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(71) **Applicant** (for all designated States except US): **THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL** [US/US]; 308 Bynum Hall, Campus Box 4105, Chapel Hill, NC 27599-4105 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants** (for US only): **BANES, Albert** [US/US]; 1821 Coleman Loop Road, Hillsborough, NC 27278 (US). **QI, Jie** [US/US]; 118 South Fields Circle, Chapel Hill, NC 27516 (US). **TSUZAKI, Mari** [US/US]; 201 Standish Drive, Chapel Hill, NC 27517 (US). **BYNUM, Donald, K.** [US/US]; 3938 St. Marks Road, Durham, NC 27707 (US). **BANES, Ashley** [US/US]; 5357 Mill Run, Raleigh, NC 27612 (US). **DMOCHOWSKI, Jacob** [US/US]; 9555 Hunting Court, Matthews, NC 28105 (US). **PATTERSON, Megan** [US/UY]; 106 Arlen Park Drive, Chapel Hill, NC 27516 (US).

(74) **Agent:** **TAYLOR, Arles, A., Jr.**; Jenkins, Wilson, Taylor & Hunt, P.A., Suite 1200, University Tower, 3100 Tower Boulevard, Durham, NC 27707 (US).

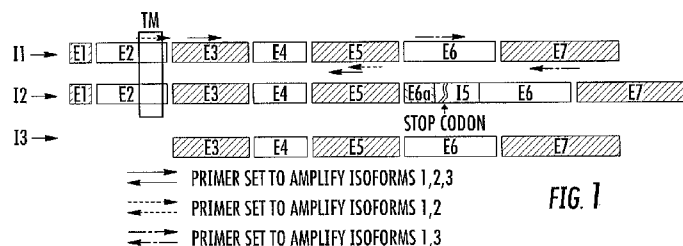
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(54) Title: TENOMODULIN (TNMD) ISOFORMS AND USES THEREOF



(57) **Abstract:** The presently disclosed subject matter relates to tenomodulin (TNMD) and its isoforms. Also described are diagnostic and therapeutic uses of tenomodulin (TNMD) and its isoforms, such as in connective tissues such as tendon. More particularly, provided herein are methods of detecting a tenomodulin polypeptide, methods of detecting a nucleic acid molecule that encodes a tenomodulin polypeptide, and methods of modulating the biological activity of a tenomodulin polypeptide.

DESCRIPTION

TENOMODULIN (TNMD) ISOFORMS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/431,576, filed January 11, 2011, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

- 10 The presently disclosed subject matter relates to tenomodulin (TNMD) and its isoforms. Also described are diagnostic and therapeutic uses of tenomodulin (TNMD) and its isoforms, such as in connective tissues such as tendon.

BACKGROUND

- 15 Tenomodulin (Tnmd) is a highly expressed candidate marker gene for tenocytes. However, the function of tenomodulin in tendons has previously been unclear. Tnmd, similar to chondromodulin I (ChM-I), contains two extracellular domains: BRICHOS and a C-terminal cysteine-rich domain. The
- 20 BRICHOS domain has been found in eight different protein families with functions associated with cancer, dementia and respiratory diseases (Sanchez-Pulido et al, 2002). The domain itself is thought to have a chaperone function, but the mechanism of action is still unclear. Scientific knowledge in the tendon field could be advanced by elucidating the functions
- 25 of Tnmd and any splice variants of this highly expressed tendon marker gene. Such knowledge could be useful as a biomarker for tendons and ligaments.

- There is diagnostic value in having a biomarker that can be reliably assessed that would indicate a degree of damage to ligaments and tendons.
- 30 Such a biomarker could be used to evaluate the healing recovery process and yield a quantitative marker that can be used to track convalescence and be concordant with gain in mobility, strength and return to work. This is of use diagnostically as well.

What are needed, then, are new strategies and compositions, such as biomarkers, for facilitating diagnosis of connective tissue, tendon and ligament injuries and evaluating the health of the same. The presently disclosed subject matter addresses this and other needs in the art.

5

SUMMARY

The presently disclosed subject matter provides tenomodulin polypeptides and polynucleotide sequences encoding the same.

10 In some embodiments, provided herein is an isolated nucleic acid encoding a tenomodulin polypeptide. In some embodiments, the nucleic acid sequence can be selected from the group consisting of SEQ ID NOs: 1-3 and a nucleic acid sequence having at least a 90% sequence identity to one of SEQ ID NOs: 1-3, optionally over its entire length. In some embodiments, the nucleic acid sequence can encode a protein comprising
15 an amino acid sequence of one of SEQ ID NOs: 4-6. In some embodiments, provided herein is a recombinant vector comprising one or more of these nucleic acid sequences. In some embodiments, provided herein is a recombinant host cell or stem cell containing one or more of these nucleic acid sequences.

20 In some embodiments, provided herein is an isolated tenomodulin polypeptide, optionally one or more of isoforms 1, 2 or 3. In some embodiments, an isolated tenomodulin polypeptide can comprise an amino acid sequence of one of SEQ ID NOs: 4-6 or a sequence having a 90% or greater sequence identity to one of SEQ ID NOs: 4-6, optionally over its
25 entire length. In some embodiments, provided herein is a recombinant cell expressing the polypeptide. In some embodiments, the polypeptide can be modified to be in detectably labeled form.

In some embodiments, provided herein is an isolated and purified antibody capable of specifically binding to the polypeptide. In some
30 embodiments, the antibody can be capable of modulating the biological activity of the polypeptide. In some embodiments, provided herein is a hybridoma cell line which produces the antibody.

In some embodiments, provided herein is a method of producing an antibody immunoreactive with a tenomodulin polypeptide, the method comprising: transfecting a recombinant host cell with a nucleic acid molecule which encodes a tenomodulin polypeptide; culturing the host cell under
5 conditions sufficient for expression of the polypeptide; recovering the polypeptide; and preparing an antibody to the polypeptide; and optionally detecting and/or isolating an antibody to one or more of the tenomodulin isoforms.

In some embodiments, provided herein is a method of detecting a
10 tenomodulin polypeptide, the method comprising immunoreacting the polypeptide with an antibody to form an antibody-polypeptide conjugate; and detecting the conjugate. In some embodiments, the detecting of the tenomodulin polypeptide further comprises detecting a tenocyte based on the detecting of the tenomodulin polypeptide. In some embodiments, the
15 detecting of the tenomodulin polypeptide comprises quantitating or staging onset or progression of a connective tissue disease, optionally dense connective tissues, optionally, tendons or ligaments.

In some embodiments, provided herein is a method of detecting a nucleic acid molecule that encodes a tenomodulin polypeptide in a biological
20 sample containing nucleic acid material, the method comprising: hybridizing a nucleic acid molecule having a sequence complementary to at least a portion of a nucleic acid sequence encoding a tenomodulin polypeptide under stringent hybridization conditions to the nucleic acid material of the biological sample, thereby forming a hybridization duplex; and detecting the
25 hybridization duplex. In some embodiments, the detecting of the tenomodulin polypeptide further comprises detecting a tenocyte based on the detecting of the nucleic acid molecule. In some embodiments, the detecting of the tenomodulin polypeptide comprises quantitating or staging onset or progression of a connective tissue disease, optionally dense
30 connective tissues, optionally, tendons or ligaments

In some embodiments, provided herein is an assay kit for detecting the presence of a tenomodulin polypeptide or antibody to tenomodulin isoforms in a biological sample, the kit comprising a first container

comprising a first antibody capable of immunoreacting with a tenomodulin polypeptide. In some embodiments, the assay kit can further comprise a second container containing a second antibody that immunoreacts with the first antibody. In some embodiments, the first antibody and the second
5 antibody comprise monoclonal antibodies. In some embodiments, the first antibody is affixed to a solid support. In some embodiments, the first and second antibodies each comprise an indicator. In some embodiments, the indicator is a radioactive label or an enzyme.

In some embodiments, provided herein is an assay kit for detecting
10 the presence, in a biological sample, of an antibody immunoreactive with a tenomodulin polypeptide, the kit comprising a tenomodulin polypeptide that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay, optionally comprising a label for the polypeptide, optionally wherein the label can be detected by an imaging
15 technique, such as but not limited to MRI or CT.

In some embodiments, provided herein is an assay kit for detecting the presence, in biological samples, of a tenomodulin polypeptide, the kit comprising a first container that contains a nucleic acid molecule identical or complementary to a segment of at least ten contiguous nucleotide bases of
20 the nucleic acid molecule encoding a tenomodulin peptide.

In some embodiments, provided herein is a method of modulating the biological activity of a tenomodulin polypeptide in a biological sample, the method comprising contacting the biological sample with an agent for modulating expression, activity or both expression and activity of a
25 tenomodulin polypeptide. In some embodiments, the biological sample is a tissue present in a subject. In some embodiments, the biological sample is a tendon, ligament or other dense connective tissue. In some embodiments, the modulating of the tenomodulin polypeptide biological activity regulates the growth of collagen fibrils, optionally wherein a ratio of large and small
30 fibrils is controlled to a more native state. In some embodiments, the tissue present in a subject is an abnormal tissue. In some embodiments, the modulation of tenomodulin polypeptide expression reduces growth of the abnormal tissue, optionally by blocking angiogenesis.

In some embodiments, provided herein is a method of screening candidate substances for an ability to modulate activity, expression or both activity and expression of a tenomodulin polypeptide, the method comprising: providing a test sample comprising a polypeptide of
5 tenomodulin; administering a test molecule to the test sample; and determining the effect of the test molecule on the activity, expression or both activity and expression of the polypeptide. In some embodiments, the test molecule is selected from the group consisting of a polypeptide, a nucleic acid, optionally an siRNA to one or more of the tenomodulin isoform mRNAs,
10 an exogenous vector coding for a nucleic acid oligonucleotide or polypeptide, a carbohydrate, a lipid, an amino acid, a fatty acid, a steroid, and a low molecular weight organic molecule. In some embodiments, determining the effect of the test molecule on the activity, expression or both activity and expression of the polypeptide comprises measuring a first
15 activity, expression or both activity and expression level of the polypeptide prior to administering the test molecule to the test sample, measuring a second activity, expression or both activity and expression level of the polypeptide after administering the test molecule to the test sample, and comparing the first and second levels. In some embodiments, the test
20 sample comprises a cell. In some embodiments, the polypeptide is provided to the cell from an exogenous source. In some embodiments, the cell expresses the polypeptide. In some embodiments, the cell is a recombinant cell. In some embodiments, the test sample comprises a non-human animal. In some embodiments, the animal is a genetically modified animal.

25 In some embodiments, the presently disclosed subject matter provides a method of diagnosing a tendon injury, the method comprising: providing a subject; collecting a biological sample from the subject; and detecting the expression of a tenomodulin gene in the sample. In some
30 embodiments, the subject is a human subject. In some embodiments, the biological sample is selected from the group consisting of tendon, connective tissue and ligament. In some embodiments, detecting a tenomodulin in the sample comprises detecting the expression of a tenomodulin gene. In some embodiments, the tenomodulin gene is selected from the group

consisting of isoform 1, isoform 2 and isoform 3. In some embodiments, a expression of a tenomodulin gene, as compared to a healthy subject, is indicative of a tendon injury. In some embodiments, detecting a tenomodulin in the sample comprises detecting the concentration of a tendomodulin polypeptide. In some embodiments, an altered concentration of a tenomodulin polypeptide, as compared to a healthy subject, is indicative of a tendon injury.

It is an object of the presently disclosed subject matter to provide tenomodulin (TNMD) and its isoforms. Also described are diagnostic and therapeutic uses of TNMD and its isoforms, such as in connective tissues such as tendon.

An object of the presently disclosed subject matter having been stated hereinabove, and which is achieved in whole or in part by the presently disclosed subject matter, other objects will become evident as the description proceeds when taken in connection with the accompanying Appendix as best described hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing of human tenomodulin (Tnmd) isoform gene constructs and the locations of primers used in the experiments of the instant disclosure. Tnmd gene construct isoform 1 (I1) is the full length Tnmd, and includes exons 1-7 (E1-E7). Isoform 2 (I2) contains exons 1-7 (E1-E7) as well as an intron (intron 5 (I5)) between exons 5 (E5) and 6 (E6), with a premature stop codon. Isoform 3 (I3) lacks exons 1 (E1) and 2 (E2).

Figures 2A and 2B are bar charts illustrating the differential expression of Tnmd isoforms in various human tissues. Human flexor digitorum profundus (FDP) and flexor carpi radialis (FCR) tendons, tibial bone, medial collateral ligament, smooth muscle, cardiac muscle, and soft tissues (liver, kidney and brain) were assessed for Tnmd isoforms.

Figure 3 is an image of a Western blot of human tenomodulin. Left and middle panels were from the same blot on COS-7 cell lysate, probed with anti-GFP and anti-tenomodulin antibodies, respectively. Lanes 1-5 are

COS-7 cells, GFP-transfected, GFP-fused isoform I transfected, GFP-fused isoform II transfected and GFP-fused isoform III transfected, respectively. Right panel was probed with anti-tenomodulin antibody. Lanes 1 and 2 were human FCR tenocyte cell lysate from two patients, and lane 3 was FCR tendon lysate.

Figure 4 is a bar chart depicting the differential expression of Tnmd isoforms in various human tendons. The relative expression levels of isoforms 1, 2 and 3 are shown for flexor digitorum profundus (FDP) adhesion, FDP, bone ulna, bone distal phalanx (BDP), tendon sheath, extensor tendon (ET) synovium, porcine Achilles tendon (PAT), and equine superficial digital flexor tendon (ESDFT).

Figures 5A and 5B are bar charts depicting the results of tenomodulin RNAi assays. Knockdown of tenomodulin reduced cell proliferation (Figure 5A) and up-regulated the expression of myostatin (MSTN) and scleraxis (Scx) (Figure 5B). MSTN expression was increased by >60% and Scx was increased by >90%. * Significant at $p < 0.05$.

Figure 6 is a schematic illustration of a regulatory pathway of Tnmd.

Figure 7 is a bar chart depicting the results of a kinetic study to determine the relative expression levels of Tnmd isoform 1 after continuous stretching. * Significant at $p < 0.05$ (Student t-test).

Figure 8 is a bar chart depicting the results of a kinetic study to determine the relative expression levels of Tnmd isoforms 1 and 2 after stretching 3% strain for 1 hour/day for 3 days. * Significant at $p < 0.05$ (Student t-test).

Figure 9 is a bar chart illustrating the relative expression of Tnmd isoforms 1, 2 and 3 in the porcine and equine.

Figure 10 is a bar chart depicting the expression of Tnmd isoform 3 between control and strain groups. * Significant at $p < 0.05$.

Figure 11 is a bar chart depicting the expression of Tnmd isoform 3 between control and wound groups. ** Significant at $p < 0.07$.

Figure 12 is a bar chart depicting the relative expression of Tnmd isoform 1 in Achilles tendons of wild type (wt), P2Y1 knock-out (KO), P2Y2 KO, and P2Y1/P2Y2 double knock-out (DKO) mice.

Figure 13 is a bar chart depicting the relative expression of Tnmd isoform 1 when tenocytes were treated with IL-1b.

Figure 14 is a schematic illustrating the sequence alignment of highly conserved nucleotide sequence for tenomodulin from equine (SEQ ID NO: 7), porcine (SEQ ID NO: 8) and human (SEQ ID NO: 9).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 is a nucleotide sequence for human tenomodulin isoform 1 cDNA.

10 SEQ ID NO: 2 is a nucleotide sequence for human tenomodulin isoform 2 cDNA.

SEQ ID NO: 3 is a nucleotide sequence for human tenomodulin isoform 3 cDNA.

15 SEQ ID NO: 4 is human tenomodulin isoform 1 deduced amino acid sequence.

SEQ ID NO: 5 is human tenomodulin isoform 2 deduced amino acid sequence.

SEQ ID NO: 6 is human tenomodulin isoform 3 deduced amino acid sequence.

20 SEQ ID NO: 7 is a highly conserved equine nucleotide sequence for tenomodulin.

SEQ ID NO: 8 is a highly conserved porcine nucleotide sequence for tenomodulin.

25 SEQ ID NO: 9 is a highly conserved human nucleotide sequence for tenomodulin.

SEQ ID NOs: 10 through 37 are forward and reverse primer sequences used in the tenomodulin studies disclosed herein.

DETAILED DESCRIPTION

30 I. General Consideration

The presently disclosed subject matter pertains to Tenomodulin (Tnmd, also called Tendin), which is a type II transmembrane glycoprotein. Tenomodulin is highly expressed in developing tendon as well as in mature

tendons. Along with scleraxis (scx), Tnmd is a candidate marker gene for tenocytes. It has been reported to have anti-angiogenic properties but a knockout mouse model did not substantiate that claim. It has homology to chondromodulin-I. Tenomodulin is highly conserved across species, as depicted in Figure 14 which illustrates a nucleotide sequence alignment for tenomodulin from equine (SEQ ID NO: 7), porcine (SEQ ID NO: 8) and human (SEQ ID NO: 9).

Single nucleotide polymorphisms of TNMD have been associated with obesity and macular degeneration in patients. In the presently disclosed subject matter, three Tnmd isoforms with deduced molecular weights of 37.1 kDa (isoform 1), 20.3 kDa (isoform 2) and 25.4 kDa (isoform 3) were identified and verified by western blot and qPCR. The term "tenomodulin polypeptide" encompasses all three isoforms. Overexpression of each Tnmd isoform followed by immunofluorescence imaging showed different intracellular localizations. Results of qPCR demonstrated differential expression of each Tnmd isoform in patient specimens taken from Flexor carpi radialis, biceps tendon and Flexor digitorum profundus tendons. Knockdown of Tnmd increased the expression of both scleraxis (scx) and myostatin (MSTN), indicating a negative feedback loop between Tnmd and its regulators. Knockdown of all Tnmd isoforms simultaneously, also reduced tenocyte proliferation. I-TASSER protein 3D conformation modeling predictions indicated each Tnmd isoform had different functions. The presently disclosed subject matter has led to better understanding of tendon development, response to injury and pathologies, all of which has provided for new therapeutic treatments for tendon diseases.

An aspect of the presently disclosed subject matter is that either normal or pathologic states can modulate the degree of expression of Tnmd and levels of Tnmd proteins that can be useful for diagnostic purpose. There is diagnostic value in having a biomarker that can be reliably assessed that would indicate a degree of damage to ligaments and tendons. Moreover, Tnmd can be used to evaluate the healing recovery process and yield a quantitative marker that can be used to track convalescence and be concordant with gain in mobility, strength and return to work. This is of use

diagnostically.

Also disclosed herein is that knocking down Tnmd isoforms with RNAi increases expression of the transcription factor, scleraxis (Scx), which has been shown to regulate Tnmd expression *in vivo* in mouse embryos and *in vitro* in treated tenocytes.

A regulatory pathway of Tnmd is also disclosed herein. Tnmd isoforms can serve as new biomarkers and more importantly, as novel therapeutic targets in tendon injury and diseases, given the effect on cell proliferation and varying intracellular localizations.

II. Definitions

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the presently disclosed subject matter belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the presently disclosed subject matter, representative methods, devices, and materials are now described.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a tumor cell" includes a plurality of such tumor cells, and so forth.

As used herein, the term "about," when referring to a value or to an amount of mass, weight, time, volume, concentration, or percentage is meant to encompass variations of in some embodiments, $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods.

As used herein, the term "and/or" when used in the context of a listing of entities, refers to the entities being present singly or in combination. Thus, for example, the phrase "A, B, C, and/or D" includes A, B, C, and D

individually, but also includes any and all combinations and subcombinations of A, B, C, and D.

The term "comprising", which is synonymous with "including," "containing," or "characterized by" is inclusive or open-ended and does not
5 exclude additional, unrecited elements or method steps. "Comprising" is a term of art used in claim language which means that the named elements are present, but other elements can be added and still form a construct or method within the scope of the claim.

As used herein, the phrase "consisting of" excludes any element,
10 step, or ingredient not specified in the claim. When the phrase "consists of" appears in a clause of the body of a claim, rather than immediately following the preamble, it limits only the element set forth in that clause; other elements are not excluded from the claim as a whole.

As used herein, the phrase "consisting essentially of" limits the scope
15 of a claim to the specified materials or steps, plus those that do not materially affect the basic and novel characteristic(s) of the claimed subject matter.

With respect to the terms "comprising", "consisting of", and "consisting essentially of", where one of these three terms is used herein, the presently
20 disclosed and claimed subject matter can include the use of either of the other two terms.

As used herein, "significance" or "significant" relates to a statistical analysis of the probability that there is a non-random association between two or more entities. To determine whether or not a relationship is
25 "significant" or has "significance", statistical manipulations of the data can be performed to calculate a probability, expressed as a "p value". Those p values that fall below a user-defined cutoff point are regarded as significant. In some embodiments, a p value less than or equal to 0.05, in some embodiments less than 0.01, in some embodiments less than 0.005, and in
30 some embodiments less than 0.001, are regarded as significant. Accordingly, a p value greater than or equal to 0.05 is considered not significant.

As used herein, the term "subject" refers to any organism for which application of the presently disclosed subject matter would be desirable. The subject treated in the presently disclosed subject matter in its many embodiments is desirably a human subject, although it is to be understood that the principles of the presently disclosed subject matter indicate that the presently disclosed subject matter is effective with respect to all vertebrate species, including mammals, which are intended to be included in the term "subject". Moreover, a mammal is understood to include any mammalian species in which treatment is desirable, particularly agricultural and domestic mammalian species.

More particularly provided is the treatment of mammals such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economic importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also provided is the treatment of birds, including the treatment of those kinds of birds that are endangered, kept in zoos, as well as fowl, and more particularly domesticated fowl, *i.e.*, poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economic importance to humans. Thus, contemplated is the treatment of livestock, including, but not limited to, domesticated swine (pigs and hogs), ruminants, horses, poultry, and the like.

III. Tenomodulin Isoforms and their Biological Properties

Tenomodulin (Tnmd) is a highly expressed, candidate marker gene for tenocytes. However, the function of tenomodulin in tendons has previously been unclear. The instant disclosure confirms the existence of three different Tnmd transcripts in human tissues. Moreover, the instant disclosure shows for the first time the different functional properties of each isoform. The overexpression of each Tnmd isoform showed different distributions of Tnmd isoforms, which supports the finding that each Tnmd

isoform plays distinct functions in tendons and/or other tissues.

Expression of myostatin and scleraxis (scx) was reduced in myostatin knock-out mice and addition of myostatin protein to the culture medium increased the expression of scx and Tnmd (Mendias et al, 2008). Therefore, applicants tested the notion that there is a negative feedback relationship between Tnmd and myostatin. Knockdown of Tnmd increased the expression of myostatin and scx (likely through the upregulation of myostatin). A potential regulatory pathway of Tnmd is depicted in Figure 6 based on the reports and data disclosed herein. Tnmd isoforms can serve as new biomarkers and as novel therapeutic targets in tendon injury and diseases, given the effect on cell proliferation and varying intracellular localizations.

Disclosed herein is the identification of tenomodulin isoforms in tissues of humans and other species. As disclosed herein, the full-length isoform (I1) has an extracellular C-terminal anti-angiogenic sequence with a furin cleavage site, proliferative sequence and membrane spanning domain. TNMD isoform 1 (I1) is 37.1 kDa, of which the nucleotide sequence is provided herein as SEQ ID NO: 1 and the deduced amino acid sequence as SEQ ID NO: 4. TNMD isoform 2 (I2) is 20.3 kDa, of which the nucleotide sequence is provided herein as SEQ ID NO: 2 and the deduced amino acid sequence as SEQ ID NO: 5. TNMD isoform 3 (I3) is 25.4kDa, of which the nucleotide sequence is provided herein as SEQ ID NO: 3 and the deduced amino acid sequence as SEQ ID NO: 6. Also provided herein are SEQ ID NOs: 7, 8 and 9, which are highly conserved tenomodulin nucleotide sequences from equine, porcine and humans, respectively.

Thus, disclosed herein are isolated nucleic acids encoding a tenomodulin polypeptide, such as SEQ ID NOs: 1-3, as discussed further hereinbelow. Also disclosed herein are isolated nucleic acid sequences coding for a protein comprising an amino acid sequence of one of SEQ ID NOs: 4-6. Also disclosed herein are recombinant vectors comprising a nucleic acid sequence encoding a tenomodulin peptide. Also disclosed herein are recombinant host cells or stem cells containing a nucleic acid sequence disclosed herein.

The presently disclosed subject matter also provides tenomodulin polypeptides, optionally one or more of isoforms 1, 2 or 3, or a combination thereof, optionally substantially pure polypeptides. In some embodiments a substantially pure tenomodulin polypeptide can comprise an amino acid
5 sequence of one of SEQ ID NOs: 4-6 or a sequence having a 90% or greater sequence identity to one of SEQ ID NOs: 4-6, optionally over its entire length. In some aspects, such a peptide can have a sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 4-6. In some aspects, such a peptide can be expressed in
10 a recombinant cell. In some aspects, such a peptide can be modified to be in detectably labeled form.

Also disclosed herein is information pertaining to the differential expression of Tnmd isoforms in several orthopedically relevant tissues. See Example 3. Tnmd is highly expressed and rapidly up and down-regulated in
15 strained tenocytes, suggesting that it has some early response role and perhaps regulatory function, belying the nuclear localization. Given its high expression level and rapid response to strain, Tnmd can be an early marker of tenocyte damage, which could be assessed by imaging at surgery, to gauge tissue damage.

The presently disclosed subject matter discloses that Tnmd is
20 expressed in tenocytes and that wounding or straining a tendon can decrease both mRNA expression and protein localization at the wound site. While not wishing to be bound by any particular theory of operation, it appears that Tnmd down-regulation in injured tendon promotes
25 angiogenesis and subsequent healing. This is the first report of Tnmd modulation in a bioartificial tendon with wound and strain. The presently disclosed subject matter indicates that the Scx as well as P2Y1 and P2Y2 receptors, and ATP, ADP pathways, regulate Tnmd expression but not the reverse.

30 Tenomodulin Nucleic Acids and Polypeptides

The presently disclosed subject matter provides for isolated nucleic acid sequences encoding a tenomodulin polypeptide. In some embodiments, the isolated nucleic acid sequences comprise a nucleic acid

sequence of one of SEQ ID NOs: 1-3. In some embodiments, the nucleic acid sequence consists essentially of one of SEQ ID NOs: 1-3. In some embodiments, the nucleic acid sequence consists of one of SEQ ID NOs: 1-3. In some embodiments, the isolated nucleic acid sequence is at least
5 90%, 95%, 96%, 97%, 98%, or 99% identical to all or a portion of SEQ ID NOs:1-3.

As used herein, the terms "tenomodulin" and "TNMD", include nucleic acids encoding tenomodulin polypeptides and tenomodulin polypeptides themselves. The terms "tenomodulin" and "TNMD" include invertebrate
10 homologs; preferably, "tenomodulin" and "TNMD" nucleic acids and polypeptides are isolated from eukaryotic sources. "Tenomodulin" and "TNMD" further include vertebrate homologs of tenomodulin family members, including, but not limited to, mammalian homologs. Representative mammalian homologs include, but are not limited to, murine
15 and human homologs. Isoforms are also included, such as but not limited to isoforms from particular tissue sources.

The terms "tenomodulin gene product", "TNMD gene product", "tenomodulin protein", "TNMD protein", "tenomodulin polypeptide", and "TNMD polypeptide" refer to peptides having amino acid sequences which
20 are substantially identical to native amino acid sequences from the organism of interest and which are biologically active in that they comprise all or a part of the amino acid sequence of a tenomodulin, or cross-react with antibodies raised against a tenomodulin polypeptide, or retain all or some of the biological activity of the native amino acid sequence or protein. Such
25 biological activity can include immunogenicity.

The terms "tenomodulin gene product", "TNMD gene product", "tenomodulin protein", "TNMD protein", "tenomodulin polypeptide", and "TNMD polypeptide" also include analogs of tenomodulin molecules. By "analog" is intended that a DNA or peptide sequence can contain alterations
30 relative to the sequences disclosed herein, yet retain all or some of the biological activity of those sequences. Analogs can be derived from genomic nucleotide sequences as are disclosed herein or from other organisms, or can be created synthetically. Those skilled in the art will

appreciate that other analogs, as yet undisclosed or undiscovered, can be used to design and/or construct tenomodulin analogs. There is no need for a "tenomodulin gene product", "TNMD gene product", "tenomodulin protein", "TNMD protein", "tenomodulin polypeptide", and "TNMD polypeptide" to
5 comprise all or substantially all of the amino acid sequence of a native tenomodulin gene product. Shorter or longer sequences are anticipated to be of use in accordance with the presently disclosed subject matter. Shorter sequences are herein referred to as "segments" or "fragments". Thus, some fragments of TNMD can be bioactive, such as but not limited to an anti-angiogenic C-terminal fragment as disclosed herein. Indeed the presently
10 disclosed subject matter includes any potential bioactivity of any fragment of a TNMD polypeptide.

The terms "tenomodulin gene product", "TNMD gene product", "tenomodulin protein", "TNMD protein", "tenomodulin polypeptide", and
15 "TNMD polypeptide" also include fusion or recombinant tenomodulin polypeptides and proteins comprising sequences of the presently disclosed subject matter. Methods of preparing such proteins are known in the art.

The terms "tenomodulin gene", "TNMD gene", "tenomodulin gene sequence", "TNMD gene sequence", "tenomodulin gene segment", and
20 "TNMD gene segment" refer to any DNA sequence that is substantially identical to a polynucleotide sequence encoding a tenomodulin gene product, protein or polypeptide as defined above, and can also comprise any combination of associated control sequences. The terms also refer to RNA, or antisense sequences, complementary to such DNA sequences. As used
25 herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Furthermore, a DNA segment encoding a tenomodulin polypeptide refers to a DNA segment that contains tenomodulin coding sequences, yet is isolated away from, or purified free from, total genomic DNA of a source species, such as *Homo sapiens*. Included within the term "DNA segment" are DNA segments and
30 smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phages, viruses, and the like.

The term "substantially identical", when used to define either a

tenomodulin gene product or amino acid sequence, or a tenomodulin gene or nucleic acid sequence, means that a particular sequence varies from the sequence of a natural tenomodulin by one or more deletions, substitutions, or additions, the net effect of which is to retain at least some of biological activity of the natural gene, gene product, or sequence. Such sequences include "mutant" sequences, or sequences in which the biological activity is altered to some degree but retains at least some of the original biological activity.

Alternatively, DNA analog sequences are "substantially identical" to specific DNA sequences disclosed herein if: (a) the DNA analog sequence is derived from coding regions of the natural tenomodulin gene; or (b) the DNA analog sequence is capable of hybridization of DNA sequences of (a) under stringent conditions and which encode biologically active tenomodulin gene product (including fragments); or (c) the DNA sequences are degenerate as a result of alternative genetic code to the DNA analog sequences defined in (a) and/or (b). Substantially identical analog proteins will be greater than about 60% identical to the corresponding sequence of the native protein. Sequences having lesser degrees of identity but comparable biological activity are considered to be equivalents. In determining nucleic acid sequences, all subject nucleic acid sequences capable of encoding substantially similar amino acid sequences are considered to be substantially similar to a reference nucleic acid sequence, regardless of differences in codon sequences or substitution of equivalent amino acids to create biologically functional equivalents.

Sequence identity or percent similarity of a DNA or peptide sequence can be determined, for example, by comparing sequence information using the GAP computer program, available from the University of Wisconsin Geneticist Computer Group. The GAP program utilizes the alignment method of Needleman et al. (1970), as revised by Smith et al. (1981). Briefly, the GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids) that are similar, divided by the total number of symbols in the shorter of the two sequences. Representative parameters for the GAP program are the default parameters,

which do not impose a penalty for end gaps. See Schwartz et al. (1979); Gribkov et al. (1986).

Tenomodulin gene products which have functionally equivalent codons are covered by the presently disclosed subject matter. The term
 5 "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the ACG and AGU codons for serine. Thus, when referring to the sequence examples presented in SEQ ID NOs: 1-3 applicants provide substitution of functionally equivalent codons of Table 1 into the sequence examples of SEQ ID NOs: 1-3. Thus, applicants are in
 10 possession of amino acid and nucleic acids sequences which include such substitutions but which are not set forth herein in their entirety for convenience.

TABLE 1 - Functionally Equivalent Codons

15	Amino Acids		Codons	
	Alanine	Ala A	GCA GCC GCG GCU	
	Cysteine	Cys C	UGC UGU	
	Aspartic Acid	Asp D	GAC GAU	
	Glumatic acid	Glu E	GAA GAG	
20	Phenylalanine	Phe F	UUC UUU	
	Glycine	Gly G	GGA GGC GGG GGU	
	Histidine	His H	CAC CAU	
	Isoleucine	Ile I	AUA AUC AUU	
	Lysine	Lys K	AAA AAG	
25	Leucine	Leu L	UUA UUG CUA CUC CUG CUU	
	Methionine	Met M	AUG	
	Asparagine	Asn N	AAC AAU	
	Proline	Pro P	CCA CCC CCG CCU	
	Glutamine	Gln Q	CAA CAG	
30	Arginine	Arg R	AGA AGG CGA CGC CGG CGU	
	Serine	Ser S	ACG AGU UCA UCC UCG UCU	
	Threonine	Thr T	ACA ACC ACG ACU	
	Valine	Val V	GUA GUC GUG GUU	
	Tryptophan	Trp W	UGG	
35	Tyrosine	Tyr Y	UAC UAU	

In some embodiments, the presently disclosed subject matter provides a nucleic acid that is complementary to a nucleic acid encoding a tenomodulin polypeptide. Nucleic acid sequences which are "complementary" are those, which are base-paired according to the standard Watson-Crick complementarity rules. As used herein, the term "complementary sequences" means nucleic acid sequences which are substantially complementary, as can be assessed by the same nucleotide comparison set forth above, or is defined as being capable of hybridizing to the nucleic acid segment in question under relatively stringent conditions such as those described herein. A particular example of a provided complementary nucleic acid segment is an antisense oligonucleotide.

One technique in the art for assessing complementary sequences and/or isolating complementary nucleotide sequences is hybridization. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of about 30°C, typically in excess of about 37°C, and optionally in excess of about 45°C. Stringent salt conditions will ordinarily be less than about 1,000 mM, typically less than about 500 mM, and optionally less than about 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. See e.g., Wethmur & Davidson (1968) J Mol Biol 31:349-370. Determining appropriate hybridization conditions to identify and/or isolate sequences containing high levels of homology is well known in the art. See e.g., Sambrook et al. (1989).

For the purposes of specifying conditions of high stringency, representative conditions are salt concentration of about 200 mM and temperature of about 45°C. One example of such stringent conditions is hybridization at 4XSSC, at 65°C, followed by a washing in 0.1XSSC at 65°C for one hour. Another exemplary stringent hybridization scheme uses 50% formamide, 4XSSC at 42°C. As used herein, "stringent conditions" means

conditions of high stringency, for example 6XSSC, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, 0.1% sodium dodecyl sulfate, 100 µg/ml salmon sperm DNA and 15% formamide at 68°C. Nucleic acids having sequence similarity are detected by hybridization under
5 low stringency conditions, for example, at 50°C and 10XSSC (0.9 M NaCl/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC. Sequence identity can be determined by hybridization under stringent conditions, for example, at 50°C or higher and 0.1XSSC (9 mM NaCl/0.9 mM sodium citrate).

10 As used herein, the term "RNA" refers to a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety. The terms encompass double stranded RNA, single stranded RNA, RNAs with both double stranded and single stranded regions, isolated RNA such as
15 partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA, or analog RNA, that differs from naturally occurring RNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material. Nucleotides in the RNA molecules of the
20 presently disclosed subject matter can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of a naturally occurring RNA.

RNA interference (RNAi) is a natural process by which living cells can
25 control which genes are expressed or suppressed. As used herein, the term "RNAi" refers to a mechanism that silences specific genes by inhibiting an RNA molecule and stopping or at least substantially reducing the expression of the protein encoded by this RNA molecule. If the target protein has a function in the cell, RNAi approaches can result in loss of that function. As
30 such, RNAi technology is an attractive therapeutic tool to modulate the expression of genes in a way to suppress disease. RNAi can be mediated by several natural and synthetic constructs, including double stranded RNA (dsRNA), or smaller dsRNA known as small interfering RNAs (siRNA), short

hairpin RNA (shRNA), microRNA (miRNA), or synthetic hammerhead ribozymes. These can be referred to as examples of RNAi molecules.

The presently disclosed subject matter further encompasses tenomodulin polynucleotides contained in a vector molecule or an expression vector and operably linked to a promoter element if necessary.

A "vector" refers to a recombinant DNA or RNA plasmid or virus that comprises a heterologous polynucleotide to be delivered to a target cell, either *in vitro* or *in vivo*. The heterologous polynucleotide can comprise a sequence of interest for purposes of therapy or biomedical or genetic research, and can optionally be in the form of an expression cassette. As used herein, a vector need not be capable of replication in the ultimate target cell or subject. The term includes cloning vectors for translation of a polynucleotide encoding sequence. Also included are viral vectors.

The term "recombinant" means a polynucleotide of genomic cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

"Heterologous" means derived from a genetically distinct entity from the rest of the entity to which it is being compared. For example, a polynucleotide, can be placed by genetic engineering techniques into a plasmid or vector derived from a different source, and is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence other than the native sequence is a heterologous promoter.

The polynucleotides of the presently disclosed subject matter can comprise additional sequences, such as additional encoding sequences within the same transcription unit, controlling elements such as promoters, ribosome binding sites, polyadenylation sites, additional transcription units under control of the same or a different promoter, sequences that permit cloning, expression, homologous recombination, and transformation of a host cell, and any such construct desirable to provide embodiments of the presently disclosed subject matter.

A "host cell" denotes a prokaryotic or eukaryotic cell that has been genetically altered, or is capable of being genetically altered by

administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both to the originally altered cell and to the progeny thereof.

Polynucleotides comprising a desired sequence can be inserted into a
5 suitable cloning or expression vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides can be introduced into host cells by any means known in the art. The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including direct
10 uptake, endocytosis, transfection, f-mating, electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is infectious, for instance, a retroviral vector). The choice of introducing vectors or polynucleotides will often depend on
15 features of the host cell.

Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. Amplified DNA can be isolated from the host cell by standard methods. See, e.g., Sambrook et al., eds. (1989), incorporated
20 herein in its entirety. RNA can also be obtained from transformed host cell, or it can be obtained directly from the DNA by using a DNA-dependent RNA polymerase.

The preparation of recombinant vectors is well known to those of skill in the art and described in many references, such as, for example,
25 Sambrook et al, (1989), incorporated herein in its entirety. It is understood that the DNA coding sequences to be expressed, in this case those encoding the tenomodulin gene products, are positioned in a vector adjacent to and under the control of a promoter. It is understood in the art that to bring a coding sequence under the control of such a promoter, one generally
30 positions the 5' end of the transcription initiation site of the transcriptional reading frame of the gene product to be expressed between about 1 and about 50 nucleotides "downstream" of (*i.e.*, 3' of) the chosen promoter.

One can also desire to incorporate into the transcriptional unit of the vector an appropriate polyadenylation site (e.g., 5'-AATAAA-3'), if one was not contained within the original inserted DNA. Typically, these poly-A addition sites are placed about 30 to 2000 nucleotides "downstream" of the coding sequence at a position prior to transcription termination.

While use of the control sequences of the specific gene will be preferred, other control sequences can be employed, so long as they are compatible with the genotype of the cell being treated. Thus, one can mention other useful promoters by way of example, including, e.g., an SV40 early promoter, a long terminal repeat promoter from retrovirus, an actin promoter, a heat shock promoter, a metallothionein promoter, and the like.

As is known in the art, a promoter is a region of a DNA molecule typically within about 100 nucleotide pairs upstream of (*i.e.*, 5' to) the point at which transcription begins (*i.e.*, a transcription start site). That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes.

Another type of discrete transcription regulatory sequence element is an enhancer. An enhancer imposes specificity of time, location and expression level on a particular coding region or gene. A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. An enhancer can function when located at variable distances from transcription start sites so long as a promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" means that an enhancer-promoter is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Approaches for operatively linking an enhancer-promoter to a coding sequence are well known in the art; the precise orientation and location relative to a coding sequence of interest is dependent, *inter alia*, upon the specific nature of the enhancer-promoter.

An enhancer-promoter used in a vector construct of the presently disclosed subject matter can be any enhancer-promoter that drives expression in a cell to be transfected. By employing an enhancer-promoter with well-known properties, the level and pattern of gene product expression
5 can be optimized.

For introduction of a vector construct that will deliver the gene to the affected cells is desired. Viral vectors can be used. These vectors can be an adenoviral, a retroviral, a vaccinia viral vector, adeno-associated virus or Lentivirus; these vectors have been successfully used to deliver desired
10 sequences to cells and tend to have a high infection efficiency. Suitable vector-target gene constructs are adapted for administration as pharmaceutical compositions, as described herein below. Viral promoters can also be of use in vectors of the presently disclosed subject matter, and are known in the art.

Commonly used viral promoters for expression vectors are derived from polyoma, cytomegalovirus, Adenovirus 2, and Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment that also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments can also
20 be used, provided there is included the approximately 250 base pair sequence extending from the Hind III site toward the Bgl I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the
25 host cell systems.

The origin of replication can be provided either by construction of the vector to include an exogenous origin, such as can be derived from SV40 or other viral source, or can be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell
30 chromosome, the latter is often sufficient.

In an alternative embodiment, the presently disclosed subject matter provides an expression vector comprising a polynucleotide that encodes a biologically active tenomodulin polypeptide in accordance with the presently

- disclosed subject matter. Optionally, an expression vector of the presently disclosed subject matter comprises a polynucleotide that encodes a polypeptide comprising SEQ ID NOs: 4-6. Optionally, an expression vector of the presently disclosed subject matter comprises a polynucleotide
- 5 comprising the nucleotide sequence comprising SEQ ID NOs: 1-3. Optionally, an expression vector of the presently disclosed subject matter comprises a polynucleotide operatively linked to an enhancer-promoter. Optionally, an expression vector of the presently disclosed subject matter comprises a polynucleotide operatively linked to a prokaryotic promoter.
- 10 Alternatively, an expression vector of the presently disclosed subject matter comprises a polynucleotide operatively linked to an enhancer-promoter that is a eukaryotic promoter and the expression vector further comprises a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid and within a transcriptional unit of the encoded polypeptide.
- 15 In yet another embodiment, the presently disclosed subject matter provides a recombinant host cell transfected with a polynucleotide that encodes a biologically active tenomodulin polypeptide in accordance with the presently disclosed subject matter. Optionally, a recombinant host cell of the presently disclosed subject matter is transfected with the polynucleotide
- 20 that encodes human tenomodulin polypeptide. Optionally, a recombinant host cell of the presently disclosed subject matter is transfected with the polynucleotide sequence encoding or set forth in any of SEQ ID NOs: 1-3. Optionally, a recombinant host cell is a mammalian cell. Optionally, the host cell is a stem cell.
- 25 In another aspect, a recombinant host cell of the presently disclosed subject matter is a prokaryotic host cell, including parasitic and bacterial cells. Optionally, a recombinant host cell of the presently disclosed subject matter is a bacterial cell, such as but not limited to a strain of *Escherichia coli*. By way of example, a recombinant host cell can comprise a
- 30 polynucleotide under the transcriptional control of regulatory signals functional in the recombinant host cell, wherein the regulatory signals appropriately control expression of the tenomodulin polypeptide in a manner to enable all necessary transcriptional and post-transcriptional modification.

The presently disclosed subject matter provides isolated polypeptides. The term "isolated" as used herein with reference to a polypeptide means the polypeptide is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, an isolated polypeptide is any polypeptide that is removed from its natural environment and is at least 60 percent pure. An isolated polypeptide can be at least about 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent pure. Typically, an isolated polypeptide will yield a single major band on a non-reducing polyacrylamide gel.

Any suitable method can be used to obtain a substantially pure polypeptide. For example, common polypeptide purification techniques such as affinity chromatography and HPLC as well as polypeptide synthesis techniques can be used. In addition, any material can be used as a source to obtain a substantially pure polypeptide. For example, tissue from wild-type or transgenic animals can be used as a source material. In addition, tissue culture cells engineered to over-express a particular polypeptide of interest can be used to obtain substantially pure polypeptide. Further, a polypeptide within the scope of the presently disclosed subject matter can be engineered to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or FLAG™ tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that could be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

The presently disclosed subject matter provides tenomodulin polypeptides. In some embodiments, the tenomodulin polypeptides comprise one of SEQ ID NOs: 4-6. In some embodiments the tenomodulin polypeptides comprise an amino acid sequence that has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a given sequence, such as one of SEQ ID NOs: 4-6.

Biologically Functional Equivalents

As mentioned above, modifications and changes can be made in the structure of the tenomodulin proteins and peptides described herein and still constitute a molecule having like or otherwise desirable characteristics. For example, certain amino acids can be substituted for other amino acids in a protein structure without appreciable loss of interactive capacity with, for example, structures in the nucleus of a cell. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence (or the nucleic acid sequence encoding it) to obtain a protein with the same, enhanced, or antagonistic properties. Such properties can be achieved by interaction with the normal targets of the native protein, but this need not be the case, and the biological activity is not limited to a particular mechanism of action. It is thus provided in accordance with the presently disclosed subject matter that various changes can be made in the sequence of the tenomodulin proteins and peptides or underlying nucleic acid sequence without appreciable loss of their biological utility or activity.

Biologically functional equivalent peptides, as used herein, are peptides in which certain, but not most or all, of the amino acids can be substituted. Thus, when referring to the nucleic acid sequence examples presented in SEQ ID NOs: 1-3 applicants provide substitution of codons that encode biologically equivalent amino acids as described herein into the sequence examples of SEQ ID NOs: 4-6. Thus, applicants are in possession of amino acid and nucleic acids sequences which include such substitutions but which are not set forth herein in their entirety for convenience.

Alternatively, functionally equivalent proteins or peptides can be created via the application of recombinant DNA technology, in which changes in the protein structure can be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man can be introduced through the application of site-directed mutagenesis techniques, *e.g.*, to introduce improvements to the antigenicity of the protein or to test mutants in order to examine a desired activity at the

molecular level.

Amino acid substitutions, such as those which might be employed in modifying the tenomodulin proteins and peptides described herein, are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. An analysis of the size, shape and type of the amino acid side-chain substituents reveals that arginine, lysine and histidine are all positively charged residues; that alanine, glycine and serine are all of similar size; and that phenylalanine, tryptophan and tyrosine all have a generally similar shape. Therefore, based upon these considerations, arginine, lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine; are defined herein as biologically functional equivalents. Other biologically functionally equivalent changes will be appreciated by those of skill in the art.

In making biologically functional equivalent amino acid substitutions, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+ 4.5); valine (+ 4.2); leucine (+ 3.8); phenylalanine (+ 2.8); cysteine (+ 2.5); methionine (+ 1.9); alanine (+ 1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte et al. (1982), herein incorporated herein by reference). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 of the original value is preferred, those, which are within ± 1 of the original value, are particularly preferred, and those within ± 0.5 of the original value are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+ 3.0); lysine (+ 3.0); aspartate (+ 3.0 $\pm$ 1); glutamate (+ 3.0 $\pm$ 1); serine (+ 0.3); asparagine (+ 0.2); glutamine (+ 0.2); glycine (0); threonine (-0.4); proline (-0.5 $\pm$ 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within $\pm$ 2 of the original value is preferred, those, which are within $\pm$ 1 of the original value, are particularly preferred, and those within $\pm$ 0.5 of the original value are even more particularly preferred.

While discussion has focused on functionally equivalent polypeptides arising from amino acid changes, it will be appreciated that these changes can be effected by alteration of the encoding DNA, taking into consideration also that the genetic code is degenerate and that two or more codons can code for the same amino acid.

Thus, it will also be understood that the presently disclosed subject matter is not limited to the particular nucleic acid and amino acid sequences of SEQ ID NOs: 1-6. Recombinant vectors and isolated DNA segments can therefore variously include the tenomodulin polypeptide-encoding region itself, include coding regions bearing selected alterations or modifications in the basic coding region, or include larger polypeptides which nevertheless comprise tenomodulin polypeptide-encoding regions or can encode biologically functional equivalent proteins or peptides which have variant

amino acid sequences.

The presently disclosed subject matter further encompasses fusion proteins and peptides wherein the tenomodulin coding region is aligned within the same expression unit with other proteins or peptides having
5 desired functions, such as for purification or immunodetection purposes.

Further, in addition to the peptidyl compounds described herein, it is also provided that other sterically similar compounds can be formulated to mimic the key portions of the peptide structure. Such compounds can be used in the same manner as the peptides of the presently disclosed subject
10 matter and hence are also functional equivalents. The generation of a structural functional equivalent can be achieved by the techniques of modeling and chemical design known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the presently disclosed subject matter.

15 Transgenic Animals

It is also provided within the scope of the presently disclosed subject matter to prepare transgenic non-human animals that express a human tenomodulin polypeptide or that have modified tenomodulin polypeptide expression. A representative transgenic animal is a mouse.

20 Techniques for the preparation of transgenic animals are known in the art. Exemplary techniques are described in U.S. Patent No. 5,489,742 (transgenic rats); U.S. Patent Nos. 4,736,866, 5,550,316, 5,614,396, 5,625,125 and 5,648,061 (transgenic mice); U.S. Patent No. 5,573,933 (transgenic pigs); 5,162,215 (transgenic avian species) and U.S. Patent No.
25 5,741,957 (transgenic bovine species), the entire contents of each of which are herein incorporated by reference.

With respect to a representative method for the preparation of a transgenic mouse, cloned recombinant or synthetic DNA sequences or DNA segments encoding a tenomodulin gene product are injected into fertilized
30 mouse eggs. The injected eggs are implanted in pseudo pregnant females and are grown to term to provide transgenic animals whose cells overexpress a tenomodulin gene product.

The presently disclosed subject matter further relates to transgenic animals with a specific "knock-out" modification. For example, the transgenic animal can be provided that under expresses a tenomodulin polypeptide.

5 In a knockout, it can be desirable for the target gene expression to be undetectable or insignificant. For example, a knockout of a target gene means that function of the gene has been substantially decreased so that expression is not detectable or only present at insignificant levels. This can be achieved by a variety of mechanisms, including introduction of a
10 disruption of the coding sequence, e.g. insertion of one or more stop codons, insertion of a DNA fragment, etc., deletion of coding sequence, substitution of stop codons for coding sequence, etc.

Different approaches can also be used to achieve the "knockout". A chromosomal deletion of all or part of the native gene can be induced,
15 including deletions of the non-coding regions, particularly the promoter region, 3' regulatory sequences, enhancers, or deletions of gene that activate expression of target genes. A functional knock-out can also be achieved by the introduction of an anti-sense construct that blocks expression of the native genes (for example, see Li and Cohen, (1996)).
20 "Knockouts" also include conditional knock-outs, for example where alteration of the target gene occurs upon exposure of the animal to a substance that promotes target gene alteration, introduction of an enzyme that promotes recombination at the target gene site (e.g. Cre in the Cre-lox system), or other method for directing the target gene alteration postnatally.

25 Generation of Antibodies

In still another embodiment, the presently disclosed subject matter provides an antibody immunoreactive with a polypeptide of the presently disclosed subject matter. Optionally, an antibody of the presently disclosed subject matter is a monoclonal antibody. Techniques for preparing and
30 characterizing antibodies are well known in the art (see e.g., Harlow & Lane (1988)).

In some embodiments, provided herein is an isolated and purified antibody capable of specifically binding to a tenomodulin polypeptide. In

some embodiments, an antibody can be capable of modulating the biological activity of a tenomodulin polypeptide.

Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogen comprising a polypeptide or polynucleotide of the presently disclosed subject matter, and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Because of the relatively large blood volume of rabbits, a rabbit is a representative choice for production of polyclonal antibodies.

As is well known in the art, a given polypeptide or polynucleotide can vary in its immunogenicity. It is often necessary therefore to couple the immunogen (e.g., a polypeptide or polynucleotide) of the presently disclosed subject matter) with a carrier. Exemplary carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers.

Approaches for conjugating a polypeptide or a polynucleotide to a carrier protein are well known in the art and include glutaraldehyde, N-maleimidobenzoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

As is also well known in the art, immunogenicity to a particular immunogen can be enhanced by the use of non-specific stimulators of the immune response known as adjuvants. Exemplary adjuvants include complete Freund's adjuvant, incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen used of the production of polyclonal antibodies varies, inter alia, upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen, e.g., subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal. The production of polyclonal antibodies is monitored by sampling blood of the immunized animal at various points following immunization. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored.

In another aspect, the presently disclosed subject matter provides a process of producing an antibody immunoreactive with a tenomodulin polypeptide, the process comprising the steps of (a) transfecting recombinant host cells with a polynucleotide that encodes that polypeptide; 5 (b) culturing the host cells under conditions sufficient for expression of the polypeptide; (c) recovering the polypeptide; and (d) preparing antibodies to the polypeptide.

A monoclonal antibody of the presently disclosed subject matter can be readily prepared through use of well-known techniques such as the 10 hybridoma techniques exemplified in U.S. Patent No 4,196,265 and the phage-displayed techniques disclosed in U.S. Patent No. 5,260,203, the contents of which are herein incorporated by reference.

A typical technique involves first immunizing a suitable animal with a selected antigen (e.g., a polypeptide or polynucleotide of the presently 15 disclosed subject matter) in a manner sufficient to provide an immune response. Rodents such as mice and rats are representative animals. Spleen cells from the immunized animal are then fused with cells of an immortal myeloma cell. Where the immunized animal is a mouse, a representative myeloma cell is a murine NS-1 myeloma cell.

20 The fused spleen/myeloma cells are cultured in a selective medium to select fused spleen/myeloma cells from the parental cells. Fused cells are separated from the mixture of non-fused parental cells, for example, by the addition of agents that block the de novo synthesis of nucleotides in the tissue culture media. This culturing provides a population of hybridomas 25 from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants for reactivity with antigen-polypeptides. The selected clones can then be propagated indefinitely to provide the monoclonal antibody.

30 By way of specific example, to produce an antibody of the presently disclosed subject matter, mice are injected intraperitoneally with between about 1-200 μ g of an antigen comprising a polypeptide of the presently disclosed subject matter. B lymphocyte cells are stimulated to grow by

injecting the antigen in association with an adjuvant such as complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*). At some time (e.g., at least two weeks) after the first injection, mice are boosted by injection with a
5 second dose of the antigen mixed with incomplete Freund's adjuvant.

A few weeks after the second injection, mice are tail bled and the sera titered by immunoprecipitation against radiolabeled antigen. The process of boosting and titering can be repeated until a suitable titer is achieved. The spleen of the mouse with the highest titer is removed and the spleen
10 lymphocytes are obtained by homogenizing the spleen with a syringe.

Mutant lymphocyte cells known as myeloma cells are obtained from laboratory animals in which such cells have been induced to grow by a variety of well-known methods. Myeloma cells lack the salvage pathway of nucleotide biosynthesis. Because myeloma cells are tumor cells, they can
15 be propagated indefinitely in tissue culture, and are thus "immortal". Numerous cultured cell lines of myeloma cells from mice and rats, such as murine NS-1 myeloma cells, have been established.

Myeloma cells are combined under conditions appropriate to foster fusion with the normal antibody-producing cells from the spleen of the
20 mouse or rat injected with the antigen/polypeptide of the presently disclosed subject matter. Fusion conditions include, for example, the presence of polyethylene glycol. The resulting fused cells are hybridoma cells. Like myeloma cells, hybridoma cells grow indefinitely in culture.

Hybridoma cells are separated from unfused myeloma cells by
25 culturing in a selection medium such as HAT media (hypoxanthine, aminopterin, and thymidine). Unfused myeloma cells lack the enzymes necessary to synthesize nucleotides from the salvage pathway because they are killed in the presence of aminopterin, methotrexate, or azaserine. Unfused lymphocytes also do not continue to grow in tissue culture. Thus,
30 only cells that have successfully fused (hybridoma cells) can grow in the selection media.

Each of the surviving hybridoma cells produces a single antibody. These cells are then screened for the production of the specific antibody

immunoreactive with an antigen/polypeptide of the presently disclosed subject matter. Single cell hybridomas are isolated by limiting dilutions of the hybridomas. The hybridomas are serially diluted many times and, after the dilutions are allowed to grow, the supernatant is tested for the presence of the monoclonal antibody. The clones producing that antibody are then cultured in large amounts to produce an antibody of the presently disclosed subject matter in convenient quantity.

By use of a monoclonal antibody of the presently disclosed subject matter, specific polypeptides and polynucleotide of the presently disclosed subject matter can be recognized as antigens, and thus identified. Once identified, those polypeptides and polynucleotide can be isolated and purified by techniques such as antibody-affinity chromatography. In antibody-affinity chromatography, a monoclonal antibody is bound to a solid substrate and exposed to a solution containing the desired antigen. The antigen is removed from the solution through an immunospecific reaction with the bound antibody. The polypeptide or polynucleotide is then easily removed from the substrate and purified.

Detecting a Polynucleotide or a Polypeptide

Alternatively, the presently disclosed subject matter provides a process of detecting a polypeptide of the presently disclosed subject matter, wherein the process comprises immunoreacting the polypeptides with antibodies prepared according to the process described above to form antibody-polypeptide conjugates, and detecting the conjugates.

In yet another embodiment, the presently disclosed subject matter provides a process of detecting messenger RNA transcripts that encode a polypeptide of the presently disclosed subject matter, wherein the process comprises hybridizing the messenger RNA transcripts with polynucleotide sequences that encode the polypeptide to form duplexes; and detecting the duplex. Alternatively, the presently disclosed subject matter provides a process of detecting DNA molecules that encode a polypeptide of the presently disclosed subject matter, wherein the process comprises hybridizing DNA molecules with a polynucleotide that encodes that polypeptide to form duplexes; and detecting the duplexes.

The detection and screening assays disclosed herein can optionally be used as a prognosis tool and/or diagnostic aid. Tenomodulin polypeptides and tenomodulin polypeptide encoding nucleic acids can be used in clinical setting as a prognostic and/or diagnostic indicator for screening for levels of expression of tenomodulin polypeptides, or alterations in native sequences.

As discussed further below in the Examples, Tnmd is highly expressed and rapidly up- and down-regulated in strained tenocytes, suggesting that it has some early response role and putative regulatory function. Given its high expression level and rapid response to strain, Tnmd can be an early marker of tenocyte damage, which for example could be assessed by imaging at surgery, to gauge tissue damage in a subject. Thus, in some embodiments, screening for tenomodulin levels in a tissue of a subject believed to have suffered an injury can be used to diagnose tenocyte damage and/or strain upon a tendon. In some embodiments, a level of tenomodulin in a subject can be a level that is altered or changed as compared to tenomodulin in an un-injured tissue in the same subject, or as compared to a level of tenomodulin from a different subject that has not suffered an injury. In some embodiments, a normal or non-altered or changed level of tenomodulin in a tissue of a subject can indicate that the subject and/or tissue has not suffered an injury. In some embodiments, the altered or changed level can be a decreased level. In some embodiments, the altered or changed level can be an increased level.

In some embodiments, tenomodulin can be used as a biomarker to evaluate the healing and recovery process in a subject recovering from an injury and yield a quantitative marker that can be used to track convalescence and be concordant with gain in mobility, strength and return to work.

In some embodiments, the presently disclosed subject matter can provide a method of diagnosing a tendon injury, wherein the method can comprise providing a subject, collecting a biological sample from the subject, and detecting a tenomodulin in the sample. In some embodiments, the subject can be a human subject. In some embodiments, the biological

sample is selected from the group consisting of tendon, connective tissue and ligament. Detection of the expression of a tenomodulin gene or the concentration of a tenomodulin peptide can be performed as disclosed herein. In such a method, an altered or changed expression of a
5 tenomodulin gene, as compared to a healthy subject, is indicative of a tendon injury. In some embodiments, an altered or changed concentration of a tenomodulin polypeptide, as compared to a healthy subject, is indicative of a tendon injury. In some embodiments, the altered or changed expression or concentration can be a reduced expression or concentration.
10 In some embodiments, the altered or changed expression or concentration can be an increased expression or concentration.

The presently disclosed subject matter provides a process of screening a biological sample for the presence of a tenomodulin polypeptide. A biological sample to be screened can be a biological fluid such as
15 extracellular or intracellular fluid, or a cell or tissue extract or homogenate. A biological sample can also be an isolated cell (e.g., in culture) or a collection of cells such as in a tissue sample or histology sample. A tissue sample can be suspended in a liquid medium or fixed onto a solid support such as a microscope slide. In accordance with a screening assay process, a
20 biological sample is exposed to an antibody immunoreactive with the polypeptide whose presence is being assayed. Typically, exposure is accomplished by forming an admixture in a liquid medium that contains both the antibody and the candidate polypeptide. Either the antibody or the sample with the polypeptide can be affixed to a solid support (e.g., a column
25 or a microtiter plate). Additional details of methods for such assays are known in the art. The presence of polypeptide in the sample is detected by evaluating the formation and presence of antibody-polypeptide conjugates. Techniques for detecting such antibody-antigen conjugates or complexes are well known in the art and include but are not limited to centrifugation,
30 affinity chromatography and the like, and binding of a secondary antibody to the antibody-candidate receptor complex, including but not limited an ELISA-based approach (see e.g., Harlow & Lane).

In one embodiment, detection is accomplished by detecting an indicator affixed to the antibody. Exemplary and well-known indicators include radioactive labels (e.g., ^{32}P , ^{125}I , ^{14}C), a second antibody or an enzyme such as horseradish peroxidase. Techniques for affixing indicators to antibodies are known in the art.

In another aspect, the presently disclosed subject matter provides a process of screening a biological sample for the presence of antibodies immunoreactive with a tenomodulin polypeptide. By way of example and not limitation, an assay kit can be provided for detecting the presence, in a biological sample, of an antibody immunoreactive with a tenomodulin polypeptide, the kit comprising a tenomodulin polypeptide that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay, optionally comprising a label for the polypeptide, optionally wherein the label can be detected by an imaging technique, such as but not limited to MRI or CT.

In another aspect, the presently disclosed subject matter provides a process of screening a biological sample for the presence of a tenomodulin polypeptide that reacts with an antibody that is immunoreactive for tenomodulin. By way of example and not limitation, an assay kit can be provided for detecting the presence, in a biological sample, of a tenomodulin polypeptide, the kit comprising an antibody immunoreactive against a tenomodulin polypeptide, with the antibody present in an amount sufficient to perform at least one assay, optionally comprising a label for the antibody, optionally wherein the label can be detected by an imaging technique, such as but not limited to MRI or CT.

A DNA or RNA molecule and particularly a DNA segment or polynucleotide can be used for hybridization to a DNA or RNA source or sample suspected of encoding a tenomodulin polypeptide; such molecules are referred to as "probes," and such hybridization is "probing". Such probes can be made synthetically. The probing is usually accomplished by hybridizing the oligonucleotide to a DNA source suspected of possessing a tenomodulin gene product. In some cases, the probes constitute only a single probe, and in others, the probes constitute a collection of probes

based on a certain amino acid sequence or sequences of the polypeptide and account in their diversity for the redundancy inherent in the genetic code.

Other molecules which are neither DNA nor RNA but are capable of hybridizing in a similar manner and which are designed structurally to mimic the DNA or RNA sequence of a tenomodulin gene product are also provided. Here, a suitable source to examine is capable of expressing a polypeptide of the presently disclosed subject matter and can be a genomic library of a cell line of interest. Alternatively, a source of DNA or RNA can include total DNA or RNA from the cell line of interest. Once the hybridization process has identified a candidate DNA segment, a positive clone can be confirmed by further hybridization, restriction enzyme mapping, sequencing and/or expression and testing.

Alternatively, such DNA molecules can be used in a number of techniques including their use as: (1) diagnostic tools to detect sequences in DNA derived from patient's cells; (2) reagents for detecting and isolating other members of the polypeptide family and related polypeptides from a DNA library potentially containing such sequences; and (3) primers for hybridizing to related sequences for the purpose of amplifying those sequences.

As set forth above, in certain aspects, DNA sequence information provided by the presently disclosed subject matter allows for the preparation of probes that specifically hybridize to encoding sequences of a selected tenomodulin gene product. In these aspects, probes of an appropriate length are prepared based on a consideration of the encoding sequence for a polypeptide of the presently disclosed subject matter. The ability of such probes to specifically hybridize to other encoding sequences lends them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

“Primers” of the presently disclosed subject matter are designed to be “substantially” complementary to each strand of the genomic locus to be amplified. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions that allow the agent
5 for polymerization to perform. In other words, the primers should have sufficient complementarity with the 5' and 3' sequences flanking the transition to hybridize therewith and permit amplification of the genomic locus.

Oligonucleotide primers of the presently disclosed subject matter are
10 employed in the amplification method that is an enzymatic chain reaction that produces exponential quantities of polymorphic locus relative to the number of reaction steps involved. Typically, one primer is complementary to the negative (-) strand of the polymorphic locus and the other is complementary to the positive (+) strand. Annealing the primers to
15 denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA polymerase I (Klenow) and nucleotides, results in newly synthesized + and - strands containing the target polymorphic locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension
20 results in exponential production of the region (*i.e.*, the target polymorphic locus sequence) defined by the primers. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

The oligonucleotide primers of the presently disclosed subject matter
25 can be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and can be synthesized as described by Beaucage et al. (1981). One method for synthesizing oligonucleotides on a
30 modified solid support is described in U.S. Patent No. 4,458,066.

Any nucleic acid specimen, in purified or non-purified form, can be utilized as the starting nucleic acid or acids, providing it contains, or is suspected of containing, a nucleic acid sequence containing the polymorphic

locus. Thus, the method can amplify, for example, DNA or RNA, including messenger RNA, wherein DNA or RNA can be single stranded or double stranded. In the event that RNA is to be used as a template, enzymes, and/or conditions optimal for reverse transcribing the template to DNA would be utilized. In addition, a DNA-RNA hybrid that contains one strand of each can be utilized. A mixture of nucleic acids can also be employed, or the nucleic acids produced in a previous amplification reaction herein, using the same or different primers can be so utilized. The specific nucleic acid sequence to be amplified, *i.e.*, the polymorphic locus, can be a fraction of a larger molecule or can be present initially as a discrete molecule, so that the specific sequence constitutes the entire nucleic acid. It is not necessary that the sequence to be amplified is present initially in a pure form; it can be a minor fraction of a complex mixture, such as contained in whole human DNA.

DNA utilized herein can be extracted from a body sample, such as blood, tissue material (*e.g.*, ligament or connective tissue), and the like by a variety of techniques such as that described by Maniatis *et. al.* (1982). If the extracted sample is impure, it can be treated before amplification with an amount of a reagent effective to open the cells, or animal cell membranes of the sample, and to expose and/or separate the strand(s) of the nucleic acid(s). This lysing and nucleic acid denaturing step to expose and separate the strands will allow amplification to occur much more readily.

The deoxyribonucleotide triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90-100°C from about 1 to 10 minutes, preferably from 1 to 4 minutes. After this heating period, the solution is allowed to cool, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art. The agent for polymerization can also be added together with the other reagents if it is heat stable. This synthesis (or amplification) reaction can occur at room temperature up to a temperature above which

the agent for polymerization no longer functions. Thus, for example, if DNA polymerase is used as the agent, the temperature is generally no greater than about 40°C. Most conveniently the reaction occurs at room temperature.

- 5 The agent for polymerization can be any compound or system that will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, E. coli DNA polymerase I, Klenow fragment of E. coli DNA polymerase, polymerase muteins, reverse transcriptase, other enzymes, including
- 10 heat-stable enzymes (*i.e.*, those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation), such as Taq polymerase. Suitable enzyme will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each polymorphic locus
- 15 nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths.

- The newly synthesized strand and its complementary nucleic acid strand will form a double-stranded molecule under hybridizing conditions
- 20 described herein and this hybrid is used in subsequent steps of the method. In the next step, the newly synthesized double-stranded molecule is subjected to denaturing conditions using any of the procedures described above to provide single-stranded molecules.

- The steps of denaturing, annealing, and extension product synthesis
- 25 can be repeated as often as needed to amplify the target polymorphic locus nucleic acid sequence to the extent necessary for detection. The amount of the specific nucleic acid sequence produced will accumulate in an exponential fashion. See McPherson et al., (1991).

- The amplification products can be detected by Southern blot analysis
- 30 with or without using radioactive probes. In one such method, for example, a small sample of DNA containing a very low level of the nucleic acid sequence of the polymorphic locus is amplified, and analyzed via a Southern blotting technique or similarly, using dot blot analysis. The use of

non-radioactive probes or labels is facilitated by the high level of the amplified signal. Alternatively, probes used to detect the amplified products can be directly or indirectly detectably labeled, for example, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the probe, or will be able to ascertain such, using routine experimentation.

Sequences amplified by the methods of the presently disclosed subject matter can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as dideoxy sequencing, PCR, oligomer restriction (Saiki et al. (1985)), and the like. Molecular techniques for DNA analysis have been reviewed (Landgren et al. (1988)).

Preferably, the method of amplifying is by PCR, as described herein and in U.S. Patent Nos. 4,683,195; 4,683,202; and 4,965,188 each of which is hereby incorporated by reference; and as is commonly used by those of ordinary skill in the art. Alternative methods of amplification have been described and can also be employed as long as a tenomodulin locus amplified by PCR using primers of the presently disclosed subject matter is similarly amplified by the alternative approach. Such alternative amplification systems include but are not limited to self-sustained sequence replication, which begins with a short sequence of RNA of interest and a T7 promoter. Reverse transcriptase transcribes the RNA into cDNA and degrades the RNA, followed by reverse transcriptase polymerizing a second strand of DNA.

Another nucleic acid amplification technique is nucleic acid sequence-based amplification (NASBATM) which uses reverse transcription and T7 RNA polymerase and incorporates two primers to target its cycling scheme. NASBATM amplification can begin with either DNA or RNA and finish with either, and amplifies to about 108 copies within 60 to 90 minutes.

Alternatively, nucleic acid can be amplified by ligation-activated transcription (LAT). LAT works from a single-stranded template with a single

primer that is partially single-stranded and partially double-stranded. Amplification is initiated by ligating a cDNA to the promoter oligonucleotide and within a few hours, amplification is about 10⁸ to about 10⁹ fold. The QB replicase system can be utilized by attaching an RNA sequence called
5 MDV-1 to RNA complementary to a DNA sequence of interest. Upon mixing with a sample, the hybrid RNA finds its complement among the specimen's mRNAs and binds, activating the replicase to copy the tag-along sequence of interest.

Another nucleic acid amplification technique, ligase chain reaction
10 (LCR), works by using two differently labeled halves of a sequence of interest that are covalently bonded by ligase in the presence of the contiguous sequence in a sample, forming a new target. The repair chain reaction (RCR) nucleic acid amplification technique uses two complementary and target-specific oligonucleotide probe pairs, thermostable polymerase
15 and ligase, and DNA nucleotides to geometrically amplify targeted sequences. A 2-base gap separates the oligo probe pairs, and the RCR fills and joins the gap, mimicking normal DNA repair.

Nucleic acid amplification by strand displacement activation (SDA) utilizes a short primer containing a recognition site for Hinc II with short
20 overhang on the 5' end which binds to target DNA. A DNA polymerase fills in the part of the primer opposite the overhang with sulfur-containing adenine analogs. Hinc II is added but only cuts the unmodified DNA strand. A DNA polymerase that lacks 5' exonuclease activity enters at the site of the nick and begins to polymerize, displacing the initial primer strand
25 downstream and building a new one which serves as more primer.

SDA produces greater than about a 10⁷-fold amplification in 2 hours at 37°C. Unlike PCR and LCR, SDA does not require instrumented temperature cycling. Another amplification system useful in the method of the presently disclosed subject matter is the QB Replicase System.
30 Although PCR is the preferred method of amplification if the presently disclosed subject matter, these other methods can also be used. Thus, the term "amplification technique" as used herein and in the claims is meant to encompass all the foregoing methods.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will
5 be readily appreciated by those of ordinary skill in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1,000mM, typically less than 500mM, and preferably less than 200mM. However, the combination of
10 parameters is much more important than the measure of any single parameter. See, e.g., Wethmur & Davidson (1986).

Accordingly, a nucleotide sequence of the presently disclosed subject matter can be used for its ability to selectively form duplex molecules with complementary stretches of a tenomodulin gene or a tenomodulin
15 polypeptide gene product. Depending on the application envisioned, one employs varying conditions of hybridization to achieve varying degrees of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, one typically employs relatively stringent conditions to form the hybrids. For example, one selects relatively
20 low salt and/or high temperature conditions, such as provided by 0.02M-0.15M salt at temperatures of about 50°C to about 70°C including particularly temperatures of about 55°C, about 60°C and about 65°C. Such conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

25 Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate polypeptide coding sequences from related species, functional equivalents, or the like, less stringent hybridization conditions are typically needed to allow formation of
30 the heteroduplex. Under such circumstances, one employs conditions such as 0.15M-0.9M salt, at temperatures ranging from about 20°C to about 55°C, including particularly temperatures of about 25°C, about 37°C, about 45°C, and about 50°C. Cross-hybridizing species can thereby be readily identified

as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased
5 temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it is advantageous to employ a nucleic acid sequence of the presently disclosed subject matter in combination with an appropriate means, such as a label, for determining hybridization. A wide
10 variety of appropriate indicator reagents are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one likely employs an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the
15 case of enzyme tags, calorimetric indicator substrates are known which can be employed to provide a reagent visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described
20 herein are useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the sample containing test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under
25 desired conditions. The selected conditions depend inter alia on the particular circumstances based on the particular criteria required (depending, for example, on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Following washing of the hybridized surface so as to remove nonspecifically bound probe
30 molecules, specific hybridization is detected, or even quantified, by means of the label.

The materials for use in the method of the presently disclosed subject matter are ideally suited for the preparation of a screening kit. Such a kit

can comprise a carrier having compartments to receive in close confinement one or more containers such as vials, tubes, and the like, each of the containers comprising one of the separate elements to be used in the method. For example, one of the containers can comprise an amplifying
5 reagent for amplifying a tenomodulin polypeptide-encoding DNA, such as the necessary enzyme(s) and oligonucleotide primers for amplifying target DNA from the subject.

In another aspect, the presently disclosed subject matter provides assay kits for detecting the presence of a polypeptide of the presently
10 disclosed subject matter in biological samples, where the kits comprise a first antibody capable of immunoreacting with the polypeptide. The assay kits of the presently disclosed subject matter can further comprise a second container containing a second antibody that immunoreacts with the first antibody. The antibodies used in the assay kits of the presently disclosed
15 subject matter can be monoclonal antibodies. The first antibody can be affixed to a solid support. The first and second antibodies can comprise an indicator, such as but not limited to a radioactive label or an enzyme.

The presently disclosed subject matter also provides an assay kit for screening agents. Such a kit can contain a polypeptide of the presently
20 disclosed subject matter. The kit can additionally contain reagents for detecting an interaction between an agent and a polypeptide of the presently disclosed subject matter.

In an alternative aspect, the presently disclosed subject matter provides assay kits for detecting the presence, in biological samples, of a
25 polynucleotide that encodes a polypeptide of the presently disclosed subject matter, the kits comprising a first container that contains a second polynucleotide identical or complementary to a segment of at least 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or more contiguous nucleotide bases of, as an example, any of SEQ ID NOs: 1-3.

30 In some embodiments, one or more PCR probes can be used that hybridize specifically to one or more exons present in a tenomodulin gene product. For examples, one or more probes can be used to detect a nucleic acid encoding a tenomodulin polypeptide.

In some embodiments of the presently disclosed subject matter, two pairs of isolated oligonucleotide primers are provided. These sets of primers are optionally derived from a tenomodulin polypeptide exon. The primers direct amplification of a target polynucleotide prior to sequencing. In another embodiment of the presently disclosed subject matter isolated allele specific oligonucleotides (ASO) are provided.

In another embodiment, the presently disclosed subject matter provides assay kits for detecting the presence, in a biological sample, of antibodies immunoreactive with a polypeptide of the presently disclosed subject matter, the kits comprising a tenomodulin polypeptide that immunoreacts with the antibodies.

In some embodiments, provided is an assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a tenomodulin polypeptide. In some embodiments, the kit comprises a tenomodulin polypeptide as disclosed herein that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay, optionally comprising a label for the polypeptide, optionally wherein the label can be detected by an imaging technique. Representative, non-limiting imaging techniques include magnetic resonance imaging (MRI) and/or computed tomography (CT). Thus, by way of additional, non-limiting example, the assay kit can comprise reagents that would recognize tenomodulin isoforms via an opaque tag that can be imaged with MRI or CT.

IV. Methods of Screening Compounds for the Ability to Modulate Tenomodulin Polypeptide Expression and/or Activity

Tenomodulin polypeptide modulators can be identified by: providing a test sample comprising a tenomodulin polypeptide; administering a test molecule to the test sample; and determining the effect of the test molecule on the activity of the polypeptide. A test molecule can be any molecule having any chemical structure. For example, a test molecule can be a polypeptide, carbohydrate, lipid, amino acid, nucleic acid, fatty acid, or steroid. In addition, a test molecule can be lipophilic, hydrophilic, plasma membrane permeable, or plasma membrane impermeable. In some

embodiments, the test molecule is selected from the group including but not limited to a polypeptide, a nucleic acid oligonucleotide, optionally an siRNA to one or more of the tenomodulin isoform mRNAs, an exogenous vector coding for a nucleic acid oligonucleotide or polypeptide, a carbohydrate, a lipid, an amino acid, a fatty acid, a steroid, and a low molecular weight organic molecule.

The presently disclosed subject matter provides several assays that can be used to identify tenomodulin polypeptide modulators. Such assays involve monitoring at least one of the biological responses mediated by a tenomodulin polypeptide in cells expressing a polypeptide having tenomodulin polypeptide activity such as cells containing an exogenous nucleic acid molecule that expresses a polypeptide having tenomodulin polypeptide activity. Representative tenomodulin polypeptide responses are disclosed herein. Thus, a tenomodulin polypeptide modulator can be identified using an assay in cells transfected with a nucleic acid molecule that expresses a polypeptide having tenomodulin polypeptide activity.

In accordance with the presently disclosed subject matter there are also provided methods for screening candidate compounds for the ability to modulate *in vivo* tenomodulin polypeptide levels and/or activity. Representative modulators of tenomodulin polypeptide levels can comprise modulators of transcription or expression. Compositions that modulate (i.e. increase or decrease) the transcription or expression of tenomodulin polypeptide-encoding genes have application for the modulation of the biological activity of tenomodulin polypeptide.

Thus provided herein is a method for discovery of compounds that modulate the expression levels of tenomodulin polypeptide-encoding genes. The general approach is to screen compound libraries for substances which increase or decrease expression of tenomodulin polypeptide-encoding genes. Exemplary techniques are described in U.S. Patent Nos. 5,846,720 and 5,580,722, the entire contents of each of which are herein incorporated by reference.

While the following terms are believed to be well understood by one of skill in the art, the following definitions are set forth to facilitate explanation

of the invention.

“Transcription” means a cellular process involving the interaction of an RNA polymerase with a gene that directs the expression as RNA of the structural information present in the coding sequences of the gene. The process includes, but is not limited to the following steps: (a) the transcription initiation, (b) transcript elongation, (c) transcript splicing, (d) transcript capping, (e) transcript termination, (f) transcript polyadenylation, (g) nuclear export of the transcript, (h) transcript editing, and (i) stabilizing the transcript. “Expression” generally refers to the cellular processes by which a biologically active polypeptide is produced from RNA.

“Transcription factor” means a cytoplasmic or nuclear protein which binds to such gene, or binds to an RNA transcript of such gene, or binds to another protein which binds to such gene or such RNA transcript or another protein which in turn binds to such gene or such RNA transcript, so as to thereby modulate expression of the gene. Such modulation can additionally be achieved by other mechanisms; the essence of “transcription factor for a gene” is that the level of transcription of the gene is altered in some way.

In accordance with the presently disclosed subject matter there is provided a method of identifying a candidate compound or molecule that is capable of modulating the transcription level of a gene encoding a tenomodulin polypeptide and thus is capable of acting in the modulation of tenomodulin polypeptide effects. Such modulation can be direct, *i.e.*, through binding of a candidate molecule directly to the nucleotide sequence, whether DNA or RNA transcript, or such modulation can be achieved via one or more intermediaries, such as proteins other than tenomodulin polypeptide which are affected by the candidate compound and ultimately modulate tenomodulin polypeptide transcription by any mechanism, including direct binding, phosphorylation or dephosphorylation, *etc.*

This method comprises contacting a cell or nucleic acid sample with a candidate compound or molecule to be tested. These samples contain nucleic acids which can contain elements that modulate transcription and/or translation of a tenomodulin polypeptide gene, such as a promoter or putative upstream regulatory region (representative of such as disclosed

herein), and a DNA sequence encoding a polypeptide which can be detected in some way. Thus, the polypeptide can be described as a "reporter" or "marker." Optionally, the candidate compound directly and specifically transcriptionally modulates expression of the tenomodulin polypeptide-
5 encoding gene.

The DNA sequence is coupled to and under the control of the promoter, under conditions such that the candidate compound or molecule, if capable of acting as a transcriptional modulator of the gene encoding tenomodulin polypeptide, causes the polypeptide to be expressed and so
10 produces a detectable signal, which can be assayed quantitatively and compared to an appropriate control. Candidate compounds or molecules of interest can include those which increase or decrease, *i.e.*, modulate, transcription from the regulatory region. The reporter gene can encode a reporter known in the art, such as luciferase, or it can encode tenomodulin
15 polypeptide.

In certain embodiments of the presently disclosed subject matter the polypeptide so produced is capable of complexing with an antibody or is capable of complexing with biotin. In this case the resulting complexes can be detected by methods known in the art. The detectable signal of this
20 assay can also be provided by messenger RNA produced by transcription of said reporter gene. Exactly how the signal is produced and detected can vary and is not the subject of the presently disclosed subject matter; rather, the presently disclosed subject matter provides the nucleotide sequences and/or putative regulatory regions of tenomodulin polypeptide for use in such
25 an assay. The molecule to be tested in these methods can be a purified molecule, a homogenous sample, or a mixture of molecules or compounds. Further, in representative embodiments, the DNA in the cell can comprise more than one modulatable transcriptional regulatory sequence.

In accordance with the presently disclosed subject matter there is
30 also provided a rapid and high throughput screening method that relies on the methods described above. This screening method comprises separately contacting each of a plurality of substantially identical samples. In such a screening method the plurality of samples preferably comprises more than

about 10^4 samples, or more preferably comprises more than about 5×10^4 samples.

V. Method of Modulating the Expression and/or Activity of Tenomodulin in a Sample or Subject

Also disclosed herein are methods of modulating the biological activity of a tenomodulin polypeptide in a biological sample. In some embodiments, the method comprises contacting the biological sample with an agent for modulating expression, activity or both expression and activity of a tenomodulin polypeptide. In some embodiments, the biological sample is a tissue present in a subject. In some embodiments, the biological sample is a tendon, ligament or other dense connective tissue.

In some embodiments, the modulating of the tenomodulin polypeptide biological activity regulates the growth of collagen fibrils, optionally wherein a ratio of large and small fibrils is controlled to a more native state.

In some embodiments, the tissue present in a subject is an abnormal tissue. In some embodiments, the modulation of tenomodulin polypeptide expression reduces growth of the abnormal tissue. While it is not desired to be bound by any particular theory of operation, in some embodiments, it is believed that the modulation of tenomodulin polypeptide expression reduces growth of the abnormal tissue by blocking angiogenesis. In some embodiments the agent for modulating expression, activity or both expression and activity of a tenomodulin polypeptide is selected from the group including but not limited a polypeptide (including a tenomodulin polypeptide as disclosed herein), a nucleic acid oligonucleotide, optionally an siRNA to one or more of the tenomodulin isoform mRNAs, a vector coding for a nucleic acid oligonucleotide or polypeptide, a carbohydrate, a lipid, an amino acid, a fatty acid, a steroid, and a low molecular weight organic molecule. Thus, a tenomodulin polypeptide or biologically active fragment thereof can be administered as the agent, such as but not limited to an anti-angiogenic C-terminal fragment.

In some embodiments, the presently disclosed subject matter takes advantage of RNAi technology (for example shRNA, siRNA and miRNA

molecules and ribozymes) to cause the down regulation of cellular genes, a process referred to as RNA interference (RNAi). As used herein, "RNA interference" (RNAi) refers to a process of sequence-specific post-transcriptional gene silencing mediated by a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecules, miRNA molecules or synthetic hammerhead ribozymes. See generally Fire et al., *Nature* 391:806-811, 1998, and U.S. Patent No. 6,506,559. The process of RNA interference (RNAi) mediated post-transcriptional gene silencing is thought to be an evolutionarily conserved cellular defense mechanism that has evolved to prevent the expression of foreign genes (Fire, *Trends Genet* 15:358-363, 1999).

As used herein, the terms "inhibit", "suppress", "down regulate", "knock down", and grammatical variants thereof are used interchangeably and refer to an activity whereby gene expression or a level of an RNA encoding one or more gene products is reduced below that observed in the absence of a composition of the presently disclosed subject matter.

With respect to the therapeutic methods of the presently disclosed subject matter, a preferred subject is a vertebrate subject. A preferred example of a vertebrate is a warm-blooded vertebrate. A preferred example of a warm-blooded vertebrate is a mammal. A preferred example of a mammal is a human. Additionally, as used herein and in the claims, the term "patient" can include both human and animal patients, and thus, veterinary therapeutic uses are provided in accordance with the presently disclosed subject matter.

A "therapeutic composition" or a "pharmaceutical composition" as described herein preferably comprises a composition that includes a pharmaceutically acceptable carrier. In some embodiments, the presently disclosed subject matter provides pharmaceutical compositions comprising an agent (such as but not limited to polypeptide or polynucleotide of the presently disclosed subject matter) and a physiologically acceptable carrier. In some embodiments, constructs will be conjugated to a carrier, for example a nanoparticle or an antibody to direct its delivery to the target cells. The carrier (e.g. nanoparticle) conjugated to the agent can be injected in an

acceptable pharmaceutical diluent. In some embodiments, an agent is delivered to a target cell by a delivery vehicle, such as but not limited to a viral vector, an antibody, an aptamer, or a nanoparticle.

5 A composition of the presently disclosed subject matter is typically administered parenterally in dosage unit formulations containing standard, well-known nontoxic physiologically acceptable carriers, adjuvants, and vehicles as desired. The term "parenteral" as used herein includes intravenous, intra-muscular, intra-arterial injection, or infusion techniques.

10 Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol.

15 Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as
20 oleic acid find use in the preparation of injectables.

Preferred carriers include neutral saline solutions buffered with phosphate, lactate, Tris, and the like. Of course, one purifies the vector sufficiently to render it essentially free of undesirable contaminants, such as defective interfering adenovirus particles or endotoxins and other pyrogens
25 such that it does not cause any untoward reactions in the individual receiving the vector construct. A preferred means of purifying the vector involves the use of buoyant density gradients, such as cesium chloride gradient centrifugation.

30 A transfected cell can also serve as a carrier. By way of example, a cell can be removed from an organism, transfected with a polynucleotide of the presently disclosed subject matter using methods set forth above and then the transfected cell returned to the organism (e.g. injected intra-vascularly).

EXAMPLES

The following Examples have been included to illustrate modes of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

10 MATERIALS AND METHODS FOR EXAMPLES 1-10

Tendon collection from patients. Flexor carpi radialis (FCR) and biceps tendons were collected at surgery from discarded tissue (UNC Memorial Hospital, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America). Specimens for nucleic acid extraction were placed in labeled plastic tubes and snap frozen in liquid nitrogen in the operating room then transferred to a -80°C freezer until processed. Specimens for cell isolation were placed in DMEM-H medium with 20 mM HEPES pH 7.4, and antibiotics in preparation for cell isolation (Banes et al, 1988). Porcine Achilles tendons were collected at a local abattoir and cells isolated and cultured utilizing the same techniques as for the human cells. COS-7 cells were obtained from the UNC cell repository (Cell Culture facility, University of North Carolina at Chapel Hill) and cultured as for tenocytes.

Isolation and maintenance of tenocytes. Tenocytes from the deep collagenous material were freed by collagenase then trypsin digestion, washed, collected then transferred to medium (Banes et al, 1988; Garvin et al, 2003; Qi et al, 2006). Cells were washed in complete DMEM-H medium with 15 mM glutamine, 20 mM sodium pyruvate, 20 mM HEPES pH 7.2, 0.1 mM ascorbate-2 phosphate and antibiotics (1% sodium penicillin G and streptomycin sulfate, and 0.1 % Fungizone). Cells were plated on type 1 collagen-coated polystyrene culture dishes at 25k cells/cm² and grown to 90% confluence prior to passage with 0.1% trypsin. Cells were used from passages 1-3.

Immunohistochemistry. Two millimeter cubed tendon segments of each tendon type were harvested at surgery, oriented in a plastic histologic block container so that a longitudinal cross-section could be sectioned then embedded in optimal cutting temperature (OCT) compound and frozen at -20°C. Five micron sections were cut using a microtome and placed on an albumin-coated glass slide (Histology Services Co., Stone Mountain, Georgia, United States of America). Tenocytes from FCR or biceps tendons were grown to passage 2, plated on collagen-coated coverslips. Tnmd was stained with anti-N or C-terminal Tnmd antibodies (Sigma, Santa Cruz Biotechnology, Santa Cruz, California, United States of America) at a 1:200 dilution at 4°C overnight. After washing three times with phosphate buffered saline (PBS, pH 7.2), Tnmd was visualized with an ALEXA FLOUR® 568 conjugated goat anti-rabbit IgG (Molecular Probes, Inc., Eugene, Oregon, United States of America) at 1: 500 dilution. Images were taken with an epifluorescence microscope (Olympus BX60, OPELCO, Dulles, Virginia, United States of America) or a LeicaSP2 AOBS laser scanning confocal microscope at 0.25 micron optical sections (Leica Microsystem, Inc., Exton, Pennsylvania, United States of America) with a 40x or 60x oil immersion objective.

Quantitative PCR. Total RNA was isolated using an RNeasy® mini kit (QIAGEN®, Valencia, California, United States of America), according to the manufacturer's protocol. Complementary DNA was synthesized with SuperScript® II (Invitrogen, Carlsbad, California, United States of America). The expression levels of target genes were determined by SYBR green real time PCR using 18S rRNA as an internal control (Ambion, Austin, Texas, United States of America) with a Brilliant® SYBR green QPCR master mix kit from Stratagene (La Jolla, California, United States of America).

The PCR conditions were as follows: 30 cycles of 94°C for 30 seconds, 60°C for 60 seconds, and 72°C for 30 seconds (optimized for each major reaction set). Tenomodulin isoforms 1 and 2 share the same N-terminal sequence and isoforms 1 and 3 share the same C-terminal sequence.

Due to the overlap in sequences among each isoform, three pairs of primers were designed in order to determine the expression levels of each isoform. Primer set 1, located in exons 3 and 5, amplified all isoforms. Primer set 2, located in exons 2 and 5, amplified isoforms 1 and 2. Primer set 3, located in exons 6 and 7, amplified isoforms 1 and 3. The signal level of the primer 1 pair minus that of the primer 3 pair yielded the expression level of isoform 2; the signal level of the primer 2 pair minus that of the primer 3 pair yielded the expression level of isoform 3. Primer sequences were as follows: Primer set 1, 5'-ATTCAGAAGCGGAAATGGCACTGA-3' (forward; SEQ ID NO: 10) and 5'-TAGGCTTTTCTGCTGGGACCCAA-3' (reverse; SEQ ID NO: 11); Primer set 2, 5'-ACTTCTGGCCGGAGGTACCCAA-3' (forward; SEQ ID NO: 12) and 5'-TGCTGGGACCCAAATCACTGACT-3' (reverse; SEQ ID NO: 13); and Primer set 3, 5'-CCCTCAAGTGAAAGTAGAGAAGA-3' (forward; SEQ ID NO: 14) and 5'-CCTCCTTGGTAGCTGTATGGA-3' (reverse; SEQ ID NO: 15).

The signal levels were normalized to the length of PCR products for each pair of primers. Primers for scleraxis (scx) and myostatin (MSTN) were 5'-AAGAAAAGCCAGCGCAGAAAGTTC-3' (SEQ ID NO: 16)/ 5'-TCTGCACCTTCTGCCTCAGCAA-3' (SEQ ID NO: 17); and 5'-GATGGCTCTTTGGAAGATGACGAT-3' (SEQ ID NO: 18)/ 5'-GTCTTCACATCAATGCTCTGCCAA-3' (SEQ ID NO: 19), respectively.

Western blot. Tendon specimens were pulverized to a powder at the temperature of liquid nitrogen (100~200mg wet weight) then nucleic acids extracted or cells were extracted in 8M urea, 50mM Tris-HCl, pH 8.0, 1 mM dithiothreitol, 1 mM EDTA and the supernatant fluids collected. Cell lysates were collected in RIPA buffer (25mM Tris-HCl, pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitors. Aliquots (20µg total protein/lane) of the samples were mixed with 2X SDS-PAGE sample buffer (100mM Tris-HCl, pH 6.8, 4% SDS, 10% β-mercaptoethanol, 20% glycerol) and separated in a 12.5% gel SDS-PAGE, then transferred to a nitrocellulose membrane. After preincubation with

blocking buffer (10% nonfat milk, 0.1% Tween 20 in Tris-buffered saline (TBS)), the membrane was probed with the specific polyclonal anti-Tnmd antibody against either N-terminal or C-terminal peptide sequences in Tnmd at 4°C overnight, followed by incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG secondary antibody. Immunoreactive signals were detected by enhanced chemiluminescence (ECL) and blots digitally recorded and analyzed by image analysis for molecular weight determinations. To determine if Tnmd is secreted from the cells, the medium of transfected cells was replaced with serum-free medium at 24 h post-transfection. Cells were further cultured for 16 h and the medium was collected and concentrated 20 times before running a western blot as described above.

Over-expression of Tnmd isoforms I, II and III. cDNA of human Tnmd isoforms I, II and III were amplified from human tendon total RNA using primers listed as follows: isoform I: 5'-aaaaCTCGAGctATGGCAAAGAATCCTCCAGAGAA-3' (forward; SEQ ID NO: 20) and 5'-aaaaGGATCCGACCCTCCCCAGCATGCGGGC-3' (reverse; SEQ ID NO: 21); Isoform II: 5'-aaaaCTCGAGctATGGCAAAGAATCCTCCAGAGAA-3' (forward; SEQ ID NO: 22) and 5'-aaaaGGATCCCTGTCTTAGAAAAATAGGAGGAGGAT-3' (reverse; SEQ ID NO: 23); Isoform III: 5'-aaaaCTCGAGctATGGAGCACACTTTCTACAGCAAT-3' (forward; SEQ ID NO: 24) and 5'-aaaaGGATCCGACCCTCCCCAGCATGCGGGC-3' (reverse; SEQ ID NO: 25). PCR products were purified with a QIAquick® PCR purification kit (QIAGEN) and cut with Xho I and BamH I. The digested PCR products were purified and ligated with Xho I/BamH I digested pEGFP-C1 vector with T4 DNA ligase, respectively. The cloning constructs were confirmed with DNA sequencing. Representative human tenocytes from the listed tendons were transfected with the constructs for isoforms 1, 2 or 3. Cell images were taken with an epifluorescence microscope (Olympus BX60, OPELCO) or a LeicaSP2 AOBS laser scanning confocal microscope at 0.25 micron optical sections (Leica Microsystem, Inc., Buffalo Grove, Illinois, United States of America) with a 40x oil immersion objective.

Knockdown of Tnmd Isoforms with siRNA. Pre-designed siRNAs targeting human Tnmd exon 5 were purchased from Ambion (Grand Island, New York, United States of America), which knocked down all three human Tnmd isoforms. siRNAs at a final concentration of 40 nM were transfected
5 into human tenocytes with Lipofectamine® 2000 (Invitrogen) according to the manufacturer's protocol. The control group was transfected under the same conditions but with scrambled siRNAs. Cells were collected at 48 hours post-transfection for qPCR analysis.

Cell proliferation assay. A cell proliferation assay was carried out with
10 a BrdU cell proliferation assay kit (Millipore, New Jersey, United States of America). Twenty-four hours post-transfection, 0.4 µL of 500x BrdU reagent was added to each well in a six-well culture plate and cells were incubated for another 24 h. Cells were fixed with the fixing solution and stained with anti-BrdU antibody and DAPI. BrdU-positive cells (proliferating cells) were
15 visualized with ALEXA FLOUR® 568 (Molecular Probes, Inc.) conjugated goat anti-mouse IgG. Triplicate assays were performed and over 500 cells per well were counted. The overall percentage of proliferating cells divided by the total cell number was calculated for each RNAi experiment.

Statistical analysis. All experiments were repeated at least three
20 times. Statistical analyses were performed using Student's t-test. A value of $P < 0.05$ was deemed as significant (*).

EXAMPLE 1

Identification of Tenomodulin Isoforms in Human Tissues

25

Tenomodulin (Tnmd) is a type II transmembrane protein with 7 exons, and principle homology to chondromodulin-1. It is a putative tendon marker gene but has been identified in ligament and in non-orthopaedic tissues such as the eye. As disclosed herein, the full-length isoform (I1) has an
30 extracellular C-terminal anti-angiogenic sequence with a furin cleavage site, proliferative sequence and membrane spanning domain. The Human Genome Project was data mined in an effort to identify any TNMD isoforms, the results of which yielded the discovery of the three TNMD isoforms

disclosed herein. Experiments were then conducted to determine if different tendon tissues express different amounts of the three isoforms, particularly I1 and isoform 3 (I3) with anti-angiogenic C-termini which would be antithetical to healing tissues.

5 The human genome was scanned for Tnmd sequences, revealing three isoforms (I1, 37.1 kDa; I2, 20.3kDa, I3, 25.4kDa). Human Flexor digitorum profundus and Flexor carpi radialis tendons, tibial bone, medial collateral ligament and soft tissues; liver, kidney and brain, were collected at surgery or purchased, then cDNAs prepared for quantitative polymerase
10 chain reaction (PCR) for amplification of Tnmd isoforms 1, 2, 3 and ribosomal mRNA as a control. Cells were cultured from porcine Achilles tendon (AT) specimens for immunochemical analysis of tenomodulin staining with antibody to N and C termini.

 Data mining the human genome revealed that Tnmd has three
15 isoforms (I1, I2, I3), as depicted in Figure 1. Isoform 1 was determined to be the full-length isoform with a transmembrane segment at exon 2, a furin cleavage sequence in exon 6, a C-terminal anti-angiogenic sequence at exon 7 and proliferative portion in the extracellular space. Isoform 2 has a more N-terminal transmembrane spanning segment at exon 2, is truncated
20 at the furin-cleavage site at exon 6, and has no anti-angiogenic portion. Isoform 3 has no membrane spanning segment but does have the anti-angiogenic C-terminus. See Figure 1.

EXAMPLE 2

25 Identification of Tenomodulin Isoforms in Tissues of Multiple Species

 Similar to the experiments and results in Example 1, three distinct Tnmd transcripts (Figure 1) were expressed in tendon tissue from multiple human specimens and tendon types as well as porcine and equine (equine
30 data not shown) and from isolated tenocytes. Here again, data mining in the GeneCards™ database revealed that Tnmd has three isoforms with deduced molecular weights of 37.1 kDa for isoform I; 20.3 kDa for isoform II; and 25.4 kDa for isoform III (GeneCards™ database; Rebhan et al, 1997).

Full-length isoform I and truncated isoform II have a transmembrane domain in exon 2, while isoform III, lacking exons 1 and 2, has no transmembrane domain.

5

EXAMPLE 3

Differential Expression of Tenomodulin Isoforms in Human Tissues

Next, the expression of the three Tnmd isoforms was assessed in various tissues. Human Flexordigitorum profundus (FDP) and Flexor capri
 10 radialis (FCR) tendons, tibial bone, medial collateral ligament, smooth muscle, cardiac muscle, and soft tissues (liver, kidney and brain) were assessed for Tnmd isoforms. Results are displayed in Figures 2A and 2B.

Summarily, bone and tendon were enriched in Tnmd expression whereas the other tissues showed comparatively weak expression.
 15 Differential expression of the three isoforms of Tnmd are shown in Table 2 as percentages.

Table 2

Differential expression of Tnmd isoforms in several
 20 orthopedically relevant tissues.

Tissue	Tnmd isoform		
	I1	I2	I3
FCR	5.6%	4.9%	89.6%
FDP	<1%	72.5%	27.4%
Meniscus	NA	2.9%	97.1%
Bone	<1%	18.4%	80.6%
Ligament	NA	25.8%	74.2%

Expression of Tnmd was confirmed in porcine and equine tenocytes through immunochemical staining with antibody to the C-terminus. Robust staining was observed at the internal leaflet of the plasma membrane, especially in cells grown on glass versus silicone membrane. Slot blot analysis also showed detectable protein secreted in the supernatant fluid from cultured tenocytes.

DISCUSSION OF EXAMPLES 1, 2 and 3

Tnmd is a chondromodulin-like protein, enriched in tenocytes. Disclosed herein for the first time, Tnmd exists in at least three isoforms, two of which may exhibit anti-angiogenesis properties at the C-terminal portion (I1) and (I3). Due to the conserved sequence of Tnmd across species, applicants confirmed expression in human, porcine, and equine tenocytes. While it is not desired to be bound by any particular theory of operation, it is expected that tenocytes in healing tendon would down-regulate expression of Tnmd to allow vasculogenesis to promote healing. Pathologic Tnmd expression would likely promote tendon failure via poor nutrition leading to weak tendons.

EXAMPLE 4

Identification of cellular localization of Tnmd

Immunostaining of Tnmd in human tendon sections revealed that Tnmd was highly expressed in human Flexor carpi radialis (FCR) and biceps tendons. Tnmd immunostaining showed perinuclear and cytoplasmic localization. No obvious nuclear localization was observed. Unexpectedly, no obvious plasma membrane localization was seen either. Overexpression of Tnmd isoforms in COS-7 cells showed different distribution patterns (images not shown). GFP-Tnmd I showed robust cytoplasmic puncta, most of which were associated with the nuclear envelope. Most of the GFP-Tnmd isoform II protein was associated with the nuclear envelope while GFP-Tnmd III distributed in the cytoplasm with no nuclear envelope localization. In

human tenocytes, both TNMD I and II showed much less nuclear envelope association. In porcine tenocytes, isoforms I and II showed dramatically different patterns: Isoform I did not show nuclear envelope localization, formed much larger puncta in the cytoplasm while isoform II was diffusely distributed in the cytoplasm and showed clear nuclear envelope localization.

EXAMPLE 5

Analysis of Tnmd proteins

Three different protein sizes (33 kDa, 45 kDa and 64 kDa) of Tnmd were detected in human tendon tissue with an anti-Tnmd C-terminus antibody in the western blot (Figure 3). One single major band was detected in cultured human biceps and Flexor carpi radialis tenocytes (42 kDa). These results indicate that the processing of Tnmd mRNA and post-translational modification of Tnmd proteins can be altered in cultured tenocytes *in vitro*. In the overexpression experiments, GFP-fused Tnmd isoforms were transfected in COS-7 cells and expression was confirmed with anti-GFP western blots (Figure 3, left panel). Re-probing of the same membrane with anti-Tnmd C-terminal antibody showed immunopositive bands at the same positions as in the anti-GFP blot, indicating concordance between the GFP signal and the immunochemical identity of the tenomodulin proteins. The re-probing result also indicated that the C-terminal recognizing antibody did not recognize Tnmd isoform II to the same degree as for isoforms I and III (Figure 3, middle panel). The membrane was also probed with an anti-N-terminal antibody, but high levels of non-specific bands were detected. The anti-Tnmd C-terminal antibody also detected two bands in COS-7 cell lysates, which are most likely Tnmd isoforms I and III based on their locations on the gel (Figure 3). Since the antibody does not recognize isoform II to a great degree, the bands shown in Figure 3 (right panel) are most likely Tnmd isoforms I and III.

Tenocytes were cultured in serum-free medium for 16 h and the supernatant fluids collected, concentrated and subjected to Western blot analysis. Results showed that no detectable Tnmd isoforms were detected in the supernatant fluids, indicating that Tnmd was not secreted under the

conditions of the experiment.

EXAMPLE 6

Differential expression of Tnmd isoforms in human tendon tissues

- 5 To determine the relative expression levels of the three Tnmd isoforms in human tendons, three pairs of primers were used in qPCR, as discussed above. Primer set 1 yielded the expression level of all isoforms. Expression levels of isoforms II and III were determined by subtracting the signal for primer sets 2 or 3 from that for primer set 1, respectively.
- 10 Differential expression of Tnmd isoforms was found in different human tendons (Figure 4). While applicants do not wish to be bound by any particular theory of operation, these differences in expression levels of Tnmd isoforms can contribute to the functional variation in different tendons.

15

EXAMPLE 7

Knockdown of Tnmd decreased cell proliferation

- Reduced cell number in patellar tendon was reported in Tnmd knockout mice (Docheva et al, 2005). However, Tnmd isoform III was likely still functional in the knockout mice. The controversial results between *in vivo* and *in vitro* studies suggest that it is important to confirm the *in vivo* results in an *in vitro* study. Results of a cell proliferation assay (BrdU insertion) performed by applicants and reported herein demonstrated that Tnmd siRNA-treated human tenocytes, in which each of the three isoforms of Tnmd were suppressed, showed a reduction in cell proliferation from 24% to 18%, a 25% reduction (Figures 5A and 5B). These results confirm that the regulation of Tnmd can have a subsequent impact on cell proliferation. The qPCR results also showed that suppressing Tnmd expression upregulated the expression of both myostatin (>60%) and scleraxis (>90%), purported regulators of the Tnmd gene.

30

DISCUSSION OF EXAMPLES 4, 5, 6 and 7

Tenomodulin (Tnmd) is a highly expressed, candidate marker gene for tenocytes. However, the function of tenomodulin in tendons has

previously been unclear. The instant disclosure confirms the existence of three different Tnmd transcripts in human tissues. Moreover, the instant disclosure shows for the first time the different functional properties of each isoform. The overexpression of each Tnmd isoform showed different distributions of Tnmd isoforms, which supports the finding that each Tnmd isoform plays distinct functions in tendons and/or other tissues.

Expression of myostatin and scleraxis (scx) was reduced in myostatin knock-out mice and addition of myostatin protein to the culture medium increased the expression of scx and Tnmd (Mendias et al, 2008). Therefore, applicants tested that notion that there is a negative feedback relationship between Tnmd and myostatin. Knockdown of Tnmd increased the expression of myostatin and scx (likely through the upregulation of myostatin). A proposed regulatory pathway of Tnmd is depicted in Figure 6. Tnmd isoforms can serve as new biomarkers and more importantly, as novel therapeutic targets in tendon injury and diseases, given the effect on cell proliferation and varying intracellular localizations.

EXAMPLE 8

Strain Modulates Tenomodulin Isoform 1 Expression and Nuclear Localization in Porcine Tenocytes

Tenomodulin is robustly expressed in developing tendons and ligaments. Studies were designed and conducted to test whether physiologic levels of strain to tenocytes cultured *in vitro* would regulate Tnmd expression to maintain tenocyte phenotype.

Porcine Achilles tendons (PAT) of 2-yr-old pigs were collected at an abbatoire, minced and treated with collagenase to release cells. Cells were cultured in DMEM-H, supplemented with ascorbate, glutamine, pyruvate and antibiotics with 10% fetal bovine serum. Cells at passages 1-4 were used to seed silicone bottomed culture plates (Bioflex®, Flexcell Corp., Hillsborough, North Carolina, United States of America) then either grown statically for 48 hours (h) or grown 48h then subjected to 3% strain at 1Hz for 1, 4 or 7 h or 1Hz, 3% strain for 1h/day for 3 days *in vitro* (n=3/group; three cell isolations).

Cells were extracted for total RNA and quantitative PCR (Q-PCR) performed for the amplification of the three Tnmd isoforms (I1, I2, I3). Cells were immunochemically stained for Tnmd using C- and N-terminal recognizing antibodies.

5 PAT grew well on the silicone surface with a generation time of about 28h. Data in Figure 7 show that expression of Tnmd I1 rapidly increased after just 1 hour (h) of strain stimulation, then decreased at 4 h and returned to control levels after 7 h of continual strain. Using the second stretching regimen described in the methods, a significant decrease in expression of I1
10 and I2 was noted, as recognized by a primer designed to detect the N terminus (Figure 8).

Surprisingly, immunochemical analysis of the strained cells showed localization of Tnmd in the nucleus with the C-terminal recognizing antibodies, but not the N terminal recognizing antibodies. While applicants
15 do not wish to be bound by any particular theory of operation, given the identification of Tnmd with only the C-terminal antibody it was concluded that either isoform 3 or a cleavage product of isoform 1 enters the nucleus since I2 does not contain the C-terminal epitope sequence.

These results indicate that Tnmd is highly expressed and rapidly up
20 and down-regulated in strained tenocytes, suggesting that it has some early response role and putative regulatory function, belying the nuclear localization. Given its high expression level and rapid response to strain, Tnmd can be an early marker of tenocyte damage, which could be assessed by imaging at surgery, to gauge tissue damage.

25

EXAMPLE 9

Tenomodulin Regulation in Equine Bioartificial Tendons *In Vitro*

Studies were designed and conducted to test whether Tnmd isoforms
30 would be down-regulated in cells adjacent to a wound to permit angiogenesis. Equine tenocytes from the superficial digital flexor tendons (SDFT) were isolated from 5 month-old, 2, 17 and 25 year-old horses during necropsy at the North Carolina State University School of Veterinary Medicine (Raleigh, North Carolina, United States of America) and Cornell

University Veterinary School (Ithaca, New York, United States of America). Cells were isolated by collagenase digestion followed by collection of tendon internal fibroblast cells. Trapezoidal shaped, three-dimensional bioartificial tendons (BATs) were created using Passage 2 SDFT cells at 1000
5 cells/microliter collagen gel with 750 µl cell-gel mixture per construct. After BATs compacted for 48h, a 2x4 mm full thickness laceration was introduced to the wound group with a 10 blade scalpel. BATs that were subjected to strain received 3% uniaxial strain at 1Hz for 1 hour. Then the wounded or strained BATs were collected and processed for quantitative PCR for Tnmd
10 isoforms (n=4/group) and immunohistochemical localization of Tnmd (n=2/group). Cells were collected at 0, 1, 4 and 8 hours post-wound and strain and assayed for Tnmd immunohistochemical localization with both N and C-terminus recognition antibodies. Results were analyzed for statistical significance using a one-tailed t-test.

15 Equine tenocytes compacted the collagen matrix and aligned with the long axis of the TRAP BAT shape in the lower third of the BAT. Tnmd was detected in isolated cells and in cells in BATs with both Tnmd antibodies (directed to N and C-termini). Isoform 1 was the dominant Tnmd mRNA expressed in equine tissue and cells; I2 was slightly expressed, and I3 was
20 about 20% of I1 (Figure 9). Tnmd expression was decreased by 1 hour after cessation of strain (Figure 10). Tnmd localization was observed associated with the plasma membrane and in the cytoplasm. Tnmd expression also decreased in samples taken from the wound region compared to the same region from non-wounded specimens (Figure 11). Approximately 10
25 minutes passed between wounding and collection for the 0 hour group, and Tnmd was diminished significantly during this time suggesting an early response regulatory function. Tnmd localization was reduced in the region proximal to the wound, but was present more distant from the wound.

These studies indicated that Tnmd was expressed in equine
30 tenocytes and that wounding or straining a three-dimensional bioartificial tendon *in vitro* decreased both mRNA expression and protein localization at the wound site. While not wishing to be bound by any particular theory of operation, applicants concluded that Tnmd down-regulation in injured tendon

promotes angiogenesis and subsequent healing. This is the first report of Tnmd modulation in a bioartificial tendon with wound and strain.

EXAMPLE 10

5 Regulation of Tnmd expression

Provided herein is evidence that the Tnmd gene is regulated by Scx via the purinoceptors P2Y1 (ADP-responsive) and the P2Y2 (ATP-responsive). Mechanical loading such as laminar shear stress, compression, tension and osmotic swelling causes the release of ATP in
10 almost every cell type examined to date, including tenocytes [Cotrina et al., 1998; Lazarowski, 1997; Tsuzaki et al., 2004; Bowler et al, 2001; Wiebe et al., 1999; Ostrom et al., 2000]. ATP release occurs by transport through connexin hemichannels, pannexins, maxi-ion and volume-regulated anion channels, and the P2X7 receptor [Abraham et al, 1993; Reisin et al, 1994];
15 [Schwiebert et al., 1995] [Beigi et al., 1999] [Cotrina et al., 1998] [Corriden et al., 2010]. Cells *in vitro*, including tenocytes, generally secrete ATP on the order of 10-150 pM on average and up to nM levels in some cells despite rapid ATP to ADP to adenosine degradation by ecto-NTPases [Lazarowski et al., 1997; Zimmerman et al., 1999]; [Tsuzaki et al., 2004] [Tsuzaki et al.,
20 2005]. Purinoceptors are selective, metabotropic, G protein coupled, P2Y class, or ionotropic, ligand-gated ion channels of the P2X class, the latter of which reacts nonselectively with ATP [Communi et al., 1997]. The P2Y mammalian receptor family has 8 members signaling through heterotrimeric G proteins in the Gi, Gq11 and Gs families, which activate phospholipase C
25 (PLC) and generates IP3-dependent [Ca²⁺]_i [Communi et al., 2000; Communi et al., 1997] [Ralevic et al., 1998]. P2Y2 reacts with ATP or UTP and P2Y1 reacts primarily with ADP but 30 fold less with ATP [Communi et al., 2000; Communi et al., 1997]. ADP can activate adenylyl cyclase and increase cAMP via degradation of ATP to ADP by ectonucleotidases that are
30 expressed and have been measured in tendon cells [Tsuzaki et al., 2004; Communi et al., 2000]. Increased cAMP concentration down-regulates collagen I expression [Kream et al., 1993; Duncan et al., 1999] [Riquet et al., 2000]. ATP can inhibit IL-1b-induced MMP mRNA and protein expression

and COX 2 expression and PGE2 secretion [Francke et al., 1998; Jones et al., 2005] [Tsuzaki et al., 2003].

The full-length Tnmd was expressed 11 fold more in Achilles tendons (AT) isolated from P2Y2 *-/-* mice (ATP receptor-less, Figure 12) and 3 fold in the P2Y1 (ADP-receptor-less) knockout mouse Achilles tendons (Figure 12). Moreover, Tnmd was increased 5 fold in the double knockout (P2Y1 *-/-* and P2Y2 *-/-*) mouse AT, indicating that the P2Y1 receptor was likely dominant in regulating the gene. These results were verified by treating tenocytes *in vitro* with interfering RNAs to the P2Y1 and P2Y2 receptors. Treatment of tenocytes with IL-1b increased then decreased Tnmd expression (Figure 13). Expression of Col1a1, Col3a1, Col14a1, FN, and decorin were not affected by Tnmd RNAi, whereas that for Scx was increased about 2 fold. Taken together, these data indicate that the Scx as well as P2Y1 and P2Y2 receptors, and ATP, ADP pathways, regulate Tnmd expression but not the reverse.

EXAMPLE 11

Modulating or ablating Tnmd to regulate cell function

Dissecting the function(s) of Tnmd and its isoforms can provide an important link between normal tendon physiology and abnormal function in healing, applied strain and response to overuse *in vivo*. Provided herein is a pathway analysis of the factors that modulate this important tendon biomarker, including the purinoceptor pathways involving ATP and ADP receptors, and the transcription factor scleraxis. Use of RNAi mixtures and over-expression, to combinations of Tnmd isoforms +/- P2Y1 and P2Y2 regulation, followed by testing angiogenesis capabilities, cell migration, proliferation and matrix expression in wounded cells and/or strained cells (2D and 3D) can determine which basic cellular functions are disturbed when Tnmd is modulated or ablated. Based on the instant disclosure one of ordinary skill in the art can regulate the expression of tenomodulin isoforms by over-expression and gene silencing of Tnmd isoforms, and the candidate regulatory purinoceptors, ADP-responsive P2Y1 and ATP-responsive P2Y2

receptors, then test tenocyte response in strain and wound experiments *in vitro*.

Overexpression of each Tnmd isoform is achieved by cloning human TNMD isoforms 1, 2 and 3 using pcDNA3 and pEGFP-C1 vectors (Qi et al., 2008). cDNA of human TNMD isoforms 1, 2 and 3 are amplified from human, tendon (flexor carpi radialis, FCR) total RNA using primers listed as follows:

TNMD Isoform 1, pcDNA3, aaaaGGATCCATGGCAAAGAATCCTCCAGAGAA (forward; SEQ ID NO: 26), aaaaCTCGAGGACCCTCCCCAGCATGCGGGC (reverse; SEQ ID NO: 27), pEGFP-C1, aaaaCTCGAGctATGGCAAAGAATCCTCCAGAGAA (forward; SEQ ID NO: 28), aaaaGGATCCGACCCTCCCCAGCATGCGGGC (reverse; SEQ ID NO: 29); TNMD Isoform 2, pcDNA3, aaaaGGATCCATGGCAAAGAATCCTCCAGAGAA (forward; SEQ ID NO: 30), aaaaCTCGAGCTGTCTTAGAAAATAGGAGGAGGAT (reverse; SEQ ID NO: 31), pEGFP-C1, aaaaCTCGAGctATGGCAAAGAATCCTCCAGAGAA (forward; SEQ ID NO: 32), aaaaGGATCCCTGTCTTAGAAAATAGGAGGAGGAT (reverse; SEQ ID NO: 33); TNMD Isoform 3, pcDNA3, aaaaGGATCCATGGAGCACACTTTCTACAGCAAT (forward; SEQ ID NO: 34), aaaaCTCGAGGACCCTCCCCAGCATGCGGGC (reverse; SEQ ID NO: 35), pEGFP-C1, aaaaCTCGAGctATGGAGCACACTTTCTACAGCAAT (forward; SEQ ID NO: 36), aaaaGGATCCGACCCTCCCCAGCATGCGGGC (reverse; SEQ ID NO: 37).

PCR products are purified with a QIAquick® PCR purification kit (QIAGEN) and cut with endonucleases Xho I and BamH I. The digested PCR products are purified with the PCR purification kit and ligated with Xho I-BamH I digested pcDNA3 or pEGFP-C1 vector DNA with T4 DNA ligase, respectively. The sequences of human TNMD isoforms are confirmed by DNA sequencing. Expression is confirmed by quantitative PCR and Western blot. Tnmd isoform expression is blocked using an RNAi approach (Qi et al., 2008). Human tenocytes are plated in each well of a 6-well plate at 200k cells per well the day before transfection in 2 mL Medium 199 (GIBCO®, Life

Technologies, Grand Island, New York, United States of America) per well, containing 10% fetal bovine serum, 1% PSF, 20 mM HEPES.

siRNAs (AMBION®, Life Technologies, Grand Island, New York, United States of America) recognizing human tenomodulin are transfected
5 into human tenocytes using Lipofectamine™ 2000 reagent according to the manufacturer's protocol. Briefly, for each well, 5 uL Lipofectamine™ 2000 reagent is mixed with 250 uL of siRNA transfection medium (Santa Cruz Biotechnology, Santa Cruz, California, United States of America) and either
10 100 pmole human tenomodulin siRNA or control group, scrambled, negative control siRNA, is mixed with another 250 uL of siRNA transfection medium. Each mixture is incubated at RT for 20 min. The siRNA-Lipofectamine™ 2000 complexes are added to each well in a 40 nM final concentration of siRNA and treated cell incubated for 72 h in a 5% CO₂ environment. At 72 h post-transfection, cells are collected; total RNA is isolated using an
15 RNeasy® kit (QIAGEN). cDNAs are synthesized with SuperScript® II enzyme (QIAGEN) and quantitative real time PCR performed with a Brilliant® SYBR® green QPCR kit (Agilent Technologies, Santa Clara, California, United States of America).

To assess potential antiangiogenesis function for Tnmd, three types
20 of experiments can be performed: 1. inhibition of endothelial cell migration, 2. inhibition of endothelial cell tube formation on Matrigel™ matrix, and 3. inhibition of VEGF-Astimulated angiogenesis on the chick chorioallantoic membrane (CAM) [Chen et al., 2010]. For the cell migration assay, human endothelial cells are plated on the top surface of a Boyden chamber
25 membrane and numbers of cells migrating through the pores are determined +/- conditioned medium from tenocytes containing Tnmd [Oshima et al., 2004]. To assess capillary tube formation on Matrigel™ matrix, endothelial cells are plated on Matrigel™ matrix in 24 well plates +/- human tenocytes in coculture wells [Oshima et al., 2004]. EC cultures are observed for
30 formation of capillary formation by day 3 post-plating and digital images collected for quantitative analysis of number of tubes, branch points and total area of tubes. Groups: n=8 EC cultures/group. Three patients' cells/group are used for each experiment (passage 2-3). Negative controls can include

supernatant fluids from Tnmd RNAi-treated cells. To assess Tnmd effects on angiogenesis *in vivo*, a modified chick chorioallantoic membrane model are used [Chen et al., 2010; Auerbach et al., 2003]. Embryonated White Leghorn chicks obtained from a North Carolina State University (Raleigh, North Carolina, United States of America) farm are set in an incubator with rocker control at 102°F with 70% humidified atmosphere. On day 8, eggs are candled and the air space region cleaned, opened and a transparent disc with confluent tenocytes are plated adjacent to a 10 nM VEGF-A- loaded Whatman filter disc (refreshed daily 5 days). Controls include VEGF-A discs alone, tenocytes discs alone, MC3T3-E1 cell control +/- VEGF-A. Digital images are collected on day 6 post-implantation in formalin-treated CAMs for capillary formation, branch points and total blood vessel area at the junctions of tenocytes and VEGF-A-loaded discs. Groups; 10 eggs per group; three patients' cells per experiment.

15

EXAMPLE 12

Cre-loxP Conditional Tnmd Knock-Out Mouse

A Tnmd Cre-loxP knock-out (KO) mouse with a β -galactosidase reporter gene, whose Tnmd ablation can be controlled to be global or specific to tendon, can provide an advance over the currently available global Tnmd ablation exon 1 mouse. Development of a novel Tnmd KO mouse can include: (1) targeting exons 3 and 4 which are common to the three human isoforms to totally ablate the mouse Tnmd gene, (2) use of the C57BL/6 genetic background to avoid any polymorphisms, (3) use of the conditional Cre-loxP knockout strategy that will allow production of a total Tnmd ablation in all tissues or a more specific ablation in tendon and tendon-like tissues, and (4) inclusion of a β -galactosidase gene that acts as a reporter gene mirroring Tnmd expression in relevant tissues. Overall, this construct yields a high degree of latitude in regulation of where and when the gene is ablated to monitor development changes globally or in dense connective tissues, tendon and ligament.

In addition, Tnmd expression in the wt or Tnmd heterozygotes (-/+) can be therapeutically increased or decreased with an antibody, given

regulation by Il-1b, or ADP, ATP or UTP compounds, to modulate the purinoceptor signaling cascade and downstream, regulate Tnmd expression, as has been done with UTP drugs and the CFTR gene in cystic fibrosis, dry eye disease, thrombosis and cardiovascular disease [Jacobson et al., 2010].

- 5 Further tests are performed concerning a role in angiogenesis, cell proliferation as well as other possible functions, such as a role in inflammation, for this tenocyte biomarker.

The overall strategy utilizes the Cre-loxP conditional Tnmd KO mouse to compare the global null KO to the conditional KO, as well as test function
10 of tendon tissue response by a wound and mechanical load challenge. A Tnmd conditional gene targeting vector is obtained from the federally-funded Knockout Mouse Project (KOMP). The vector contains an FRT-flanked splice-acceptor beta-galactosidase module in intron 2 of the Tnmd gene and loxP recombination sites flanking exons 3 and 4 of the Tnmd gene. Exons 3
15 and 4 are common to all known splice forms of human Tnmd, and deletion of exons 3 and 4 is predicted to cause a frameshift and nonsense-mediated decay of the Tnmd transcript. The Tnmd vector is electroporated into C57BL/6 mouse embryonic stem cells and targeted clones are identified by PCR and Southern blot analysis. Targeted clones are injected into albino-
20 C57BL/6 blastocysts to generate chimeras that transmit the mutant allele through the germline. Offspring carrying the Tnmd mutant allele can express beta-galactosidase in the Tnmd expression domain, and are used to analyze Tnmd expression patterns.

To create the LoxP-flanked conditional allele, the Tnmd-mutant mice
25 are mated to Flpe transgenic mice to remove the splice-acceptor beta-galactosidase and neo cassettes. The resulting animals are mated to a global Cre recombinase deleted strain to produce the null allele. Animals heterozygous for the null-allele are intercrossed to produce Tnmd-null mice for phenotyping.

30 Initial experiments are conducted to characterize the global versus the conditional Tnmd knockout mouse at P1, P14, P60, by body weight, gross connective tissue defects after Alizarin red (skeletal defects) and toluidine blue staining (tendon defects), paraffin and frozen sections for

gross hematoxylin and eosin staining and specific immunochemical staining for Tnmd, collagens 1, 3, 6, 14, decorin, Scx, Mlx, respectively, transmission electron microscopy studies for collagen fibril diameters, gene array analysis, quantitative polymerase chain reaction for expression analysis of specific matrix and regulatory genes, Tnmd protein studies, biomechanical properties of tendons and bone. Specifically, genotyped offspring that are wt, null mutation for Tnmd, or conditional KO, are identified after analysis of tail tissue by QPCR, fixed in 4% glutaraldehyde, rinsed and stained with Alizarin red or toluidine blue, photographed and analyzed for gross pathologic differences in skeletal and tendon development. More detailed analyses are performed after tissue dehydration, paraffin embedding sectioning at 6 microns, and serial sections stained with H&E, Gomori's one step for collagen, Mallory's triple stain and studied by light microscopy.

Separate mice collected for immunochemical studies are embedded in OCT, frozen then 10 micron sections made with a freezing microtome, serial sections stained with primary antibodies to tenomodulin and the matrix and regulatory proteins as above, secondary antibodies applied, then sections studied by fluorescence microscopy and photographs recorded. In addition, sections are developed for β -galactosidase, studied by light microscopy and regions of interest, specifically in tendon, photographed.

Analyses of collagen fibril diameters are made by tissue fixation in 4% glutaraldehyde, embedding in epon, thin sections made with an ultramicrotome and captured on electron microscopy grids, sections stained with 2% uranyl acetate and post-stained with 2% phosphotungstic acid, then examined with a TEM. Achilles tendon and tail tendon sections with collagen fibrils oriented longitudinally and in cross-section are photographed, fibril diameters measured by image analysis and compared to wt.

Gene array analyses are performed using cDNAs from wt, null and conditional null Achilles tendons using Affymetrix gene arrays. Biomechanical studies can be performed on skin, tibias and Achilles tendons from P60 wt and Tnmd null and conditional KO mice. Shaved, dumbbell-shaped, duplicate skin specimens are collected from the dorsum, ink-marked normal to the sagittal plane, cross-sectional area determined using a two-

camera system at the ELF-Enduratec™ 3200 materials testing machine, skin glued and clamped in sandpaper with grip to grip distance of 10 mm and a series of ink dots at mm increments made so that optical tracking could be recorded by the system. Specimens are clamped in fixtures, preloaded to
5 0.03 N and loaded to failure at 0.33 mm/s (1.67%/s; [Ansorge et al., 2008; Christner et al., 2006]. Samples can be sprayed with a fine aqueous mist during the test and maintained at 37°C.

Achilles tendons are dissected from both rear legs, muscle and bone clamped in custom fixtures as above with approximately 10 mm from grip to
10 grip, optical tracking marks made in the tendon length, the specimens preloaded to 0.1 N, stress-relaxed, then ramped to failure at 100%/s [87, 88]. Tail tendon fascicles are also tested but at a strain rate of 50%/s [87]. Tibias are dissected free of soft tissue, ends potted in epoxy, ink marked along the shaft and clamped in custom fixtures. Specimens are subjected to
15 conditioning as above then pulled to failure in the ELF 3200 load frame with the custom two camera system to record specimen dimensions at the start and during the test [Garvin et al., 2003]. Values for maximum stress and modulus of elasticity are calculated for all specimens, values subjected to statistical treatment using one-way ANOVA on cross-sectional area,
20 maximum load, maximum stress, elastic modulus compared across wt and mutants for each group.

Mice receive 150 mg/kg ketamine, 10 mg/kg xylazine in sterile saline via intramuscular injection in the right thigh, then a 1 cm, vertical incision are made medial to the posterior mid-line above the calcaneus. The Achilles
25 tendon are isolated from the underlying muscle using blunt dissection and a mid-substance tenotomy are created in the left side, with a right side sham control, and both repaired using a 6-0 monofilament polypropylene suture. As a control, right side sham surgeries are performed.

Control wt, Tnmd null, and conditional null mice are exercised by
30 treadmill running. Mice are initially trained for a period of 15 min/day at 13 meters/min for one week (Exer-6M, Open Treadmill; Columbus Instruments, Columbus, Ohio, United States of America). Then treadmill running groups are exercised at 13 meters/min, for 50 min/day, 5 days/week, 3 weeks while

control groups have free cage movement [Zhang et al., 2010]. Six specimens and contralateral sham controls are used for biomechanical measurements as above.

REFERENCES

The references listed below, as well as all references cited in the specification, including patents, patent applications, journal articles, and all database entries (e.g., GENBANK® database entries, including any annotations presented in the databases associated with the disclosed sequences), are incorporated herein by reference to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

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- 5 It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

10

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a tenomodulin polypeptide.
- 5 2. An isolated nucleic acid sequence of claim 1, comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-3 and a nucleic acid sequence having at least a 90% sequence identity to one of SEQ ID NOs: 1-3, optionally over its entire length.
- 10 3. An isolated nucleic acid sequence of claim 1, coding for a protein comprising an amino acid sequence of one of SEQ ID NOs: 4-6.
4. A recombinant vector comprising the nucleic acid sequence of one of claims 1-3.
- 15 5. A recombinant host cell or stem cell containing the nucleic acid sequence of one of claims 1-3.
6. An isolated tenomodulin polypeptide, optionally one or more of isoforms 1, 2 or 3 or a combination thereof.
- 20 7. An isolated tenomodulin polypeptide of claim 6, comprising an amino acid sequence of one of SEQ ID NOs: 4-6 or a sequence having a 90% or greater sequence identity to one of SEQ ID NOs: 4-6, optionally over its entire length.
- 25 8. A recombinant cell expressing the polypeptide of claim 7.
9. The polypeptide of claim 7, modified to be in detectably labeled form.
- 30 10. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 7.

11. The antibody of claim 10, wherein the antibody is capable of modulating the biological activity of the polypeptide.

5 12. A hybridoma cell line which produces the antibody of claim 10.

13. A method of producing an antibody immunoreactive with a tenomodulin polypeptide, the method comprising:

transfecting a recombinant host cell with a nucleic acid molecule of
10 any of claims 1-3 which encodes a tenomodulin polypeptide;

culturing the host cell under conditions sufficient for expression of the polypeptide;

recovering the polypeptide; and

preparing an antibody to the polypeptide;

15 and optionally detecting an antibody to one or more of the tenomodulin isoforms.

14. A method of detecting a tenomodulin polypeptide, the method comprising immunoreacting the polypeptide with an antibody of claim 10 or
20 an antibody prepared according the method of claim 13 to form an antibody-polypeptide conjugate; and detecting the conjugate.

15. The method of claim 14, wherein the detecting of the tenomodulin polypeptide further comprises detecting a tenocyte based on
25 the detecting of the tenomodulin polypeptide.

16. The method of claim 14, wherein the detecting of the tenomodulin polypeptide comprises quantitating or staging onset or progression of a connective tissue disease, optionally dense connective
30 tissues, optionally, tendons or ligaments

17. A method of detecting a nucleic acid molecule that encodes a tenomodulin polypeptide in a biological sample containing nucleic acid material, the method comprising:

hybridizing a nucleic acid molecule having a sequence complementary to at least a portion of a nucleic acid sequence of any one of claims 1-3 under stringent hybridization conditions to the nucleic acid material of the biological sample, thereby forming a hybridization duplex; and
5 detecting the hybridization duplex.

18. The method of claim 17, wherein the detecting of the tenomodulin polypeptide further comprises detecting a tenocyte based on the detecting of the nucleic acid molecule.

10

19. The method of claim 18, wherein the detecting of the tenomodulin polypeptide comprises quantitating or staging onset or progression of a connective tissue disease, optionally dense connective tissues, optionally, tendons or ligaments

15

20. An assay kit for detecting the presence of a tenomodulin polypeptide or antibody to tenomodulin isoforms in a biological sample, the kit comprising a first container comprising a first antibody capable of immunoreacting with a polypeptide of claim 6 or an antibody of claim 10.

20

21. The assay kit of claim 20, further comprising a second container containing a second antibody that immunoreacts with the first antibody.

25

22. The assay kit of claim 21, wherein the first antibody and the second antibody comprise monoclonal antibodies.

23. The assay kit of claim 21, wherein the first antibody is affixed to a solid support.

30

24. The assay kit of claim 21, wherein the first and second antibodies each comprise an indicator.

25. The assay kit of claim 24, wherein the indicator is a radioactive label or an enzyme.

26. An assay kit for detecting the presence, in a biological sample,
5 of an antibody immunoreactive with a tenomodulin polypeptide, the kit comprising a polypeptide of claim 6 that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay, optionally comprising a label for the polypeptide, optionally wherein the label can be detected by an imaging technique, such as but not limited to
10 MRI or CT.

27. An assay kit for detecting the presence, in biological samples, of a tenomodulin polypeptide, the kit comprising a first container that contains a nucleic acid molecule identical or complementary to a segment of
15 at least ten contiguous nucleotide bases of the nucleic acid molecule of any of claims 1-3.

28. A method of modulating the biological activity of a tenomodulin polypeptide in a biological sample, the method comprising contacting the
20 biological sample with an agent for modulating expression, activity or both expression and activity of a tenomodulin polypeptide.

29. The method of claim 28, wherein the biological sample is a tissue present in a subject.

25

30. The method of claim 28 or 29, wherein the biological sample is a tendon, ligament or other dense connective tissue.

31. The method of any one of claims 28, 29 or 30, wherein the
30 modulating of the tenomodulin polypeptide biological activity regulates the growth of collagen fibrils, optionally wherein a ratio of large and small fibrils is controlled to a more native state.

32. The method of claim 29, wherein the tissue present in a subject is an abnormal tissue.

33. The method of claim 32, wherein the modulation of tenomodulin polypeptide expression reduce growth of the abnormal tissue, optionally by blocking angiogenesis.

34. A method of screening candidate substances for an ability to modulate activity, expression or both activity and expression of a tenomodulin polypeptide, the method comprising:

providing a test sample comprising a polypeptide of claim 6 or a nucleic acid encoding a polypeptide of claim 6;

administering a test molecule to the test sample; and

determining the effect of the test molecule on the activity, expression or both activity and expression of the polypeptide.

35. The method of claim 34, wherein the test molecule is selected from the group consisting of a polypeptide, a nucleic acid oligonucleotide, optionally an siRNA to one or more of the tenomodulin isoform mRNAs, an exogenous vector coding for a nucleic acid oligonucleotide or polypeptide, a carbohydrate, a lipid, an amino acid, a fatty acid, a steroid, and a low molecular weight organic molecule.

36. The method of claim 34, wherein determining the effect of the test molecule on the activity, expression or both activity and expression of the polypeptide comprises measuring a first activity, expression or both activity and expression level of the polypeptide prior to administering the test molecule to the test sample, measuring a second activity, expression or both activity and expression level of the polypeptide after administering the test molecule to the test sample, and comparing the first and second levels.

37. The method of claim 34, wherein the test sample comprises a cell.

38. The method of claim 37, wherein the polypeptide is provided to the cell from an exogenous source.

5 39. The method of claim 37, wherein the cell expresses the polypeptide.

40. The method of claim 39, wherein the cell is a recombinant cell.

10

41. The method of claim 34, wherein the test sample comprises a non-human animal.

15 42. The method of claim 41, wherein the animal is a genetically modified animal.

43. A method of diagnosing a tendon injury, the method comprising:

20 providing a subject;
collecting a biological sample from the subject; and
detecting a tenomodulin in the sample.

44. The method of claim 43, wherein the subject is a human subject.

25

45. The method of claim 43, wherein the biological sample is selected from the group consisting of tendon, connective tissue and ligament.

30 46. The method of claim 43, wherein detecting a tenomodulin in the sample comprises detecting the expression of a tendomodulin gene.

47. The method of claim 46, wherein the tenomodulin gene is

selected from the group consisting of isoform 1, isoform 2 and isoform 3.

48. The method of claim 46, wherein an altered expression of a tenomodulin gene, as compared to a healthy subject, is indicative of a tendon injury.

49. The method of claim 43, wherein detecting a tenomodulin in the sample comprises detecting the concentration of a tendomodulin polypeptide.

10

50. The method of claim 49, wherein an altered concentration of a tenomodulin polypeptide, as compared to a healthy subject, is indicative of a tendon injury.

15

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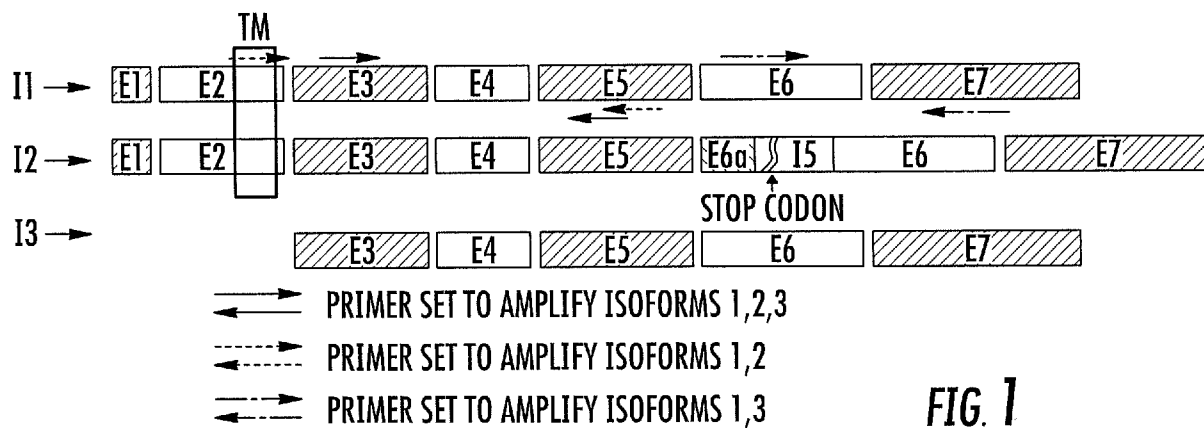


FIG. 1

FIG. 2A

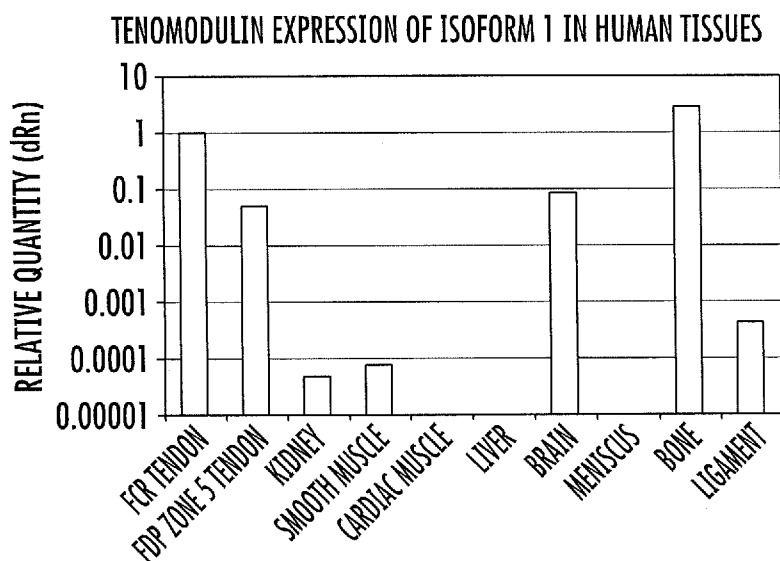
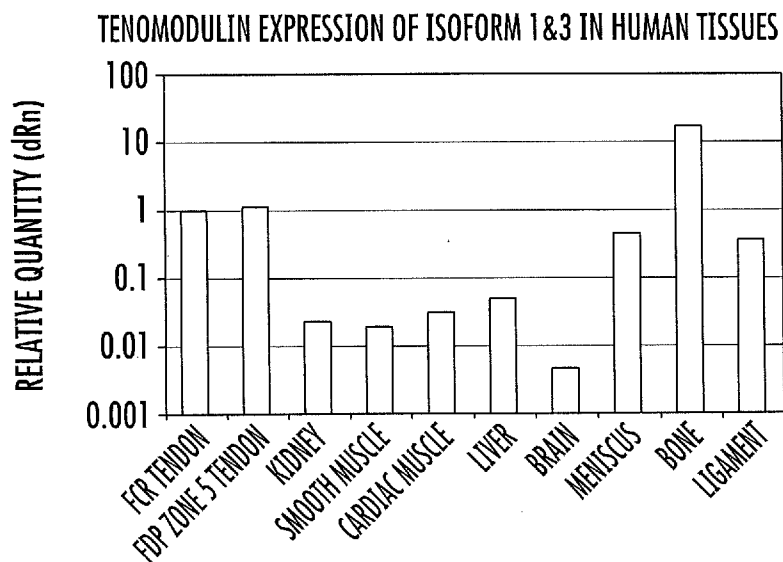


FIG. 2B



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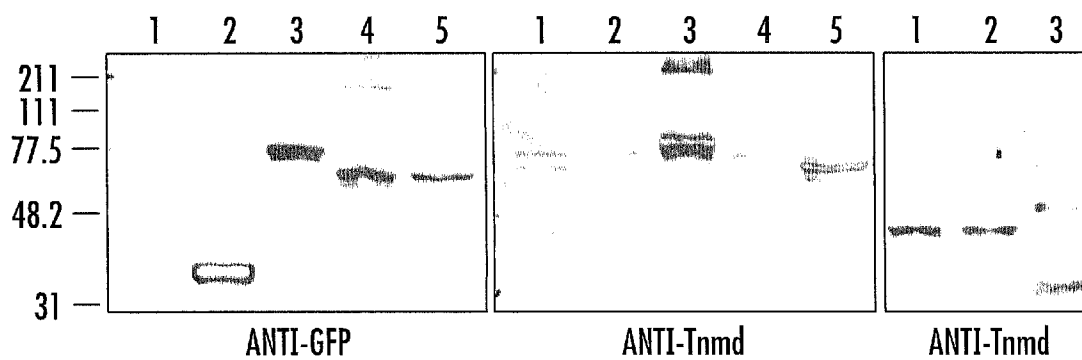


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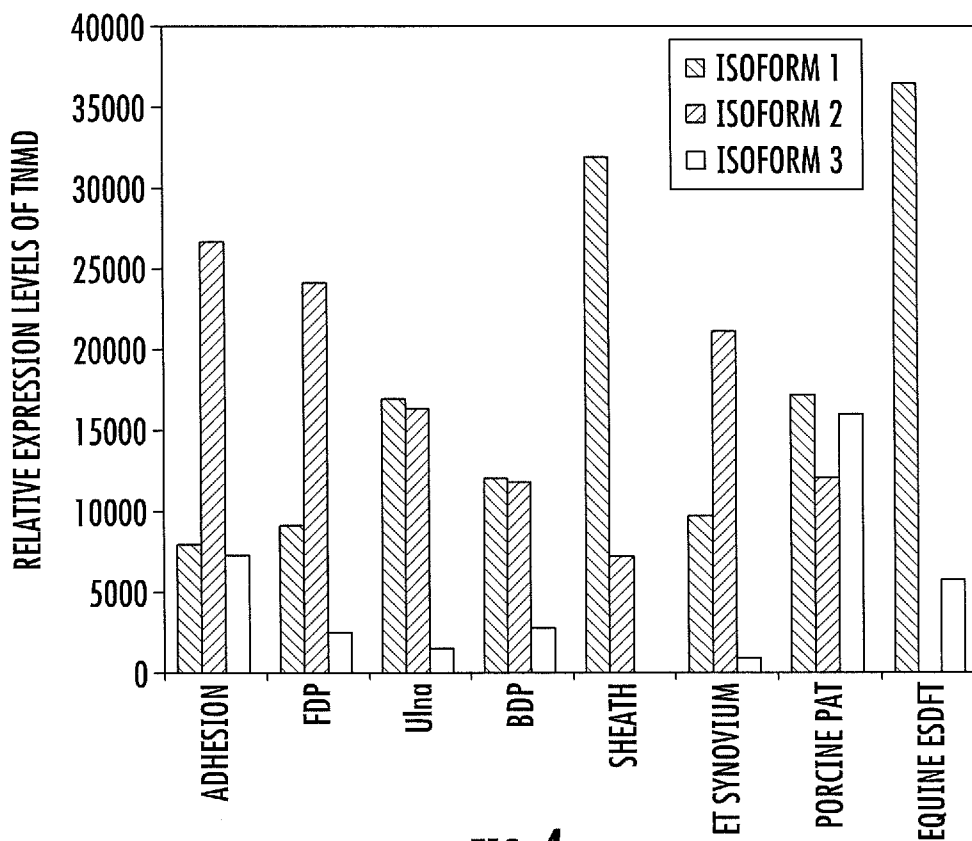
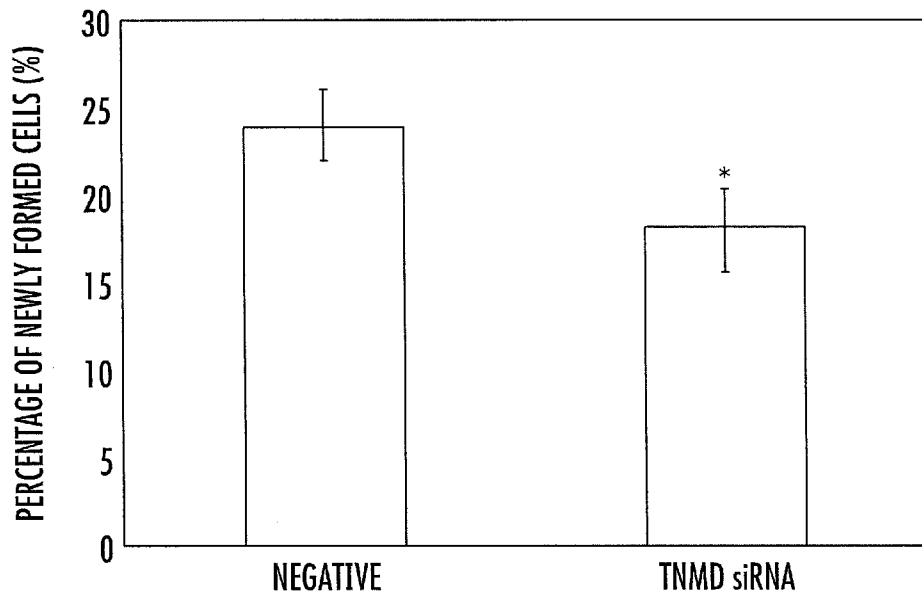
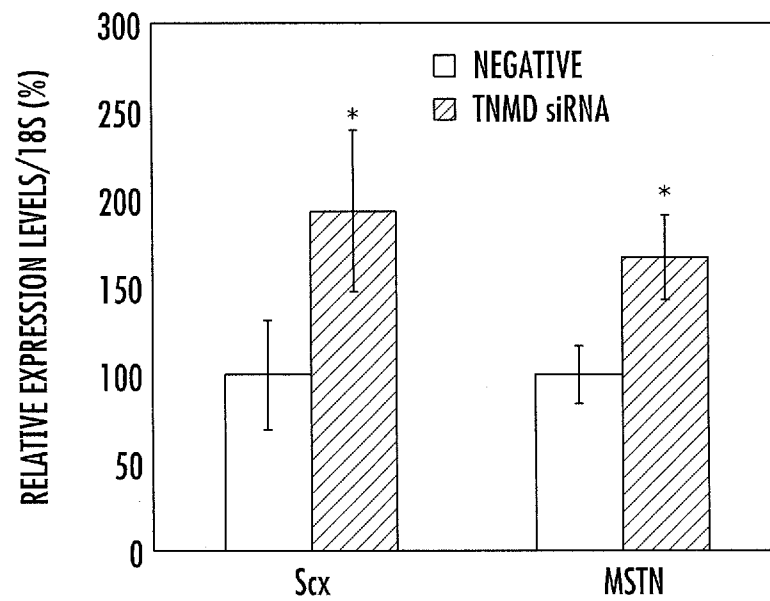
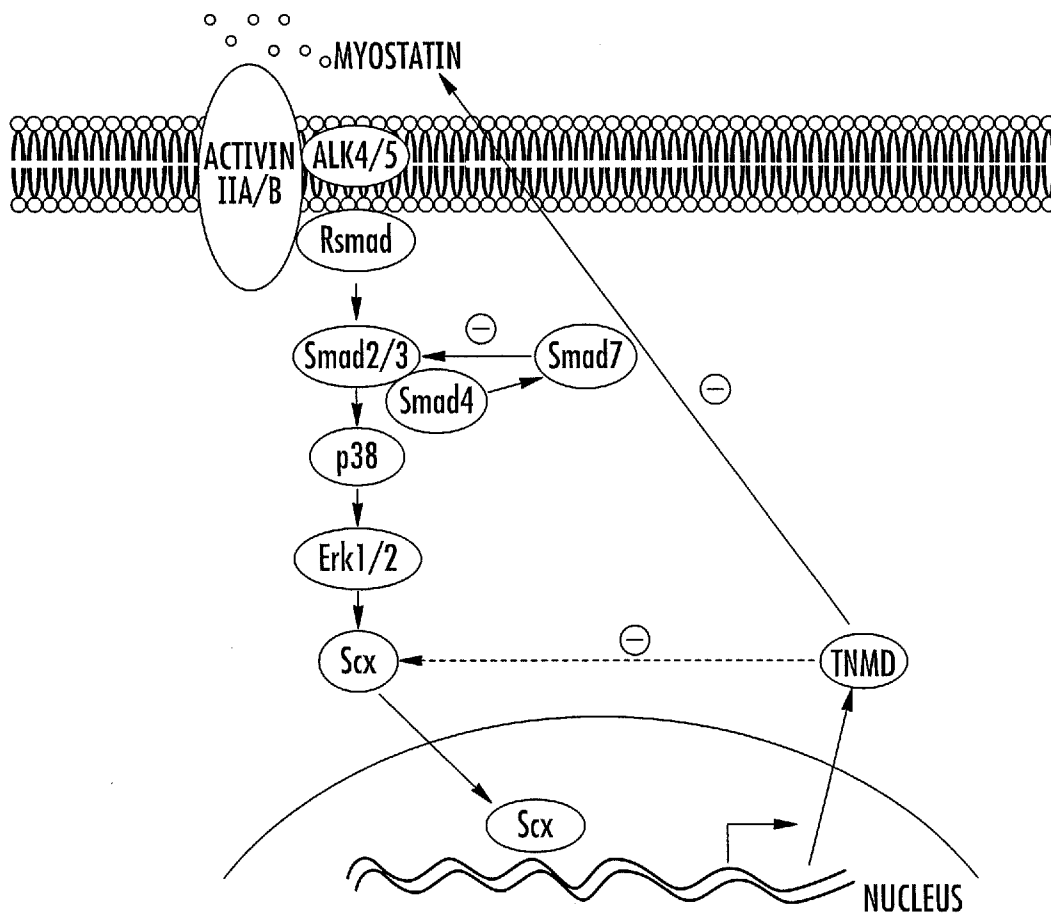


FIG. 4

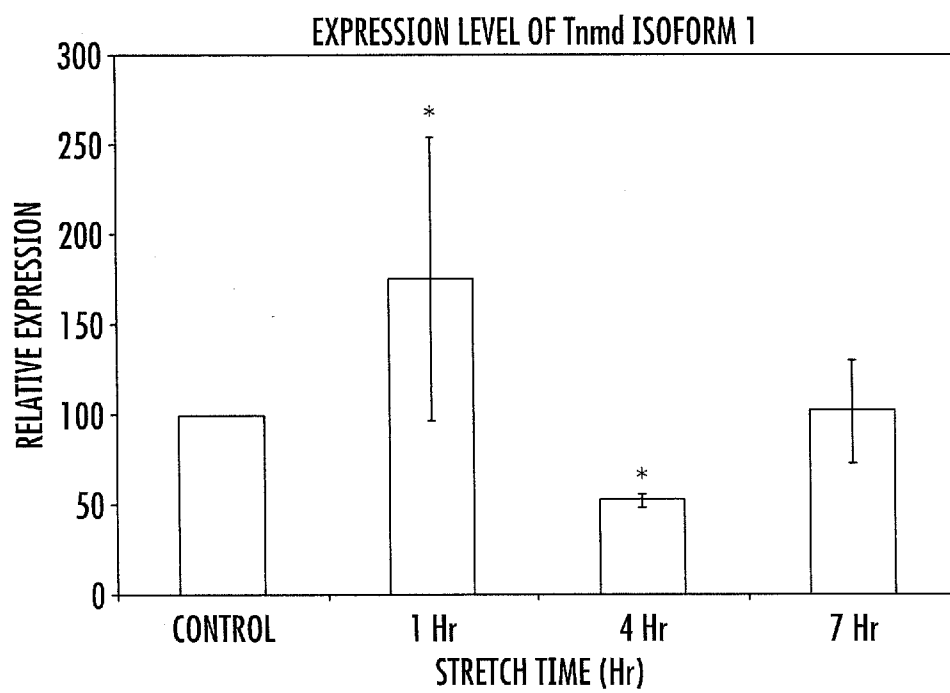
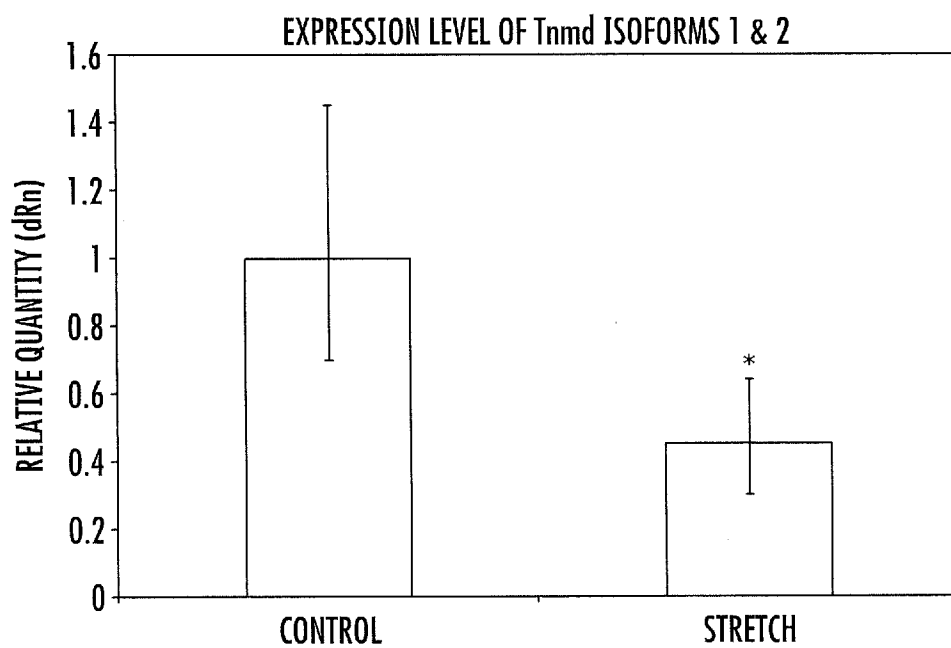
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**FIG. 5A****FIG. 5B**

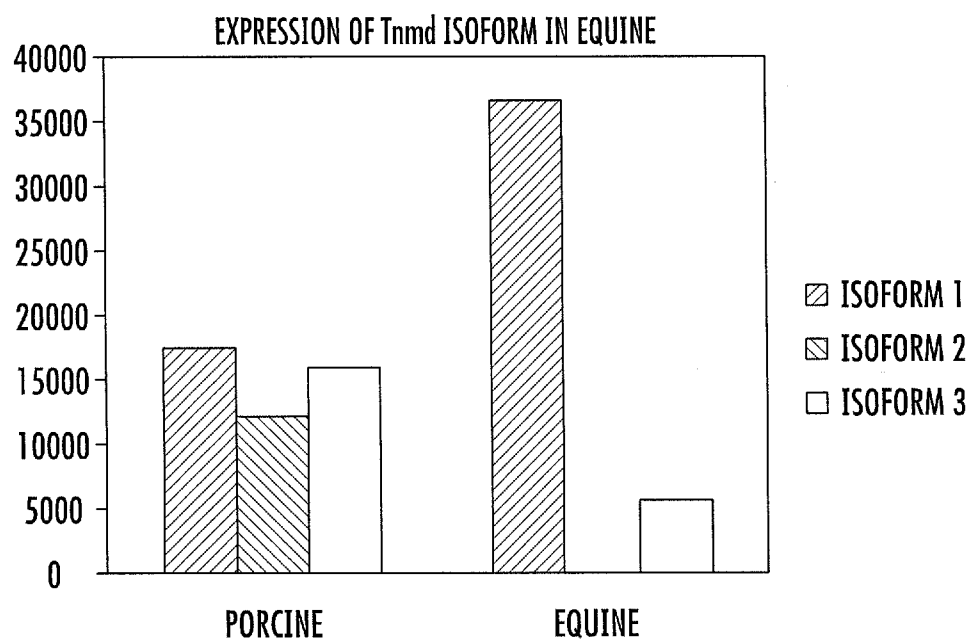
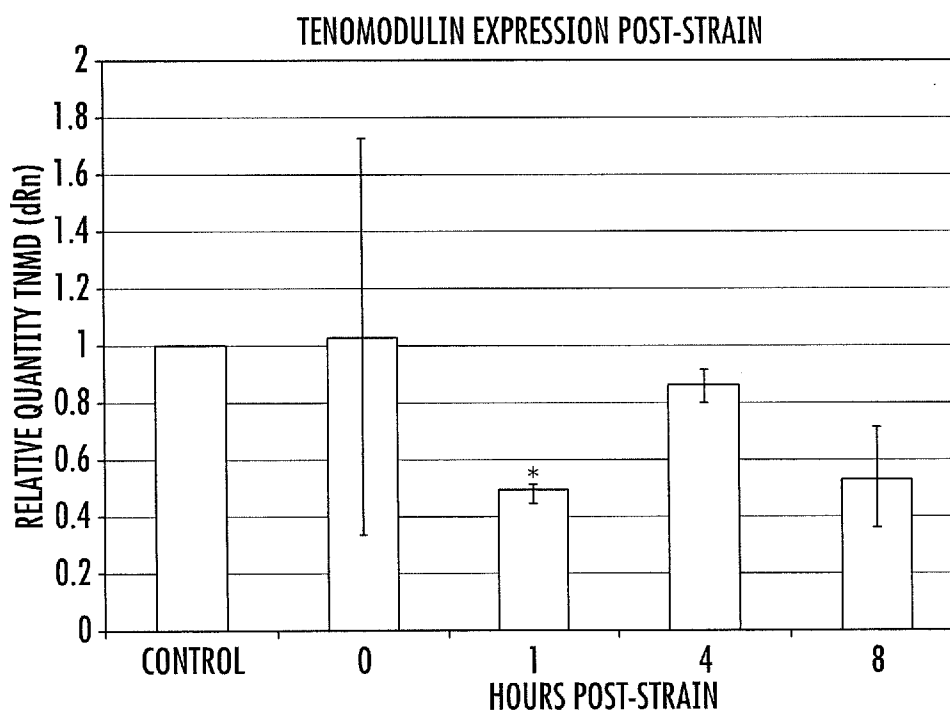
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**FIG. 6**

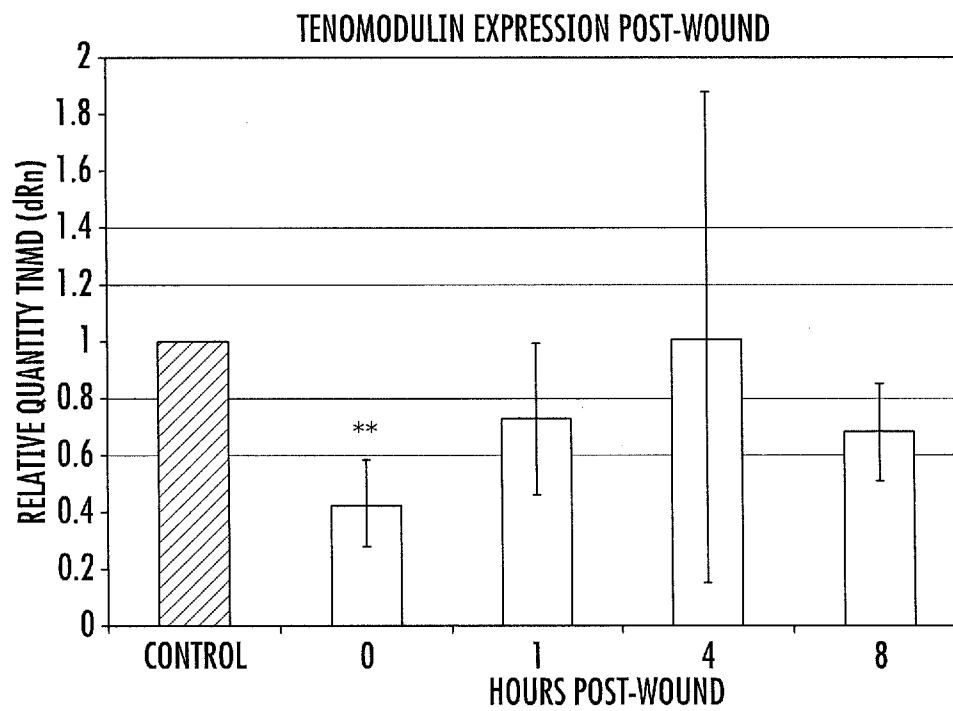
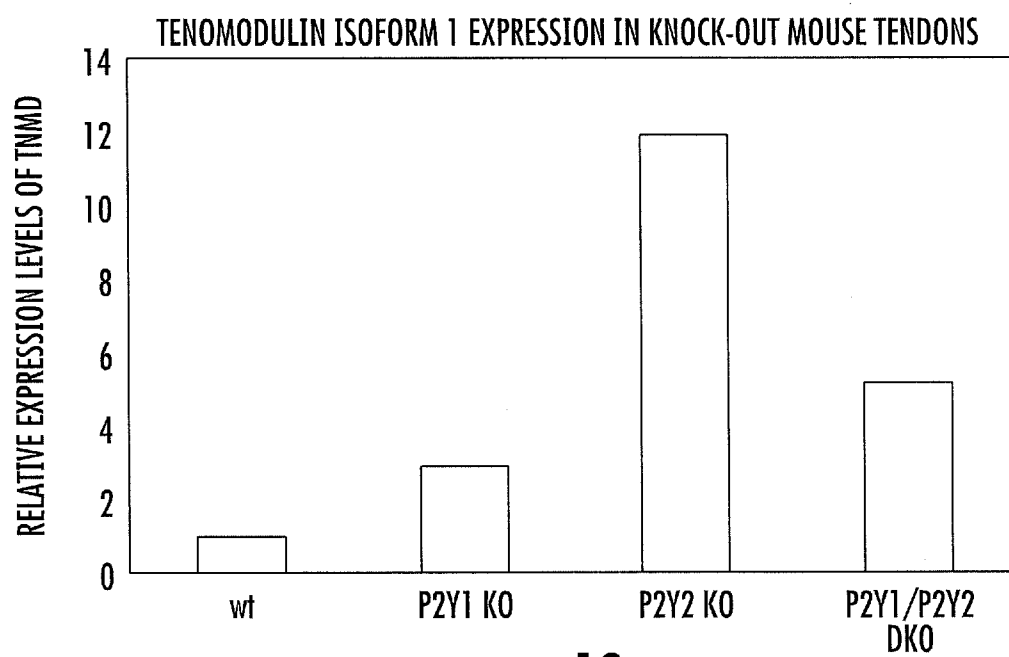
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**FIG. 7****FIG. 8**

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**FIG. 9****FIG. 10**

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**FIG. 11****FIG. 12**

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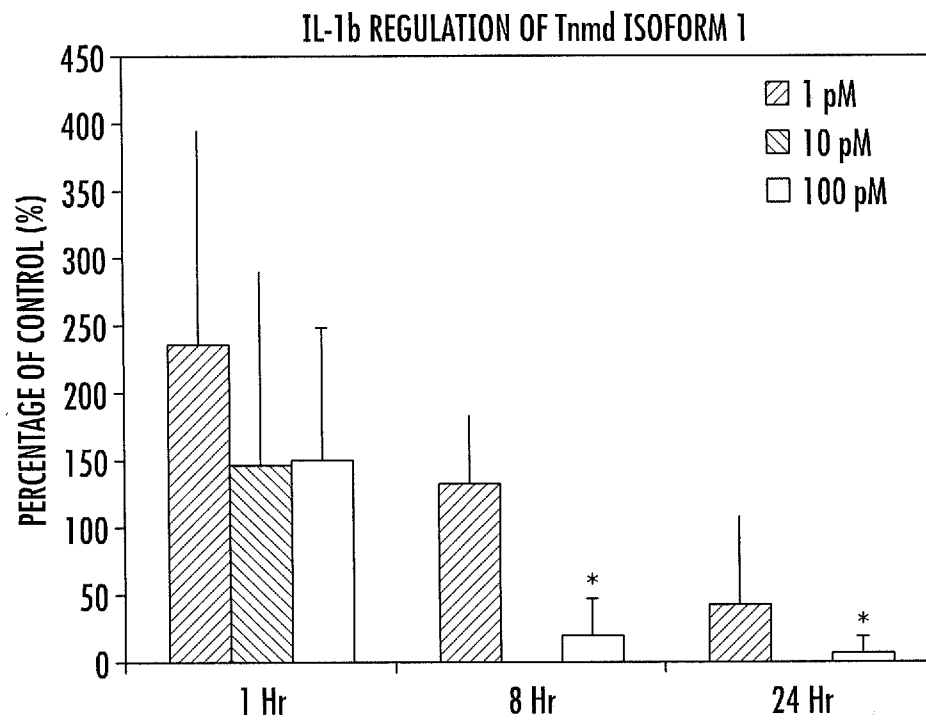
**FIG. 13**

FIG. 14

Tnmd: Highly Conserved Sequences Across Species

Horse	MAKNPPENCEDCHILNAEAFKSKICKSLKICGLVFGILALILIVLFWGKHFWEIPKK	60
Porcine	MAKNPPENCEDCHILNAEAFKSKICKSLKICGLVFGILALILIVLFWGKHFWEIPKK	60
Human	MAKNPPENCEDCHILNAEAFKSKICKSLKICGLVFGILALILIVLFWGKHFWEIPKK	60

Horse	TYDMEHTFYSNGEKKKIYMEIDPVTRTEIFRSNGTDEITLEVHDFKNGYTGIFYVGLQKC	120
Porcine	TYDMEHTFYSNGEKKKIYMEIDPVTRTEIFRSNGTDEITLEVHDFKNGYTGIFYVGLQKC	120
Human	AYDMEHTFYSNGEKKKIYMEIDPVTRTEIFRSNGTDEITLEVHDFKNGYTGIFYVGLQKC	120

Horse	FIKTIQKVIPEFSEPEEEIDENEIITITFEQSVIWPVPAEKPIENRDFLKNKSKILEICDN	180
Porcine	FIKTIQKVIPEFSEPEEEIDENEIITITFEQSVIWPVPAEKPIENRDFLKNKSKILEICDN	180
Human	FIKTIQKVIPEFSEPEEEIDENEIITITFEQSVIWPVPAEKPIENRDFLKNKSKILEICDN	180

Horse	VIMYWINETLLIYVSELQDFEEDGEDLHFFTNDDKKGIEQNEQWVQVAVKTRRARQASE	240
Porcine	VIMYWINETLLIYVSELQDFEEDGEDLHFFTNDDKKGIEQNEQWVQVAVKTRRARQASE	240
Human	VIMYWINETLLIYVSELQDFEEDGEDLHFFTNDDKKGIEQNEQWVQVAVKTRRARQASE	240

Horse	EELPINDYTGIEFDFMLDERGYCCICYCRRGNRYCRRVCEPLLGYPPYCYQGGRVIC	300
Porcine	EELPINDYTGIEFDFMLDERGYCCICYCRRGNRYCRRVCEPLLGYPPYCYQGGRVIC	300
Human	EELPINDYTGIEFDFMLDERGYCCICYCRRGNRYCRRVCEPLLGYPPYCYQGGRVIC	300

Horse	RVIMPCNWWVARMGLRV	317
Porcine	RVIMPCNWWVARMGLRV	317
Human	RVIMPCNWWVARMGLRV	317
