared in the presence and in the absence of the test agent. The polypeptide is less than 100 amino acid residues in length and comprises the amino acid sequence TAVALYDYQ (SEQ ID NO: 5). A test agent which diminishes amount of binding of TEM3 to the polypeptide is identified as a potential therapeutic agent for treating pathological vascularization.

In a tenth embodiment of the invention an isolated antibody is provided which binds to a TEM17I protein. The protein comprises a nidogen G1-like domain of TEM17M protein but lacks a signal peptide, a transmembrane domain, and a plexin-like domain. The antibody does not bind to TEM17M.

In an eleventh embodiment of the invention an isolated antibody is provided which binds to a TEM17S1 or TEM17S2 protein. The protein comprises a nidogen G1-like domain and a signal peptide of TEM17M protein, but lacks a transmembrane domain and a plexin-like domain. The antibody does not bind to TEM17M.

In a twelfth embodiment of the invention a method of detecting a serum protein indicative of pathological angiogenesis is provided. Binding of a serum

protein to an antibody which binds to a secreted or intracellular form of TEM17 is determined. Detection of the serum protein is indicative of pathological angiogenesis.

In another embodiment of the invention a method of detecting expression of TEM17I, TEM17S1, or TEM17S2 is provided. A body sample is contacted with an antibody which specifically binds to TEM17I, TEM17S1, or TEM17S2, but not to TEM17M. Antibody bound to the body sample is detected. The presence of bound antibody indicates pathological angiogenesis.

According to another aspect of the invention another method is provided for detecting expression of TEM17I, TEM17S1, or TEM17S2. A body sample is contacted with a nucleic acid primer or probe which hybridizes to a polynucleotide according to SEQ ID NO: 2, 4, or 16, but not with a polynucleotide according to SEQ ID NO: 20. Hybridized primer or probe is detected. The hybridized primer or probe indicates pathological angiogenesis.

Still another aspect of the invention is an isolated polynucleotide probe or primer which is at least 95% identical to a sequence selected from the group consisting of: nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and complements thereof.

These and other embodiments of the invention provide the art with diagnostic, drug discovery, and therapeutic tools for cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1D. Analysis of TEM17 Structure and Expression. Fig. 1A, Schematic of TEM17 variants. SP; Signal Peptide, NIDO; region sharing similarity to Nidogen G1 domain, PSI; Plexin-like domain found in Plexins, Semaphorins and Integrins, TM; Transmembrane domain. The region where IMT7 monoclonal antibody binds TEM17-M is indicated. To search for TEM binding proteins, alkaline phosphatase (AP) was fused to the extracellular domain of TEM17 (APT7) or TEM3

(APT7R). Fig. 1B, Intron/exon structure of the TEM17 gene. Exon 11 has two alternative splice acceptor sites; splice acceptor 1 (S1) gives rise to E11A and splice acceptor 2 (S2) gives rise to E11B. * Exon 10, exon 11A and exon 15 all have stop codons which disrupt the coding sequence (black in inset) of the different TEM17 variants. The secreted form of TEM17 (TEM17-S) uses the stop codon of either exon 10 or 11A, whereas the intracellular form (TEM17-I) uses exon 11A. The previously described membrane bound form (TEM17-M) uses E11B to initiate transcription, exon 14 to produce a transmembrane domain, and uses a stop codon in exon 15. Fig. 1C, Immunoprecipitation of TEM17. Left panel; An 85 kD protein was immunoprecipitated (IP) from TEM17-M transfected 293 cells (293/T7) but not control 293 cells using either a monoclonal antibody (IMT7) or a polyclonal antibody (pAb) against TEM17. IMT7 antibody was used for Immunoblotting (IB). Right panel; Proteins immunoprecipitated with IMT7 were either untreated, or treated with N-Glycosidase-F or else a cocktail of glycosidases and then immunoblotted with IMT7 monoclonal antibody. Fig. 1D, TEM17-M protein is elevated in tumor tissues. Left panel; Immunoblotting with IMT7 antibody revealed a triplet pattern that was elevated in tumors compared to matched normal tissues from five colorectal cancer patients (two representative cases are shown). A triplet was also elevated in VX2 liver tumors of rabbits compared to normal liver tissue. Note that the bottom band of the triplet (~85 kD) migrates at a position similar to that observed in 293 cells transfected with TEM17-M.

Figs. 2A-2C. TEM17 expression in tumor vessels. Fig. 2A, Immunohistochemical staining of TEM17-M in colon, lung and esophageal tumor tissues. IMT7 antibody was used to detect TEM17-M and an anti-von Willebrand factor (vWF) antibody was used as a control for vessel staining. Following the addition HRP-labeled secondary antibody, sections were stained with DAB (brown). Bar=50μm. Fig. 2B, Immunofluorescence staining of TEM17 in colon cancer. Co-localization of TEM17-M staining with the pan-endothelial marker vWF demonstrates that the endothelial cells are the predominant cell type responsible for the vessel staining observed by IH. Bar=50μm. Fig. 2C, TEM17 staining by transmission Electron microscopy. Using IMT7 antibody to detect TEM17-M, staining was

observed predominantly in the tight junctions between endothelial cells (EC), but a lower level of expression on the luminal surface of tumor endothelium was also observed (arrowheads). Bar=200nm.

Figs. 3A-3F. TEM17 and TEM3 interact with cortactin. Fig. 3A, The T7R-AP binding assay (BA) used to identify TEM17/7R interacting proteins. Note that three bands of approximately 75, 80 and 85 kD were observed in crude extracts prepared from mouse brain tissue. Fig. 3B, TEM3 binds cortactin. Extracts prepared from mouse brain were immunoprecipitated with either non-specific IgG control antibody (IP-IgG) or an anti-cortactin monoclonal antibody (IP-Cor). Immunoprecipitated proteins the expected size of cortactin were readily detected when probed with AP-TEM3 fusion protein. Fig. 3C, TEM17 binds cortactin. Extracts of 293 cells transfected with TEM17-M were immunoprecipitated with either non-specific IgG antibody (IP-IgG) or anti-TEM17 monoclonal antibody (IP-T7). Precipitated proteins were immunoblotted with either anti-TEM17 (α-T7) or anti-cortactin (α-Cor) antibodies. Note that endogenous cortactin in 293 cells migrates as a doublet of approximately 80 and 85 kD. Fig. 3D, Endogenous TEM17 and cortactin interact in tumor extracts. TEM17 was observed in cortactin (IP-Cor), but not control (IP-IgG) immunoprecipitates of extracts derived from rabbit tumor tissue (Tumor lysates). Note that only the 85 kD product appeared to co-precipitate with cortactin. Fig. 3E, Cortactin deletion constructs used to map the TEM17/7R binding domain. The cortactin repeat region is shown in grey and the SH3 domain in black. Expression of each deletion construct was verified by Immunoblotting with anti-myc antibodies. Binding of TEM3 to each of the cortactin deletions was measured using AP-T7R as a probe in the AP binding assay. Fig. 3F, 13-amino acid residue peptides spanning the region of cortactin responsible for binding TEM17/7R were spotted onto a membrane and probed with AP-TEM3. The three peptides that gave the strongest signal are shown along with their minimal consensus (red).

DETAILED DESCRIPTION OF THE INVENTION

We have identified secreted and intracellular forms of TEM17, a tumor endothelium marker which is abundantly expressed in the tumor endothelium

transcriptome. The existence of non-membrane bound forms of TEM17 permits serum assays of the marker to identify, monitor, and predict tumor growth. We have also identified a binding partner of TEM17, cortactin. Cortactin binds to TEM17 via a particular 9 amino acid region. This region can be used to target therapeutic and diagnostic agents to tumor endothelium. Moreover, the binding interaction between TEM17 and cortactin can be used in drug discovery assays. Drugs which inhibit the binding interaction may inhibit the function of one or both of TEM17 and cortactin *in vivo*. Cortactin also binds to TEM3 protein, which is homologous to TEM17, and can be used similarly in drug discovery assays.

Polypeptides according to the present invention can be full length or portions of native proteins. Portions comprise anything less than full length proteins, including proteins that have one amino acid residue less than the full length protein. Polypeptides may comprise less than 250 amino acid residues, less than 200, less than 150, less than 100, less than 75, less than 50, less than 25, less than 20, or less than 15 amino acid residues.

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson & Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant. The FASTA algorithm is described by Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO: 1) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence the ktup value), then the trimmed initial regions are examined

to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman & Wunsch, *J. Mol. Biol.48*:444 (1970); Sellers, *SIAM J. Appl. Math.26*:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default. Since the DNA sequences shown in the sequence listing represent double stranded sequences, sequences which hybridize to or are complementary to or are identical with any of the sequences shown can have the polarity and sequence of either strand of the double strand.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine. The cortactin binding region may contain one amino acid substitution, so long as it still retains binding activity.

Fusion proteins can be made using recombinant DNA techniques. One of the fusion partners can be a polypeptide comprising a cortactin binding domain. The other fusion partner can be a diagnostic or therapeutic agent. The diagnostic agent can be, for example enzyme, such as alkaline phosphatase, or a fluorescent or bioluminescent protein. Any diagnostic agent can be used which is detectable in the intended assay. Therapeutic agents can be any that the artisan wants delivered to

pathologic endothelium. These agents may be cytotoxic agents, such as biological or chemical toxins. Antibodies or parts of antibodies can be used a therapeutic agents. Diagnostic or therapeutic moieties can be attached to a polypeptide of the present invention synthetically, without recourse to recombinant DNA technology. Suitable chemical linkages will depend on the identity of the moieties to be attached. However, standard chemical linkages can be used for this purpose. The diagnostic or therapeutic moieties which are attached synthetically need not be proteins.

Diagnostic and therapeutic agents according to the invention can be delivered by any means known in the art. The delivery means will depend on the location of the site of pathological angiogenesis. For example, a skin lesion can be treated topically, while an internal tumor may be treated systemically. Suitable means of administration include without limitation: oral, vaginal, rectal, subcutaneous, intramuscular, intravenous, intranasal, intrabronchial, intraarterial, intratumoral, and transdermal. Diagnostic agents are typically chemical moieties which can be detected from outside the body without invasive procedures. Thus such moieties may be radioactive moieties which can be detected by appropriate imaging techniques. Similarly contrast dyes can be used to increase detectability using imaging techniques.

Binding assays of cortactin or cortactin polypeptides or fusion proteins to TEM17 or TEM3 or their related polypeptides or fusion proteins can be performed *in vitro* or *in vivo*. Full length proteins can be used or portions which specifically bind to the opposite binding partner. Fusion proteins or conjugates can be used in such assays. Any format for determining binding of two proteins can be used. Numerous binding assays are known in the art. The skilled artisan is able to select the most convenient assay format for his or her situation. Examples of assays include filter binding assays, pseudo-western blotting assays, co-immunoprecipitation assays, two-hybrid assays, fluorescence resonance energy transfer assays, and bioluminescence resonance energy transfer assays. Any form of TEM17 (*e.g.*, M, S1, S2, I) or TEM3 can be used. Such forms include variants having at least 95 % identity with the particular sequences disclosed herein.

Antibodies according to the invention are able to bind to unique portions of TEM17 -S1, -S2, or –I, relative to TEM17M. Using the information provided in the present invention regarding structure of these forms of the protein, one can use as an immunogen, a region of a TEM17 protein which is not contained within TEM17M. Such regions may be selected from the encoded sequences of: nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, and nucleotides 1860-1884 of SEQ ID NO: 16. Alternatively, or in conjunction, one can use such a region of a TEM17 protein to screen and/or select antibodies. Those of skill in the art will know numerous antibody binding assays which can be used preparatively or analytically to obtain antibodies which bind to one protein but not to a second protein.

Antibody-antigen binding assays can also be readily selected by the skilled artisan to detect secreted or intracellular forms of TEM17 (-S1, -S2, or -I, for example) in the blood, plasma, or serum of a patient. Antigen-antibody binding can also be detected in tissue samples (*e.g.,* immunohistochemically), stool, urine, saliva, sputum, or other convenient body sample. Similarly, nucleic acid binding or amplification reactions can be used to determine whether any of the secreted or intracellular forms of TEM17 are expressed. Such techniques include RT-PCR, *in situ* hybridization, or other hybridization or amplification techniques. Suitable probes and primers which can be employed typically hybridize to, are at least 95 % identical to, or are complementary to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and their complements.

Selective delivery of agents to tumor endothelium is a major goal of current anti-angiogenic approaches to cancer therapy. An ideal marker for such selective targeting would be highly expressed in tumor endothelium but absent or exceedingly rare in all non-tumor endothelium of adults. To date few, if any, markers have been identified that meet such strict criteria. TEM17 is of particular interest in this regard

because it was the most abundant novel transcript identified in an unbiased screen for transcripts differentially expressed in tumor endothelial cells and it was predicted to reside at the cell surface. Using antibodies we demonstrate that the TEM17 protein, like its mRNA, is overexpressed in human tumor endothelium. We also show that TEM17-M is glycosylated and present at the cell surface, though most of it appears to lie in the adhesive junctions between endothelial cells.

In an attempt to identify binding partners for TEM17, we adopted an approach that involved probing tissue lysates with AP-TEM17 or AP-TEM3 fusion proteins. Using the AP-TEM17/3 binding assay to guide biochemical purification, we identified cortactin as a protein capable of binding both TEM17 and TEM3. The minimal region of cortactin required for TEM17 binding was a 9 amino acid residue sequence located immediately adjacent to the SH3 domain. This 9 amino acid residue sequence allows construction of small molecular weight compounds (peptides or analogs) that can target tumor endothelium for diagnostic or therapeutic purposes.

The identification of a putative intracellular TEM17 variant suggests that a physiological interaction between TEM17 and cortactin could occur intracellularly. In support of this view, the cytoplasmic variant of TEM17 contains a Nidogen-like domain that may be involved in mediating protein-protein interactions (7). Regardless of whether or not cortactin is a physiological ligand of TEM17 and TEM3, its 9 amino acid binding domain is a useful tool for directing agents to tumor endothelium.

The identification of splice variants of TEM17 that are secreted is also of interest. For example, there is an urgent need for serum markers that can directly measure the response to anti-angiogenic therapies. Thus, one can use ELISAs for detection of circulating TEM17-S1 and TEM17-S2 proteins in patients with cancer and other diseases involving abnormal angiogenesis. TEM17-S1 and TEM17-S2 levels provide useful surrogate markers of angiogenesis which can be measured using monoclonal antibodies that specifically target the extracellular domain of TEM17-S.

While the invention has been described with respect to specific examples including presently preferred modes of carrying out the invention, those skilled in the art will appreciate that there are numerous variations and permutations of the above described systems and techniques that fall within the spirit and scope of the invention as set forth in the appended claims.

EXAMPLES

Example 1

Materials and Methods

TEM17 antibodies. An anti-TEM17 polyclonal antibody was made by immunizing rabbits with a recombinant protein encompassing amino acids 23 to 412 of the extracellular region of TEM17-M (Genovac). Serum was tested for anti-TEM17 reactivity by immunostaining 293 cells transfected with TEM17 plasmid, and rabbit polyclonal anti-serum was purified using protein A Sepharose. An Anti-TEM17 monoclonal antibody (clone IMT7) was raised against the peptide sequence NNLSPKTKGTPVHLGTI (SEQ ID NO: 19) that resides in the extracellular region of TEM17-M, proximal to the transmembrane domain (Imgenex).

Immunohistochemistry. Paraffin sections were deparaffinized, incubated with proteinase K (Invitrogen), heated at 95°C for 20 min in citrate buffer (pH 6), and treated with peroxidase blocking reagent (Dako). Sections were incubated with a polyclonal antibody against vWF (Dako) or monoclonal antibody against TEM17 (clone IMT7) followed by a biotin-conjugated secondary antibody (Pierce). A third layer consisting of HRP-conjugated anti-biotin (Dako) was then followed by diaminobenzidine (DAB; Sigma) staining. Sections were counterstained with hematoxylin.

Immunofluorescence. Dual-colour immunofluorescence was performed on fresh-frozen sections fixed in Leukoperm (Serotec) and stained with anti-TEM17 monoclonal (IMT7) and anti-vWF polyclonal (Dako) antibodies. vWF was detected with a FITC-conjugated anti-rabbit antibody (Jackson Immunoresearch Laboratories),

and TEM17 was detected using a biotin anti-mouse antibody (Jackson) followed by rhodamine-streptavidin (Vector).

Immunoelectron Microscopy. Fresh colorectal cancer specimens were fixed in 4% paraformaldehyde, 0.1% glutaraldehyde in phosphate-buffered saline (PBS) (pH 7.2) overnight at 4oC. Samples were rinsed in PBS, incubated with 0.25% tannic acid (Mallinckrodt) in PBS for 1 hour, rinsed again, and uncrosslinked glutaraldehyde was reduced with 50mM NH4Cl in PBS. Samples were washed in 0.1M maleate buffer prior to en bloc staining with 2% uranyl acetate in 0.1M maleate buffer. After a graded ethanol series, dehydrated samples were infiltrated and embedded with L.R. white resin. Samples were polymerized in tightly sealed gelatin capsules for 2 days at 40°C. 70-80nm thick sections were cut and picked up on Formvar-coated nickel grids. After incubating with IMT7 monoclonal antibody and 6nm gold-conjugated anti-rabbit secondary antibody (Jackson Laboratories), sections were post-fixed with 2% glutaraldehyde and stained with uranyl acetate followed by lead citrate. All grids were viewed and photographed on a Phillips CM 120 TEM operating at 80 Kv.

Immunoblotting. Samples were separated by SDS-PAGE and transferred to a PDVF membrane (Millipore). Immunoblots were probed with an anti-TEM17 antibody (IMT7), anti-myc antibody (Sigma) or anti-cortactin antibody (Upstate) followed by an HRP-conjugated anti-mouse secondary antibody (Jackson), and visualized using the ECL plus system (Amersham) according to the supplier's instructions.

Immunoprecipitation. Extracts from mouse brain tissues (Pelfreez Biologicals), 293 cells, or 293 cells transfected with TEM17-M were incubated overnight with the antibodies described above or with or with non-specific rabbit or mouse IgG as controls. Precipitated proteins were eluted from protein G-agarose beads (Roche) and detected by Immunoblotting with anti-TEM17 (IMT7) or anti-cortactin (Upstate) monoclonal antibodies. For deglycosylation, precipitated proteins were treated with N-glycosidase-F alone or a cocktail containing N-glycosidase-F, Endo-O-Glycosidase, α-2(3,6,8,9)-Neuraminidase, ß(1,4)-Galactosidase and ß-N-Acetylglycosaminidase (Sigma).

AP-TEM17/3 and cortactin deletion constructs. cDNAs encoding the extracellular regions of TEM17-M (amino acids 1-423) or TEM3 (amino acids 1-450) were PCR-amplified and directionally cloned into the AP-Tag5 vector (Genhunter) by incorporating the restriction enzyme sites NheI and HindIII (TEM17-M) or NheI and BglII (TEM3) into the PCR forward and reverse primers respectively. The cortactin deletion constructs were made by PCR amplification from full length human cortactin cDNA (accession number BC008799). A Sfi-1 restriction site was placed in the forward primer and an EcoR1 site was place in the reverse primer for directional cloning into pCMV-Myc (Becton Dickinson). All vectors generated by PCR were sequence verified to ensure they were mutation free.

AP-TEM17/7R binding assay. Samples were separated by SDS-PAGE and transferred to a PDVF membrane (Millipore). Membranes were probed directly with supernatants from 293 transfectants expressing either AP alone (control), AP-TEM17 or AP-TEM3, and then visualized using BCIP/NBT substrate (Sigma). For added sensitivity, in some experiments anti-AP antibody (Sigma) was added, followed by HRP-conjugated anti-mouse secondary antibody (Jackson). Blots were visualized on X-ray film using an ECL detection system (Amersham).

Biochemical purification of cortactin. Using the AP-TEM3 binding assay as a screen to follow activity, we fractionated protein extracts from 100 mouse brains (Pelfreez) using anion exchange (Bio-Scale Q20 column, Bio-Rad) followed by cation exchange (Bio-Scale S20 column, Bio-Rad) chromatography. This was followed by affinity chromatography using anti-AP beads (Sigma) that had been armed with the AP-TEM3 fusion protein. Finally, purified extract was loaded onto an SDS-denaturing gel, stained with Coomassie Blue, and two prominent protein bands that migrated at about ~75 and 80 kD were gel isolated. Following tryptic digestion and analysis using mass spectroscopy (Microchemistry and Proteomics analysis facility, Harvard University) both bands were identified as cortactin.

Example 2

Characterization of TEM17 gene products

While attempting to clone the full-length human TEM17 gene by PCR amplification of cDNA obtained from tumor-derived endothelial cells (3) we noticed additional PCR products that were larger than expected. Sequencing of these products revealed that various forms of TEM17 exist in tumor endothelium, each derived by alternative splicing (Fig. 1A and Table 1).

Table 1. *Exons of the various TEM17 alternative splice variants and structural domains*[a]

name	Accession[b]	Exons	SP	NIDO	PSI	TM
TEM17-M	AF279144	1B,2,3,4,5,6,7,8,9,11B,12,13,14,15	+	+	+	+
TEM17-S1	AY704670	1B,2,3,4,5,6,7,8,9,11A,12,13,14,15	+	+	-	-
TEM17-S2	AY704671	1B,2,3,4,5,6,7,8,9,10,11A,12,13,14,15	+	+	-	-
TEM17-I	AY704672	1A,2,3,4,5,6,7,8,9,11A,12,13,14,15	-	+	-	-

[a]SP; signal peptide; NIDO: nidogen domain; PSI: plexin-like domain found in Plexins, Semaphorins and Integrins; TM: Transmembrane domain
[b]Genbank accession number

Comprehensive PCR analyses and comparisons with EST and genomic sequence allowed us to compile the intron/exon structure of TEM17 shown in Fig. 1B. In one of the transcripts identified, an alternative splice acceptor site resulted in a new exon (exon11A) encoding a stop codon prior to the transmembrane domain (Fig 1B). The predicted protein product is a secreted TEM17 molecule (TEM17-S1) with an intact Nidogen-like domain. Likewise, another novel exon, exon 10, was identified and found to contain a premature stop codon producing a second secreted form of TEM17 (TEM17-S2; see Table 1). We also noticed new sequences in the EST repository which suggested that an alternative exon 1 (called E1A) might exist upstream of the original exon 1 (called E1B). Indeed, using PCR primers from sequences within this exon we were able to verify its expression in cDNA isolated from tumor-derived endothelium. Sequencing of the amplified cDNA revealed that exon 1A is used in conjunction with exon E11A such that the predicted product lacks both a signal peptide and transmembrane domain, and is expected to reside intracellularly (TEM17-I, see Table 1). To differentiate the various forms of TEM17, we now refer to the original membrane bound form as TEM17-M.

Endothelial cell surface proteins are attractive targets because of their accessibility to blood-borne therapeutics. In order to determine whether or not TEM17-M protein represents a potential target within tumor endothelium, we generated a monoclonal antibody that would specifically recognize TEM17-M but not

TEM17-I or TEM17-S. The antibody, called IMT7, was generated against a peptide sequence just outside the transmembrane domain of TEM17M (Fig. 1A). We also generated a rabbit polyclonal antibody against the entire extracellular domain of TEM17-M. Using 293 cells transfected with the gene encoding TEM17-M, we found that both the monoclonal and polyclonal antibodies were able to immunoprecipitate a product of ~85 kD (Fig. 1C). The electrophoretic behavior of this polypeptide was slower than that expected from the 54 kD predicted from its amino acid sequence. The decreased mobility was apparently due to glycosylation, as treatment with glycosidase reduced the apparent size by ~20 kD (Fig. 1C).

To determine if the expression of the TEM17 protein, like its mRNA, was elevated in colorectal tissues, we prepared protein extracts from normal colonic mucosa or colorectal tumors of five patients. Immunoblotting with TEM17 antibodies revealed elevated expression of the expected 85 kD product as well as additional 90 kD and 95 kD products in each of the tumor tissues tested (Fig. 1D and data not shown). The reason for the two larger bands observed in each of the tumor samples is not clear, but could be due to alternative splicing, post-translational glycosylation, or ubiquitination. Although the relative abundance of each of the products in the 85/90/95 kD triplet varied between samples, all three were readily immunoprecipitated from tumor extracts with either the polyclonal or the monoclonal anti-TEM17 antibodies.

Example 3

Cellular localization of TEM17 protein

To identify the cellular source of the TEM17 protein observed in the human tumor extracts, we performed a histological survey of various tumor types. As shown in Fig. 2A, immunohistochemistry revealed a vessel-like pattern of staining in colorectal cancers, while TEM17 staining was undetectable in the normal colonic mucosa (data not shown). We observed a similar pattern of staining of tumor vessels in esophagus, lung and bladder cancers (Fig. 2A and data not shown). To determine whether the endothelial cells were responsible for the vessel-like patterns of staining,

we performed co-localization studies with an antibody to the pan-endothelial marker von Willebrand Factor (vWF). As shown in Fig. 2B, TEM17 staining co-localized with vWF in tumor endothelium. Finally, we performed transmission electron microscopy using the same antibodies. TEM17-M was predominantly expressed at the tight junctions between endothelial cells, although some expression at the luminal surface was also detected (Fig. 2C).

Because the expression pattern of mTEM17 mRNA was undetectable in the tumor endothelium of mice (4), we sought other tumor model systems that would allow us to study TEM17 in the context of tumor angiogenesis in vivo. We found that the VX2 rabbit tumor system was suitable for this purpose, as a robust triplet of TEM17 polypeptides was apparent upon Western blotting of an intrahepatic tumor but not in the adjacent normal liver tissue (Fig 1D).

Example 4

Identification of a TEM17/7R binding protein

To identify TEM17 binding partners, we constructed a fusion protein in which the extracellular domain of TEM17 was joined to alkaline phosphatase (AP) (AP-T7; Fig. 1A). Because TEM3 mRNA has been shown to be highly expressed in tumor endothelium, we also fused its extracellular region to AP (AP-T7R). The AP-TEM vectors were transfected into mammalian 293 cells, and the secreted fusion proteins used to probe various tissue extracts using a modified western blotting assay. Protein extracts were resolved on denaturing gels, transferred to nitrocellulose, and blotted using AP-T7/T7R fusion proteins. When various tissue extracts were tested in this assay using AP alone, no binding was detected. However, when probed with either AP-T7 or AP-T7R, a prominent doublet of 80 and 85 kD was observed in every mouse and human tissue examined. Brain tissue appeared to be unique in that it contained a 75 kD product in addition to the 80/85 kD doublet (Fig. 3A). We chose to use mouse brain for biochemical purification because the products recognized by the AP-T7 and AP-T7R fusion proteins were particularly abundant in this tissue. Although similar patterns were observed with both AP-T7 and AP-T7R probes, the

latter yielded slightly stronger signals and were therefore used for subsequent screening assays.

Using the modified western blot assay as a screen, we biochemically fractionated and purified the 75 and 80 kD proteins using a strategy that involved tandem ion-exchange chromatography, affinity chromatography and preparative SDS-PAGE. When the 75 and 80 kD bands were excised from the gel and analyzed by MS/MS Mass spectroscopy, both were independently identified as cortactin. Interestingly, others have shown that cortactin typically migrates as a doublet of 80/85 kD with the difference in size presumably due to phosphorylation (5). Furthermore, the additional ~75 kD product observed in brain tissue presumably represents a brain-specific alternative splice variant that harbors a deletion in the cortactin repeat region (6).

To verify the interaction between endogenous cortactin and TEM17/3, we immunoprecipitated cortactin from mouse brain extracts using anti-cortactin monoclonal antibody and then probed the blot using the AP-TEM3 fusion protein. As shown in Fig. 3B, AP-TEM3 binds specifically to proteins which are the expected size of cortactin. To determine if TEM17, like TEM3, could also bind cortactin, the IMT7 monoclonal antibody was used to immunoprecipitate TEM17 from 293 cells expressing exogenous TEM17. A strong band of the expected size for endogenous cortactin was observed in these immunoprecipitates (Fig. 3C). Importantly, endogenous TEM17 also co-precipitated with cortactin in lysates derived from rabbit tumor tissues, although only the 85 kD product of the triplet was observed (Fig. 3D). Thus, cortactin appears to bind both TEM17 and TEM3.

To map the region of cortactin responsible for binding TEM17 and TEM3, we generated a series of myc-tagged deletion constructs (Fig. 4A). Following exogenous expression in 293 cells, each of the cortactin deletions could be readily detected using an anti-myc antibody (Fig. 4A). Probing the same deletion-containing extracts with the TEM3-AP fusion protein revealed that the minimal sequence required for binding was in the plexin-like domain immediately adjacent to the SH3 domain. Because the minimal region required for binding was still more than 100 amino acids, we

generated a series of overlapping 20 amino acid peptides encompassing this entire region. When each of these peptides were spotted onto nitrocellulose and blotted with the AP-TEM3 fusion protein, a minimal 9 amino acid consensus sequence that appeared to be critical for binding was identified (Fig. 4B).

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We claim:

1. An isolated and purified TEM17I protein which comprises a nidogen G1-like domain of TEM17 protein having an amino acid sequence as shown in SEQ ID NO: 1 or variants thereof having at least 95 % identity with said sequence, wherein said TEM17I protein lacks a signal peptide, a transmembrane domain, and a plexin-like domain.
2. An isolated and purified polynucleotide which encodes a TEM17I protein according to claim 1.
3. An isolated and purified TEM17I protein according to claim 1 which has the amino acid sequence shown in SEQ ID NO: 1.
4. An isolated and purified polynucleotide according to claim 2 which encodes a TEM17I protein which has the amino acid sequence shown in SEQ ID NO: 1.
5. An isolated and purified polynucleotide of claim 4 which comprises the nucleotide sequence shown in SEQ ID NO: 2.
6. An isolated and purified TEM17S protein which comprises a nidogen G1-like domain and a signal peptide of TEM17 protein having an amino acid sequence as shown in SEQ ID NO: 3 or SEQ ID NO: 15 or variants of either having at least 95 % identity with said sequence, wherein said TEM17S protein lacks a transmembrane domain and a plexin-like domain.
7. An isolated and purified polynucleotide which encodes a TEM17S protein according to claim 6.
8. An isolated and purified TEM17S protein according to claim 6 which has the amino acid sequence shown in SEQ ID NO: 3 or SEQ ID NO: 15.
9. An isolated and purified polynucleotide according to claim 7 which encodes a TEM17S protein which has the amino acid sequence shown in SEQ ID NO: 3 or SEQ ID NO: 15.
10. An isolated and purified polynucleotide of claim 9 which comprises the nucleotide sequence shown in SEQ ID NO: 4 or SEQ ID NO: 16.
11. A polypeptide of less than 100 amino acids, said polypeptide comprising an amino acid sequence selected from the group consisting of: Thr Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 5); **Xaa** Ala Val Ala Leu Tyr Asp Tyr

Gln (SEQ ID NO: 6); Thr **Xaa** Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 7); Thr Ala **Xaa** Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 8); Thr Ala Val **Xaa** Leu Tyr Asp Tyr Gln (SEQ ID NO: 9); Thr Ala Val Ala **Xaa** Tyr Asp Tyr Gln (SEQ ID NO: 10); Thr Ala Val Ala Leu **Xaa** Asp Tyr Gln (SEQ ID NO: 11); Thr Ala Val Ala Leu Tyr **Xaa** Tyr Gln (SEQ ID NO: 12); Thr Ala Val Ala Leu Tyr Asp **Xaa** Gln (SEQ ID NO: 13); and Thr Ala Val Ala Leu Tyr Asp Tyr Xaa (SEQ ID NO: 14).

12. The polypeptide of claim 11, said polypeptide consisting of the amino acid sequence TAVALYDYQ (SEQ ID NO: 5).

13. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 11 and a second polypeptide which is an enzyme.

14. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 11 and a second polypeptide which is a therapeutic agent.

15. The recombinant fusion protein of claim 14 wherein the therapeutic agent is cytotoxic.

16. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 11 and a second polypeptide which is a fluorescent protein.

17. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 11 and a second polypeptide which is a bioluminescent protein.

18. A conjugate comprising the polypeptide of claim 11 conjugated to a diagnostically detectable moiety.

19. The conjugate of claim 18 wherein the diagnostically detectable moiety is a radioactive moiety.

20. A conjugate comprising the polypeptide of claim 11 conjugated to a therapeutic moiety.

21. The conjugate of claim 20 wherein the therapeutic moiety is a radioactive moiety.

22. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 12 and a second polypeptide which is an enzyme.

23. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 12 and a second polypeptide which is a therapeutic agent.

24. The recombinant fusion protein of claim 23 wherein the therapeutic agent is cytotoxic.

25. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 12 and a second polypeptide which is a fluorescent protein.

26. A recombinant fusion protein comprising a first polypeptide which is the polypeptide of claim 12 and a second polypeptide which is a bioluminescent protein.

27. A conjugate comprising the polypeptide of claim 12 conjugated to a diagnostically detectable moiety.

28. The conjugate of claim 27 wherein the diagnostically detectable moiety is a radioactive moiety.

29. A method of delivering a therapeutic agent to tumor endothelium, comprising the step of:

administering a therapeutic agent to a patient harboring a tumor, wherein the therapeutic agent is linked to the polypeptide of claim 11.

30. The method of claim 29 wherein the therapeutic agent is a second polypeptide.

31. The method of claim 29 wherein the therapeutic agent is covalently linked to the polypeptide.

32. A method of delivering a diagnostic agent to tumor endothelium, comprising the step of:

administering a diagnostic agent to a patient harboring a tumor, wherein the diagnostic agent is linked to the polypeptide of claim 11.

33. The method of claim 32 wherein the diagnostic agent is a second polypeptide.

34. The method of claim 32 wherein the diagnostic agent is covalently linked to the polypeptide.

35. A method of screening for potential therapeutic agents which inhibit binding of cortactin to TEM17, comprising the steps of:

comparing binding of TEM17 to cortactin in the presence and in the absence of a test agent, wherein a test agent which diminishes amount

of binding of TEM17 to cortactin is identified as a potential therapeutic agent for treating pathological vascularization.

36. The method of claim 35 wherein the TEM17 and cortactin are contacted *in vitro*.

37. The method of claim 35 wherein the TEM17 and cortactin are expressed on cells which are contacted.

38. The method of claim 35 wherein the TEM17 and cortactin are portions of fusion proteins.

39. A method of screening for potential therapeutic agents which inhibit binding of cortactin to TEM3, comprising the steps of:

comparing binding of TEM3 to cortactin in the presence and in the absence of a test agent, wherein a test agent which diminishes amount of binding of TEM3 to cortactin is identified as a potential therapeutic agent for treating pathological vascularization.

40. The method of claim 39 wherein the TEM3 and cortactin are contacted *in vitro*.

41. The method of claim 39 wherein the TEM3 and cortactin are expressed on cells which are contacted.

42. The method of claim 39 wherein the TEM3 and cortactin are portions of fusion proteins.

43. A method of screening for potential therapeutic agents which inhibit the binding of cortactin to TEM17, comprising the steps of:

comparing binding of TEM17 to a polypeptide in the presence and in the absence of the test agent, wherein the polypeptide is less than 100 amino acid residues in length, said polypeptide comprising the amino acid sequence TAVALYDYQ (SEQ ID NO: 5) wherein a test agent which diminishes the amount of binding of TEM17 to the polypeptide is identified as a potential therapeutic agent for treating pathological vascularization.

44. The method of claim 43 wherein the TEM17 and the polypeptide are contacted *in vitro*.

45. The method of claim 43 wherein the TEM17 and the polypeptide are expressed on cells which are contacted.

46. The method of claim 43 wherein the TEM17 and the polypeptide are portions of fusion proteins.

47. A method of screening for potential therapeutic agents which inhibit the binding of cortactin to TEM3, comprising the steps of:

comparing binding of TEM3 to a polypeptide in the presence and in the absence of the test agent, wherein the polypeptide is less than 100 amino acid residues in length, said polypeptide comprising the amino acid sequence TAVALYDYQ (SEQ ID NO: 5), wherein a test agent which diminishes amount of binding of TEM3 to the polypeptide is identified as a potential therapeutic agent for treating pathological vascularization.

48. The method of claim 47 wherein the TEM3 and the polypeptide are contacted *in vitro*.

49. The method of claim 47 wherein the TEM3 and the polypeptide are expressed on cells which are contacted.

50. The method of claim 47 wherein the TEM3 and the polypeptide are portions of fusion proteins.

51. An isolated antibody which binds to a TEM17I protein which comprises a nidogen G1-like domain of TEM17M protein but lacks a signal peptide, a transmembrane domain, and a plexin-like domain, wherein said antibody does not bind to TEM17M.

52. The isolated antibody of claim 51 which binds to an epitope encoded by exon 11A.

53. An isolated antibody of claim 51 which binds to a TEM17I protein which has the amino acid sequence shown in SEQ ID NO: 1, wherein said antibody does not bind to TEM17M as shown in SEQ ID NO: 17.

54. The isolated antibody of claim 53 which binds to an epitope encoded by exon 11A.

55. An isolated antibody which binds to a TEM17S1 or TEM17S2 protein which comprises a nidogen G1-like domain and a signal peptide of TEM17M

protein, but lacks a transmembrane domain and a plexin-like domain, wherein said antibody does not bind to TEM17M.

56. The isolated antibody of claim 55 which binds to an epitope encoded by exon 10.

57. An isolated antibody of claim 55 which specifically binds to a TEM17S1 OR TEM17S2 protein which has the amino acid sequence shown in SEQ ID NO: 3 or SEQ ID NO: 15, wherein said antibody does not bind to TEM17M as shown in SEQ ID NO: 17.

58. The isolated antibody of claim 57 which binds to an epitope encoded by exon 10.

59. The isolated antibody of claim 57 which binds to an epitope encoded by exon 11A.

60. A method of detecting a serum protein indicative of pathological angiogenesis, comprising:

detecting binding of a serum protein to an antibody of any of claims 51-59, wherein detection of the serum protein is indicative of pathological angiogenesis.

61. A polynucleotide encoding the recombinant fusion protein of claim 13-17.

62. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 5).

63. The polypeptide of claim 11 wherein the amino acid sequence is **Xaa** Ala Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 6).

64. The polypeptide of claim 11 wherein the amino acid sequence is Thr **Xaa** Val Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 7).

65. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala **Xaa** Ala Leu Tyr Asp Tyr Gln (SEQ ID NO: 8).

66. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val **Xaa** Leu Tyr Asp Tyr Gln (SEQ ID NO: 9).

67. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala **Xaa** Tyr Asp Tyr Gln (SEQ ID NO: 10).

68. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala Leu **Xaa** Asp Tyr Gln (SEQ ID NO: 11).

69. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala Leu Tyr **Xaa** Tyr Gln (SEQ ID NO: 12).

70. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala Leu Tyr Asp **Xaa** Gln (SEQ ID NO: 13).

71. The polypeptide of claim 11 wherein the amino acid sequence is Thr Ala Val Ala Leu Tyr Asp Tyr **Xaa** (SEQ ID NO: 14).

72. A polypeptide comprising a portion of cortactin consisting of less than 100 amino acids, said polypeptide comprising the amino acid sequence TAVALYDYQ (SEQ ID NO: 5).

73. The polypeptide of claim 72 wherein the portion of cortactin consists of less than 50 amino acids.

74. The polypeptide of claim 72 wherein the portion of cortactin consists of less than 25 amino acids.

75. The polypeptide of claim 72 wherein the portion of cortactin consists of less than 15 amino acids.

76. The method of claim 35 wherein the TEM 17 protein is TEM17S1 .

77. The method of claim 35 wherein the TEM 17 protein is TEM17S2.

78. The method of claim 35 wherein the TEM 17 protein is TEM17I.

79. The method of claim 35 wherein the TEM 17 protein is TEM17M.

80. The method of claim 43 wherein the TEM 17 protein is TEM17S1 .

81. The method of claim 43 wherein the TEM 17 protein is TEM17S2.

82. The method of claim 43 wherein the TEM 17 protein is TEM17I.

83. The method of claim 43 wherein the TEM 17 protein is TEM17M.

84. A method of detecting expression of TEM17I, TEM17S1, or TEM17S2, comprising:

contacting a body sample with an antibody according to any of claims 51-59;

detecting antibody bound to the body sample, wherein the presence of bound antibody indicates pathological angiogenesis.

85. The method of claim 84 wherein the body sample is selected from the group consisting of: a tissue sample, stool, urine, saliva, sputum.

86. A method of detecting expression of TEM17I, TEM17S1, or TEM17S2, comprising

contacting a body sample with a nucleic acid primer or probe which hybridizes to a polynucleotide according to SEQ ID NO: 2, 4, 16 or their complements, but not with a polynucleotide according to SEQ ID NO: 20 or its complement;

detecting hybridized primer or probe, wherein the hybridized primer or probe indicates pathological angiogenesis.

87. The method of claim 86 wherein the nucleic acid primer or probe hybridizes to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and each of the complements thereof.

88. The method of claim 86 wherein the nucleic acid primer or probe is at least 95% identical to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and each of the complements thereof.

89. The method of claim 86 wherein the nucleic acid primer or probe is complementary to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and each of the complements thereof.

90. An isolated polynucleotide probe or primer which is at least 95% identical to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and each of the complements thereof.

91. The polynucleotide probe or primer of claim 90 which is complementary to a sequence selected from the group consisting of:

nucleotides 1-103 of SEQ ID NO: 2, nucleotides 1018-1379 of SEQ ID NO: 2, nucleotides 2606-2630 of SEQ ID NO: 2, nucleotides 1072-1433 of SEQ ID NO: 4, nucleotides 2660-2684 of SEQ ID NO: 4, nucleotides 1177-1633 of SEQ ID NO: 16, nucleotides 1860-1884 of SEQ ID NO: 16, and each of the complements thereof.

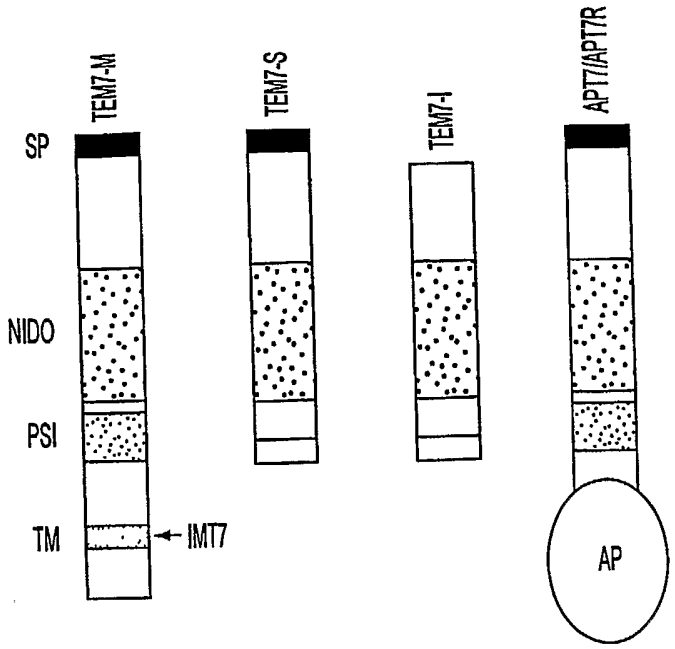
TEM7-M
TEM7-S
TEM7-I
APT7/APT7R
SP
NIDO
PSI
TM
IMT7
AP

FIG. 1A

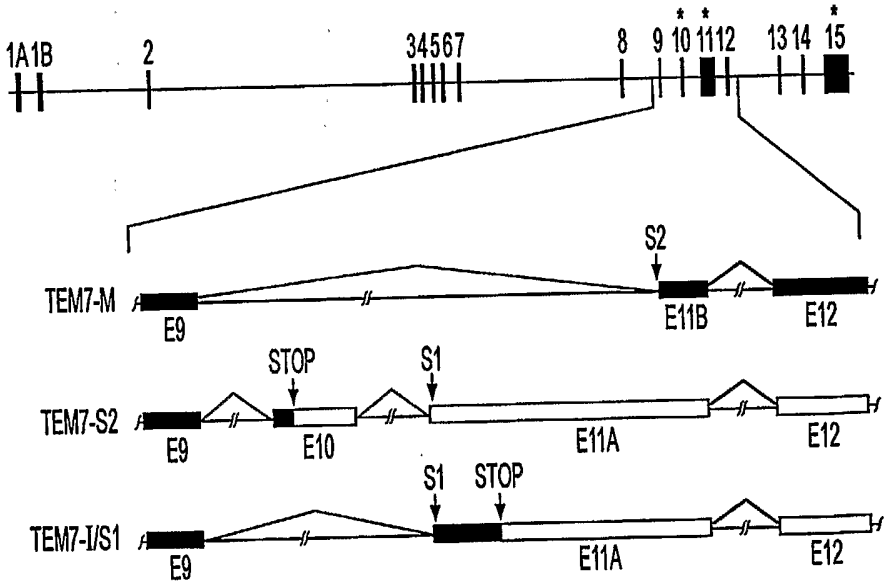
1A 1B
2
3 4 5 6 7
8
9
10
11
12
13
14
15
S2
TEM7-M
E9
E11B
E12
STOP
S1
TEM7-S2
E9
E10
E11A
E12
S1
STOP
TEM7-I/S1
E9
E11A
E12

FIG. 1B

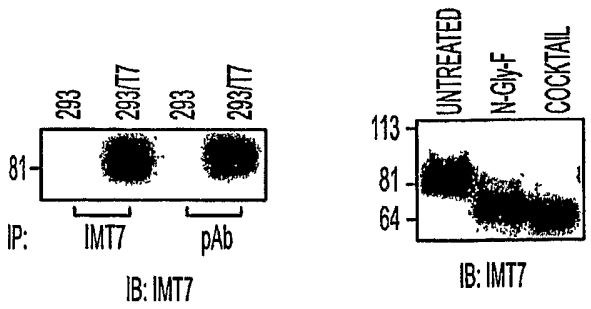
293
293/T7
293
293/T7
81
IP:
IMT7
pAb
IB: IMT7
UNTREATED
N-Gly-F
COCKTAIL
113
81
64
IB: IMT7

FIG. 1C

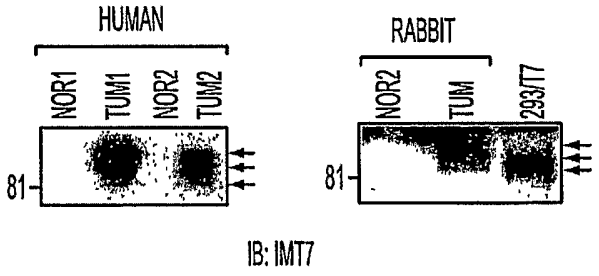
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FIG. 1D

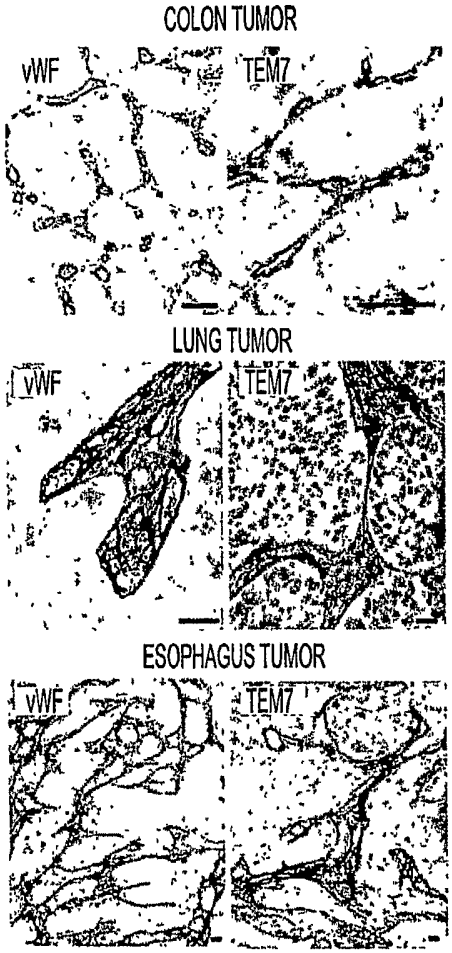
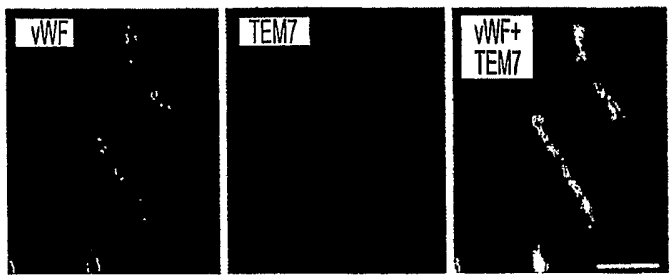


FIG. 2A



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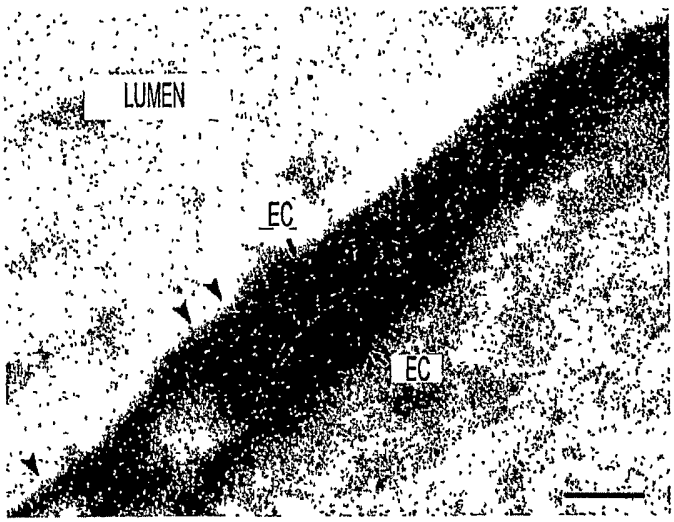

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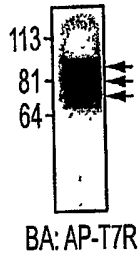

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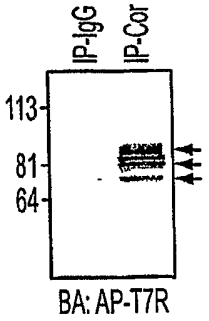

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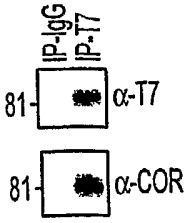

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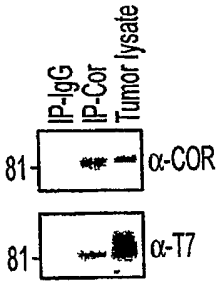

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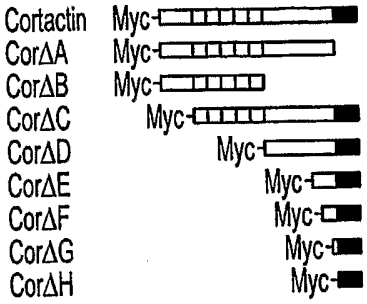

FIG. 3E

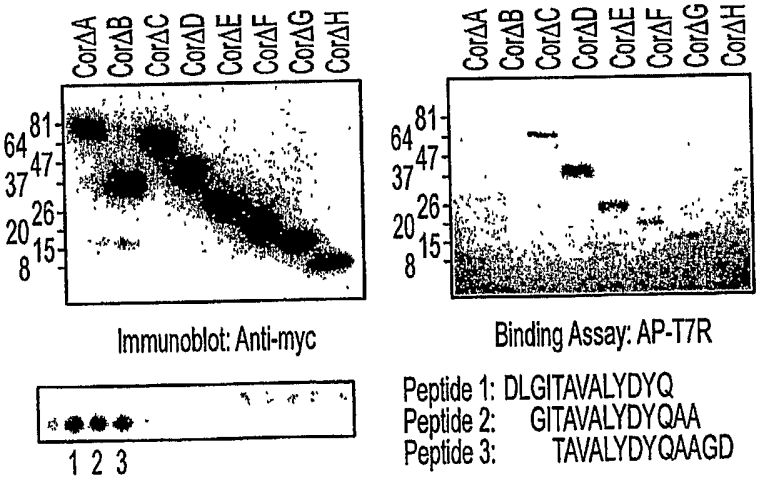

FIG. 3F

936291_3

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4	TEM17S1	2684	DNA
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6	var. 1	9	Protein
7	var. 2	9	Protein
8	var. 3	9	Protein
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14	var. 9	9	Protein
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16	TEM 17S2	2884	DNA
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18	TEM 3	529	Protein
19	immunogen	17	Protein
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Kinzler, Kenneth
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Buckhautls, Phillip

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<213> Homo sapiens

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Gln Asp Leu Gly Gly Gly Thr Leu Ala Met Asp Thr Leu Pro Asp Asn
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Arg Thr Arg Val Val Glu Asp Asn His Ser Tyr Tyr Val Ser Arg Leu
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Tyr Gly Pro Ser Glu Pro His Ser Arg Glu Leu Trp Val Asp Val Ala
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Gly His Pro Leu Arg Gln Ile Thr Ile Ala Thr Gly Gly Phe Ile Phe
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Pro Leu Met Ala Asn Phe Asn Pro Gly Tyr Ser Asp Asn Ser Thr Val
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Val Tyr Phe Asp Asn Gly Thr Val Phe Val Val Gln Trp Asp His Val
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Thr Lys Gly Thr Pro Val His Leu Gly Thr Ile Val Gly Ile Val Leu
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<213> Homo sapiens

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His Thr Glu Glu Glu Val Glu Val Asp Ser His Ala Tyr Ser His Arg
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Trp Lys Arg Asn Leu Asp Phe Leu Lys Ala Val Asp Thr Asn Arg Ala
65                  70                  75                  80
Ser Val Gly Gln Asp Ser Pro Glu Pro Arg Ser Phe Thr Asp Leu Leu
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Leu Asp Asp Gly Gln Asp Asn Asn Thr Gln Ile Glu Glu Asp Thr Asp
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Ser Val Ser Arg Asn Ser Thr Val Arg Tyr Phe Asp Asn Gly Thr Ala
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225                 230                 235                 240
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Phe Gly Tyr Lys Glu Ile Pro Val Leu Val Thr Gln Ile Ser Ser Thr
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Gly Gly Thr Leu His Ala Gly Leu Ile Val Gly Ile Leu Ile Leu Val
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Leu Ile Val Ala Thr Ala Ile Leu Val Thr Val Tyr Met Tyr His His
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