# PTD-SMAD7 THERAPEUTICS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims priority to U.S. provisional patent application USSN 61/775,252, filed March 8, 2013, which is incorporated by reference herein in its entirety.

## STATEMENT OF GOVERNMENT INTEREST

**[0002]** This invention was made with government support under grant number AR061796 awarded by the National Institutes of Health. The government has certain rights in the invention.

## SEQUENCE LISTING

[0002b] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 29, 2014, is named 089491-0303_SL.txt and is 120,505 bytes in size

## BACKGROUND

**[0003]** Oral mucositis, a severe oral ulceration, is a common adverse effect of a large dose of radiation for bone marrow transplant or craniofacial radiotherapy for cancer. Severe oral mucositis could require feeding tubes, management of severe pain, and prematurely halting radiotherapy. Excessive inflammation and epithelial ablation are key features of oral mucositis.

**[0004]** Palifermin, a KGF (human keratinocyte growth factor) recombinant protein, is approved for preventing oral mucositis in bone-marrow transplant patients. Two Palifermin clinical trials in head and neck cancer patients showed that Palifermin reduced severe oral mucositis incidence from 67% and 69% to 51% and 54%, respectively. Other oral mucositis drugs in clinical trials or pre-clinical studies include growth factors, agents for radioprotection, anti-inflammatory agents or immune modulators.

**[0005]** The modest effects of Palifermin and drugs being developed in the above mentioned categories highlight the need for identification of biomarkers for novel therapies. However, the lack of routine diagnostic biopsies or discarded tissues from oral mucositis patients has hindered this effort.

**[0006]** Cutaneous wound healing progresses through three overlapping phases: inflammation, tissue formation, and tissue remodeling. These are dynamic processes that involve interactions among the epidermis, leukocytes, extracellular matrix (ECM), and dermal fibroblasts. In response to skin injury, blood clots, infiltrated inflammatory cells and other cell types in the wound release multiple cytokines and chemokines. These cytokines

84:1259-1270, 2004). However, adenoviral vector delivery of Smad7 to balloon injury in rat carotid arteries resulted in reduced vascular healing (Mallawaarachchi *et al.*, *Arterioscler Thromb Vasc Biol* 25: 1383-1387, 2005). These studies suggest that the effects of TGF-β signaling components, such as Smad7, on wound healing are complex and highly context-specific. Additionally, the effect of Smad7 may not always be explained by its role in TGF-β signaling. For instance, Smad7 has also been shown to interact with components of the Wnt/β-catenin (Han *et al.*, *Dev Cell Biol* 11:301-312, 2006) and the TNFβ/NF-κB (Hong *et al.*, *Nat Immunol* 8:504-513, 2007) families.

## SUMMARY

**[0010]** The present technology provides a nucleic acid molecule comprising a codon-optimized human Smad7 cDNA nucleotide sequence.

**[0010a]** In one aspect, there is provided a nucleic acid molecule comprising a codon-optimized human Smad7 cDNA nucleotide sequence, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codons for serine, one or more codons for histidine, and one or more codons for arginine optimized for expression in one or more of bacteria, yeast, or mammalian cells.

**[0010b]** In some embodiments, the codon-optimized human Smad7 nucleotide sequence may include one or more codons for arginine optimized for expression in one or more of bacteria or yeast, including one or more codons for serine optimized for expression in one or more of bacteria or yeast, and/or including one or more codons for histidine optimized for expression in one or more of bacteria or yeast. In some embodiments, the codon-optimized human Smad7 nucleotide sequence may include 28 serine codons, 6 histidine codons, and 9 arginine codons optimized for expression in one or more of bacteria or yeast. In some embodiments, the codon-optimized human Smad7 nucleotide sequence may be selected from the group consisting of SEQ ID NOs: 9, 21, 23, 24, 26, 28, 30, 32-34, 36, 38, 39, 87, 89, 91, 93, 96, 97, 99, and 100~~-10, 11, 21, 23-41~~. In some embodiments, the codon-optimized human Smad7 nucleotide sequence may have about 65 to 75 percent homology to human Smad7 cDNA, may comprise a nucleotide sequence encoding an N-terminal fragment SMAD7, may comprise a nucleotide sequence encoding a C-terminal fragment of SMAD7, may comprise nucleotides encoding amino acids 2-258 of the human Smad7 protein, may comprise nucleotides encoding amino acids 259-426 of the human Smad7 protein, or may comprise nucleotides encoding amino acids 204-258 of the human Smad7 protein. In some embodiments, any of the foregoing may further comprise a nucleotide sequence encoding a protein transduction domain, such as Tat. In some embodiments, any of the foregoing may also further comprise a nucleotide sequence encoding one or more of an epitope tag or a purification tag, such as V5, glutathione-S-transferase, or 6-Histidine (SEQ ID NO: 40).

**[0011]** In some embodiments, any of the foregoing may be isolated and/or purified. In some embodiments, any one of the foregoing may also encode a polypeptide having one or more biological activities selected from the group consisting of reducing or eliminating phosphorylation of Smad2, reducing or eliminating nuclear translocation of the NF-κB p50 subunit, increasing cell proliferation, reducing apoptosis, reducing radiation-induced DNA damage, reducing inflammation, reducing angiogenesis, promoting healing in oral mucositis, promoting wound healing, and treating auto-immune disease.

[0011a] ~~In some embodiments,~~ In one aspect, there is provided a pharmaceutical compositions comprising the nucleic acid molecules as defined above and one or more pharmaceutically acceptable excipients ~~are provided~~. In some embodiments, expression vectors comprising the nucleic acid molecules above operably linked to a promoter are provided, as are host cells comprising such expression vectors, and pharmaceutical compositions comprising such vectors and host cells with one or more pharmaceutically acceptable excipients.

[0011b] In one aspect, there is provided a pharmaceutical composition comprising the protein molecule as defined herein, and one or more pharmaceutically acceptable excipients.

**[0012]** In one aspect, there is provided a protein molecule comprising a human Smad7 protein having leucine at position 216 ~~is provided~~. In some embodiments, the human Smad7 protein may be truncated at the C-terminal, or truncated at the N-terminal. In some embodiments, the truncated human Smad7 protein may include about 50% of the full-length Smad7 sequence, or may include about 13% of the full-length Smad7 sequence.

[0012a] In one aspect, there is provided a protein molecule comprising a human Smad7 protein having amino acids 2-258 of the human Smad7 protein.

[0012b] In one aspect, there is provided a protein molecule comprising a human Smad7 protein having amino acids 259-426 of the human Smad7 protein.

[0012c] In some embodiments, the human Smad7 protein may comprise or consist of amino acids 2-258, amino acids 204-258, or amino acids 259-426 of the human Smad7 protein. In some embodiments, the protein molecule may have one or more biological activities selected from the group consisting of reducing or eliminating phosphorylation of Smad2, reducing or eliminating nuclear translocation of the NF-κB p50 subunit, increasing cell proliferation, reducing apoptosis, reducing radiation-induced DNA damage, reducing inflammation, reducing angiogenesis, promoting healing in oral mucositis, promoting wound healing, and treating auto-immune disease. In some embodiments, any of the foregoing may further comprise a protein transduction domain, such as Tat. In some embodiments, any of the foregoing may also further comprise one or more of an epitope tag or a purification tag, such as V5, glutathione-S-transferase or 6-histidine (SEQ ID NO: 40). In some embodiments, a pharmaceutical composition comprising any of the foregoing, a protein molecule, and one or more pharmaceutically acceptable excipients is provided.

[0012d] In one aspect, there is provided the use of the pharmaceutical composition as defined herein in the manufacture of a medicament for the treatment or prevention of an inflammatory condition in a subject.

[0013] In another ~~aspect~~embodiment, a method is provided for treating or preventing an inflammatory condition in a subject comprising providing to the subject a therapeutically effective amount of the pharmaceutical composition described above. In some embodiments, the inflammatory condition may be one or more of a chronic wound, skin inflammation, psoriasis, or an autoimmune disease. In some embodiments, the composition may reduce inflammation through inhibition of TGF-β and NF-κB signaling.

[0013a] In one aspect, there is provided the use of the pharmaceutical composition as defined herein in the manufacture of a medicament for the prevention or treatment of a disease or disorder in a subject comprising one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage in the subject, wherein one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage is useful in preventing or treating the disease or disorder.

[0014] In another ~~aspect~~embodiment, a method is provided for preventing or treating a disease or disorder in a subject comprising one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage in the subject comprising providing to the subject a therapeutically effective amount of the pharmaceutical composition as described above, wherein one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage is useful in preventing or treating the disease or disorder. In some embodiments, the disease or disorder may include one or more of chronic wounds, acute wounds, or mucositis. In some embodiments, the chronic wounds may include one or more of diabetic ulcers, pressure ulcers, venous ulcers, or oral ulcers, the acute wounds may include one or more of trauma-induced wounds, surgical wounds, or scarring, the mucositis may include one or more of radiation-induced mucositis or chemotherapy-induced mucositis and the mucositis may include one or more of oral mucositis or gut mucositis.

[0015] It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

[0016] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The word "about" means plus or minus 5% of the stated number.

[0017] Other objects, features and advantages of the present technology will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the present technology, are given by way of illustration only, since various changes and modifications within the spirit and scope of the present technology will become apparent to those skilled in the art from this detailed description.

was used for counterstain. **FIG. 9D** provides an illustrative embodiment of *in vitro* cell migration assay for Smad7 transgenic and WT keratinocytes after Rac1 knockdown. Pairs of dotted lines delineate the scratch wound. Quantification of cell migration is presented in **FIG. 2G**.

**[0028]** **FIGS. 10A-F** provide an illustrative embodiment of data showing Smad7 increased Rac1 expression by repressing Smad and CtBP1 binding to the SBE of the Rac1 promoter. **FIG. 10A** provides an illustrative embodiment of western blot analysis for GTP-Rac1 and total Rac1 in Smad7 transgenic keratinocytes. Additional samples are shown in **FIG. 3B**. M, molecular marker. **FIG. 10B** provides a graphical representation of the quantification of GTP-Rac1, total Rac1 and Smad7 in WT and K5.Smad7 keratinocytes shown in **FIG 10A** and in FIG. 3B. The protein level in WT keratinocytes of each blot was normalized as "1". Data is presented as mean ± s.d. and two-tail Student's *t*-test was used to calculate *P* values. $^{**}P < 0.01$, $^{***}P < 0.001$. **FIGS. 10C** and **10D** provide an illustrative embodiment of western blot analysis for Smad2, Smad3, and Smad4 knockdown in NOK-SI cells. Their effects on Rac1 expression are shown in FIG. 3C. M, molecular marker. GAPDH, internal protein control by reprobing same blot. **FIG. 10F** provides an illustrative embodiment showing CtBP1 knockdown promotes NOK-SI cell migration. Pairs of dotted lines delineate the scratch wound. Quantification of cell migration and efficiency of CtBP1 knockdown are shown in **FIG. 4A** and **FIG. 4C**.

**[0029]** **FIGS. 11A-G** provide an illustrative embodiment of data showing the purification and characterization of Tat-Smad7 and Tat-Cre proteins. **FIG. 11A** shows an illustrative embodiment of a schematic representation of Tat-Smad7 protein. FIG. 11A discloses SEQ ID NOS 49 and 101, respectively, in order of appearance. **FIG. 11B** provides an illustrative embodiment of a western blot of purified Tat-Smad7 protein. **FIG. 11C** provides an illustrative embodiment of immune-staining of Tat-Smad7 protein transduction in keratinocytes. Left and middle panels: Tat-Smad7 staining (green) using a V5 antibody, counterstained with a K14 antibody (red). Cells showed Tat-Smad7 in the nucleus 5 min after transduction and in both nucleus and cytoplasm 12 hours after transduction. Right panels: Tat-Smad7 abrogated Smad2 phosphorylation (pSmad2, green). V5 (red) counterstain visualizes Tat-Smad7 transduced cells. **FIG. 11D** provides an illustrative embodiment of immune-staining showing that V5 antibody staining detects Tat-Smad7 transduction in buccal mucosa 12 hours after Tat-Smad7 topical application. A K14 antibody was used for counterstain. Scale bar, 50 μm for both panels. **FIG. 11E** provides

**[0043]** The primary obstacle to using growth factors to treat oral mucositis in cancer patients is the potential risk of promoting cancer cell growth. The majority of human oral cancers lose TGF-β signaling in tumor epithelial cells. Thus, anti-Smad-associated cell proliferation and migration by Smad7 would not be effective in cancer cells. In tumors with intact TGF-β signaling, activation of other oncogenic pathways could override TGF-β-induced tumor suppressive effects. These two scenarios could explain why there was no observation of Smad7 increasing proliferation and migration in oral cancer cells with mutant or intact TGF-β signaling components.

**[0044]** Additionally, TGF-β signaling promotes tumor invasion mainly through Smad-independent mechanisms after loss of TGF-β-induced tumor suppression. Thus, blocking TGF-β signaling by Smad7 in cancer cells could abrogate TGF-β-mediated tumor promotion effects, which behaves similarly to TGF-β inhibitors currently being used in clinical trials for advanced cancers. Further, the potent anti-inflammatory effect of Smad7 may reduce the risk of tumor progression. Therefore, long-term Smad7 application may also be helpful in cancer treatment.

**[0045]** Spontaneous tumor formation in K5.Smad7 mice has not been observed. Because Smad7 is not a secreted protein, local and short-term Smad7 protein delivery in oral mucositis treatment should have few systemic effects. In bone marrow transplant patients, whose oral epithelia do not contain cancer cells, Smad7 topical application may be suitable for both prevention and treatment of oral mucositis.

**[0046]** Although not wishing to be bound by any theory, Smad7-mediated oral mucositis healing appears to be a result of targeting multiple pathogenic processes mediated by one or more molecules (*see, e.g.*, **FIGS. 14A-B**). It is believed that one or more of these molecules (*e.g.*, TGF-β, NF-κB, CtBP1, Rac1) may also be helpful as predictive and therapeutic responsive markers of oral mucositis in patients.

## A. Nucleic acids, Vectors and Host Cells

**[0047]** The present disclosure also provides, in another embodiment, genes encoding Smad7. In addition to the wild-type *SMAD7* gene (SEQ ID NOs: ~~12,~~22, 88), as well as various codon-optimized versions (SEQ ID NOs: ~~9-11, 21, 23-41~~9, 21, 23, 24, 26, 28, 30, 32-34, 36, 38, 39, 87, 89, 91, 93, 96, 97, 99, and 100), it should be clear that the present technology is not limited to the specific nucleic acids disclosed herein. As discussed below, a "Smad7 gene" may contain a variety of different bases and yet still produce a corresponding

or 100% pure of other cellular nucleic acid molecules as determined using standard biochemical techniques, such as but not limited to agarose gel electrophoresis. As used herein, the term "isolated free of total cellular protein" means that the protein molecule is about or at least about 75% pure, 80% pure, 85% pure, 90% pure, 95% pure, 96% pure, 97% pure, 98% pure, 99% pure, or 100% pure of other cellular nucleic acid molecules as determined using standard biochemical techniques, such as but not limited to a western blot. In certain embodiments, the present technology concerns a nucleic acid sequence essentially as set forth in, and/or including any one of SEQ ID NOs: ~~9-11, 21, 23-41~~9, 21, 23, 24, 26, 28, 30, 32-34, 36, 38, 39, 87, 89, 91, 93, 96, 97, 99, and 100.

**[0051]** An isolated nucleic acid molecule may be produced using recombinant DNA technology (*e.g.*, polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications provide the desired effect (*e.g.*, production of Smad7 protein in non-human expression systems).

**[0052]** The term "essentially as set forth in one or more nucleic acid sequence (*e.g.*, SEQ ID NOs: 9-11, 21, 23-41 " means that the nucleic acid sequence substantially corresponds to at least a portion, and in some cases the entirety, of the one or more nucleic acid sequence (*e.g.*, SEQ ID NOs: ~~9-11, 21, 23-41~~9, 21, 23, 24, 26, 28, 30, 32-34, 36, 38, 39, 87, 89, 91, 93, 96, 97, 99, and 100). In some embodiments, sequences that substantially correspond to at least a portion of a nucleic acid sequence, may correspond to about, or at least about 50 nucleic acids, 75 nucleic acids, 150 nucleic acids, 200 nucleic acids, 250 nucleic acids, 300 nucleic acids, 350 nucleic acids, 400 nucleic acids, 450 nucleic acids, 500 nucleic acids, 550 nucleic acids, 600 nucleic acids, 650 nucleic acids, 700 nucleic acids, 750 nucleic acids, 800 nucleic acids, 900 nucleic acids, 1000 nucleic acids, 1100 nucleic acids, 1200 nucleic acids, or 1250 nucleic acids of one or more of the sequences described herein. In some embodiments, sequences that substantially correspond to at least a portion of a nucleic acid sequence, may correspond to about a range of about 50-1250 nucleic acids, 75-1250 nucleic acids, 150-1250 nucleic acids, 200-1250 nucleic acids, 250-1250 nucleic acids, 300-1250 nucleic acids, 350--1250 nucleic acids, 400--1250 nucleic acids, 450-1250 nucleic acids, 500-1250 nucleic acids, 550-1250 nucleic acids, 600-1250 nucleic acids, 650-1250 nucleic acids, 700-1250 nucleic acids, 750-1250 nucleic acids, 800-1250 nucleic acids, 900-1250 nucleic acids, 1000-1250 nucleic acids, 1100-1250 nucleic acids, 1200-1250

human Smad7 wild-type cDNA (SEQ ID NOs: ~~12,~~ 22, 88). In some embodiments, one or more codon-optimized nucleic acid may have about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, or 75%, homology to human Smad7 wild-type cDNA (SEQ ID NOs: 12, 22). In some embodiments, one or more codon-optimized nucleic acid may also have a nucleotide substitution in the codon for Met216 (ATG), to form the codon for Leu216 (CTG).

**[0067]** A methionine codon (Met216; ATG) that has the potential for being perceived by translation machinery (*e.g.*, such as but not limited bacteria or yeast) as an alternative open reading frame has been identified. Although not intending to be bound by theory, it is believed that the presence of the second potential open reading frame may decrease expression of the Smad7 protein. In some embodiments, one or more Smad7 nucleic acid sequences are modified at nucleotide position (646-648) to encode a human Smad7 protein where Met216 (ATG) is modified to Leu216 (CTG).

**[0068]** It has also been discovered that various truncated forms and fragments of Smad7 protein retain one or more of the activities of full-length human Smad7, such as, but not limited to, increasing proliferation, reducing or inhibiting cell death, reducing excessive inflammation, preventing DNA damage, and/or increasing cell migration, as well as treating or preventing one or more disease or disorders in which such treatment would be helpful as further discussed herein. Such activities can be assessed using one or more assays including, but not limited to, the ability to block phosphorylation of Smad2 and/or nuclear translocation of the NF-κB p50 subunit, increase cell proliferation, reduce apoptosis and/or radiation-induced DNA damage, reduce inflammation and/or angiogenesis, promote healing in oral mucositis, surgical wounds, diabetes wounds, and/or wounds associated with chronic inflammation in mice.

**[0069]** Further, in some embodiments, various truncated forms and fragments of Smad7 protein retain only a subset of the one or more of the activities of full-length human Smad7. For example, the C-terminal MH2 domain of Smad7 may primarily mediate the anti-inflammatory effect of Smad7. Smad7 peptides having this anti-inflammatory function may be sufficient and optionally an improvement for treating chronic inflammation associated conditions, such as but not limited to, oral mucositis, stomatitis, arthritis, and psoriasis, among others. The N-terminal MH1 domain may primarily mediate cell migration and/or blocking TGF-β-induced growth arrest and/or fibrotic response. Smad7 peptides having this cell migration and proliferation function may be sufficient, and

**[0076]** In some embodiments, the PTD nucleic acid sequence is a Tat nucleic acid sequence. ggccgtaaaaaacgccgtcaacgccgccgt (SEQ ID NO: 1) encoding GRKKRRQRRR (SEQ ID NO: 2), tatggccgtaaaaaacgccgtcaacgccgccgt (SEQ ID NO: 3) encoding YGRKKRRQRRR (SEQ ID NO: 4), or ggccgtaaaaaacgccgtcaa (SEQ ID NO: 5) encoding GRKKRRQ (SEQ ID NO: 6).

**[0077]** In some embodiments, the nucleic acid sequence further includes a nucleotide sequence encoding one or more of an epitope tag or a purification tag. In some embodiments, the epitope tag is V5. In some embodiments, the purification tag is one or more of glutathione-S-Transferase (GST) or 6-histidine (H6) (SEQ ID NO: 40).

**[0078]** The term "epitope tag" as used herein in reference to nucleic acid molecules refers to nucleotides encoding peptide sequences that are recognized and bound by the variable region of an antibody or fragment. In some embodiments, the epitope tag is not part of the native protein. In some embodiments, the epitope tag is removable. In some embodiments, the epitope tag is not intrinsic to the protein's native biological activity. Examples of epitope tags include, but are not limited to V5.

**[0079]** The term "purification tag" as used herein in reference to nucleic acid molecules refers to nucleotides encoding peptide sequences that facilitate the purification of the protein, but are generally not necessary for the protein's biological activity. In some embodiments, purification tags may be removed following protein purification. Examples of purification tags include, but are not limited to GST and H-6 (SEQ ID NO: 40).

### 2. Vectors for Cloning, Gene Transfer and Expression

**[0080]** Within certain embodiments, expression vectors are employed to express the Smad7 polypeptide product, which can then be purified for various uses. In other embodiments, the expression vectors are used in gene therapy. Expression requires that appropriate signals be provided in the vectors, and which include various regulatory elements, such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in host cells. Elements designed to optimize messenger RNA stability and translatability in host cells also are defined. The conditions for the use of a number of dominant drug selection markers for establishing permanent, stable cell clones expressing the products are also provided, as is an element that links expression of the drug selection markers to expression of the polypeptide.

at least about 50, at least about 75, at least about 100, at least about 125, at least about 150, at least about 200, at least about 250, at least about 300, or at least about 350, or at least about 400 amino acids from one or the other terminus are specifically provided. In some embodiments, a "truncated" molecule is biologically active, having one or more of the Smad7 activities described herein.

**[0106]** The term "fragment" as used herein in reference to polypeptide sequences refers to a molecule containing contiguous residues of a full length sequence but lacking some N-terminal and/or C-terminal residues of the full length sequence. In some embodiments, a "fragment" includes a portion of one or more of the full length sequences described herein. In some embodiments, the "fragment" does not include sequences encoding either the N-terminal or the C-terminal, but only internal fragments. In some embodiments, a "fragment" encodes a polypeptide that is biologically active, having one or more of the Smad7 activities described herein. In some embodiments, polypeptide fragments have at least about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100,150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 amino acids.

**[0107]** A specialized kind of variant is the fusion protein. This molecule generally has all or a substantial portion of the native molecule, linked at the N- or C-terminus, to all or a portion of a second polypeptide. However, in some embodiments, the fusion protein may include any one of the fragments and/or truncated (N-terminal, C-terminal) Smad7 proteins described throughout the disclosure. For example, fusions may employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of an optional functionally active domain, such as but not limited to an antibody epitope and/or a purification tag (*e.g.*, V5: GKPIPNPLLGLDST (SEQ ID NO: 41); Flag: KYKDDDDK (SEQ ID NO: 42); HA: YPYDVPDYA (SEQ ID NO: 43)). Another type of fusion includes attaching a domain that can act as the target for an activating or inactivating ligand, thereby permitting control of the fusion protein's function once delivered to a subject. Such domains include, for example, steroid ligand binding (*e.g.*, ER, PR, GR), which can be activated by small molecules, *e.g.*, 4-hydroxyl tamoxifen or RU486 that are either uniquely able to activate those steroid ligand binding domains and/or do not exist in nature and will therefore enable full control of the Smad7 function by the presence of these small molecules.

**[0108]** Another specific form of a fusion protein finding particular utility in the present technology is a fusion including a protein transduction domain (PTD), also called a cell delivery domain or cell transduction domain. Such domains have been described in the art and are generally characterized as short amphipathic or cationic peptides and peptide derivatives, often containing multiple lysine and arginine resides (Fischer, *Med. Res. Rev.* 27:755-795 (2007)). In some embodiments, the PTD is one or more variant of TAT protein from HIV (GRKKRRQRRR (SEQ ID NO: 2), YGRKKRRQRRR (SEQ ID NO: 4), or GRKKRRQ (SEQ ID NO: 6)) or alternatively, HSV VP16. Alternate forms of Tat may be used. In some embodiments, a linker may be used to connect one or more PTDs and SMad7. In some embodiments, the PTD (optionally Tat) is fused or linked in frame to the N-terminal and/or C-terminal end of any one of the Smad7 full-length, fragments, and/or truncated (N-terminal, C-terminal) proteins described throughout the disclosure. Other examples of PTDs provided by the present technology are shown in Table 1.

**TABLE 1 – PROTEIN TRANSDUCTION DOMAINS**

| | SEQ ID NO: | | SEQ ID NO: |
|---|---|---|---|
| GALFLGWLGAAGSTMGAK KKRKV | 448 | QAATATRGRSAASRPTERP RAPARSASRPRRPVE | 6530 |
| RQIKIWFQNRRMKWKK | 459 | MGLGLHLLVLAAALQGAK SKRKV | 6631 |
| RRMKWKK | 4610 | AAVALLPAVLLALLAPAAA NYKKPKL | 6732 |
| RRWRRWWRRWWRRWRR | 4711 | MANLGYWLLALFVTMWTD VGLCKKRPKP | 6833 |
| RGGRLSYSRRRFSTSTGR | 4812 | LGTYTQDFNKFHTFPQTAIG VGAP | 6934 |
| YGRKKRRQRRR | 413 | DPKGDPKGVTVTVTVTVTG KGDPXPD | 7035 |
| RKKRRQRRR | 4914 | PPPPPPPPPPPPP | 7136 |
| YARAAARQARA | 5015 | VRLPPPVRLPPPVRLPPP | 7237 |
| RRRRRRRR | 5116 | PRPLPPPRPG | 7338 |
| KKKKKKKK | 5217 | SVRRRPRPPYLPRPRPPPFFP PRLPPRIPP | 7439 |
| GWTLNSAGYLLGKINLKAL AALAKXIL | 5318 | TRSSRAGLQFPVGRVHRLL RK | 7540 |
| LLILLRRRIRKQANAHSK | 5419 | GIGKFLHSAKKFGKAFVGEI MNS | 7641 |
| SRRHHCRSKAKRSRHH | 5520 | KWKLFKKIEKVGQNIRDGII KAGPAVAVVGQATQIAK | 7742 |
| NRARRNRRRVR | 5621 | ALWMTLLKKVLKAAAKAA LNAVLVGANA | 7843 |
| RQLRIAGRRLRGRSR | 57522 | GIGAVLKVLTTGLPALISWI KRKRQQ | 7944 |
| KLIKGRTPIKFGK | 5823 | INLKALAALAKKIL | 8045 |
| RRIPNRRPRR | 5924 | GFFALIPKIISSPLPKTLLSAV | 8146 |

| TABLE 1 – PROTEIN TRANSDUCTION DOMAINS | | | |
|---|---|---|---|
| | | GSALGGSGGQE | |
| KLALKLALKALKAALKLA | 6025 | LAKWALKQGFAKLKS | 8247 |
| KLAKLAKKLAKLAK | 6126 | SMAQDIISTIGDLVKWIIQTV NXFTKK | 8348 |
| GALFLGFLGAAGSTNGAWS QPKKKRKV | 6227 | LLGDFFRKSKEKIGKEFKRI VQRIKQRIKDFLANLVPRTE S | 8449 |
| KETWWETWWTEWSQPKK KRKV | 6328 | PAWRKAFRWAWRMLKKA A | 8550 |
| LKKLLKKLLKKLLKKLLKK L | 6429 | KLKLKLKLKLKLKLKLKL | 8651 |

**[0109]** In particular embodiments, the present technology provides for sequence variants of Smad7 in which one or more residues have been altered. For example, in one embodiment, the methionine residue found at position 216 of the human Smad7 sequence is modified to a leucine residue (ATG to CTG).

## C. Methods of Treatment

**[0110]** Smad7-treatable diseases and disorders may include those including one or more of reduced cell proliferation, reduced cell migration, increased cell death, excessive inflammation, and/or DNA damage. Smad7-related diseases and disorders may include those where treatment with a Smad7 protein and biologically active fragments and derivatives thereof that have one or more activities including but not limited to increasing proliferation, reducing or inhibiting cell death, reducing excessive inflammation, preventing DNA damage, and/or increasing cell migration is helpful. Such diseases and/or disorders may include but are not limited to acute (*e.g.*, through surgery, combat, trauma) and chronic wounds (*e.g.*, ulcers, such as diabetic, pressure, venous), scarring, fibrosis, and aberrant healing, mucositis (*e.g.*, oral and/or gastro-intestinal), stomatitis, proctitis, autoimmune disease (*e.g.*, psoriasis, arthritis), and cancer.

**[0111]** In some embodiments, one or more of the diseases and or disorders described herein may be prevented, treated, and/or ameliorated by providing to a subject in need of such treatment a therapeutically effective amount of one or more of the Smad7 proteins (*e.g.*, full-length or biologically active truncated (*e.g.*, N- terminal or C-terminal) or fragment thereof) described in the disclosure. In some embodiments, the one or more Smad7 proteins are fusion proteins including a PTD domain. In some embodiments, the one or more Smad7 proteins includes Leu216. In some embodiments, the Smad7 proteins make part of a pharmaceutical composition including one or more pharmaceutically acceptable excipients.

Example 3: Tat-Smad7 alleviates radiation-induced oral mucositis

**[0233]** Smad7 transgene's ability to block multiple pathological processes of oral mucositis prompted us to explore if localized Smad7 delivery can be used to prevent and treat oral mucositis. Because Smad7 is a nuclear protein, local Smad7 delivery needs to allow Smad7 to rapidly enter into cells before saliva washes off the protein. Thus, a recombinant human Smad7 with an N-terminal Tat-tag allowing proteins to rapidly permeate the cell membrane and enter the nucleus was produced. A V5 epitope was added to the C-terminal end of the Tat-Smad7 protein to track Tat-Smad7 cell penetration (**FIGS. 11A-11D**).

**[0234]** Using its ability to block Smad2 phosphorylation, Tat-Smad7 bioactivity was tested (**FIG. 11C**). Tat-Cre recombinant protein with the same tags as a control (**FIGS. 11E-F**) was produced, and cloned into the pET101-Topo protein expression vector (Invitrogen) that contains a sequence encoding C-terminal 6XHis SEQ ID NO: 40). *Tat-Cre* was transformed into BL-21 STAR™ *E. coli* (Invitrogen) to produce Tat-Cre protein and was purified with Ni-NTA column.

**[0235]** The purity and size of both proteins was verified using SDS-PAGE electrophoresis. To evaluate transduction and activity of Tat-Smad7 protein *in vitro*, Tat-Smad7 was added to primary mouse keratinocytes. Slides were fixed in cold methanol for 5 minutes and stained for V5 and pSmad2. Tat-Cre activity was verified by digesting a 1,460 bp floxed fragment from the 7,650 bp vector pLL3.7 (Addgene). For *in vivo* treatments, 30 μL 50% glycerol/PBS as a vehicle control and Tat-Cre as a non-irrelevant protein control were used. Tat-Smad7 or Tat-Cre (in 30 μL 50% glycerol/PBS, doses and regimens are specified in each figure) was topically applied to mouse oral cavity and mice were restricted from oral intake for 1 hour.

**[0236]** For oral mucositis prevention, both Tat-Smad7 and Tat-Cre (in 50% glycerol/PBS) were topically applied to the oral cavity of 8–10 week old C3H females (Jackson Laboratory) or C57BL/6 mice daily, starting 24 hours prior to radiation through day 8 after initiation of radiation. Treated tissues were examined on day 9. Mouse tongues were harvested, fixed in 10% formalin, embedded in paraffin, and cut into 5 μm sections. Histological changes were analyzed and ulcers were measured using H&E stained slides. An additional group received Palifermin treatment with a clinical regimen, *i.e.*, 6.25 mg $kg^{-1}$ (i.p.) daily for 3 days prior to irradiation, and daily for 3 days 24 hours after the last dose of radiation.

3' Tat: Smad7-Ggccgtaaaaaacgccgtcaacgccgccgt (SEQ ID NO: 8)

The 5' Tat-Smad7 construct included a 3' V5 tag sequence, and was cloned into the pGEX-6p-1 protein expression vector (New England Biolabs) to make a GST-Tat-Smad7 fusion protein. Tat-Smad7 gene was transformed into BL-21 Star Escherichia coli (Invitrogen) to produce Tat-Smad7 protein. The protein was purified by glutathione column purification and elution, using enzymatic cleavage from the Glutathione S Transferase (GST) fusion (Precision enzyme, GE Life Sciences).

**[0245]** While creating a PTD-Smad7 fusion protein, a V5 tag at the 3' end was included to monitor Tat-Smad7 penetration into cells by immunostaining using a V5 antibody. This epitope tag can be deleted for use in the clinic (*e.g.*, by re-cloning the sequence in the absence of the V5 tag), if appropriate.

**[0246]** A PTD-Smad7 fusion protein (Tat-Smad7-V5-6H) ("6H" disclosed as SEQ ID NO: 40) was also created having a 6-Histidine (6-H) tag (SEQ ID NO: 40) for protein purification, and is shown below. Tat-Smad7-V5-6H ("6H" disclosed as SEQ ID NO: 40) has the following nucleotide sequence: 1-53 include the 5' sequence of pET-TOPO; 54-1365 include Tat-Smad7; 1366-1497 include 3' pET-TOPO containing the V5 epitope and 6xHis tag (SEQ ID NO: 40) (V5 includes 1393-1434, His tag includes1444-1461, and the Stop includes 1462-1464).

**[0247]** Tat-human Smad7, codon-optimized for protein production, cloned to pET101/D-Topo vector is shown below:

ttcccctctagaaataattttgtttaactttaagaaggaattcaggagcccttcaccatg

M

cgtaaaaaacgccgtcaacgccgccgtggtttccgtacgaaacgctcggccctggtccgt

R K K R R Q R R R G F R T K R S A L V R

cgcctgtggcgctcccgtgctccgggtggtgaagatgaagaagaaggtgctggcggcggt

R L W R S R A P G G E D E E E G A G G G

ggcggtggcggtgaactgcgtggcgagggtgcaaccgatagtcgtgcacacggtgcaggc

G G G G E L R G E G A T D S R A H G A G

L L E P G D R S H W C V V A Y W E E K T

cgtgtcggtcgcctgtactgcgtacaggaaccgtcgctggatatcttttatgacctgccg

R V G R L Y C V Q E P S L D I F Y D L P

cagggcaatggtttctgtctgggccaactgaactcagataataaatcgcagctggtgcaa

Q G N G F C L G Q L N S D N K S Q L V Q

aaagttcgctcaaaaattggctgcggtatccagctgacccgtgaagttgacggtgtctgg

K V R S K I G C G I Q L T R E V D G V W

gtatataaccgcagctcttacccgatttttatcaaaagtgccaccctggataatccggac

V Y N R S S Y P I F I K S A T L D N P D

tcccgtacgctgctggtccacaaagtatttccgggcttctcaatcaaagcgttcgattac

S R T L L V H K V F P G F S I K A F D Y

gagaaagcctactcgctgcagcgcccgaacgaccatgaattcatgcagcaaccgtggacg

E K A Y S L Q R P N D H E F M Q Q P W T

ggttttactgtgcagatctctttcgttaaaggctggggtcaatgctacacccgtcagttt

G F T V Q I S F V K G W G Q C Y T R Q F

atctcgtcctgtccgtgctggctggaagtgattttcaatagccgcaagggcgagctcaat

I S S C P C W L E V I F N S R K G E L N

tcgaagcttgaaggtaagcctatccctaaccctctcctcggtctcgattctacgcgtacc

S K L E G K P I P N P L L G L D S T R T

Ggtcatcatcaccatcaccattgagtttgatccggctgctaacaaagcccgaaagga (SEQ ID NO: 9)

G H H H H H H - (SEQ ID NO: 10)

Tat-Smad7-V5 401 pwtgftvqisfvkgwgqcytrqfisscpcwlevifnsr*kgelnskle*GKP

human Smad7 389 pwtgftvqisfvkgwgqcytrqfisscpcwlevifnsr----------

Tat-Smad7-V5 451 IPNPLLGLDST (SEQ ID NO: 11)

human Smad7 427 ----------(SEQ ID NO: 12)

Example 5: Additional assays for PTD-Smad7 protein activity

**[0250]** *Immunofluorescence (IF), immunohistochemistry (IHC), and TUNEL assay for apoptosis.* IF and IHC were performed as previously described (Han, G., Li, F., Ten Dijke, P. & Wang, X.J. Temporal smad7 transgene induction in mouse epidermis accelerates skin wound healing. *Am J Pathol* 179, 1768-1779 (2011)). Primary antibodies used were guinea pig antibody to K14 (1:400, Fitzgerald, 20R-CP200), rat antibody to CD4 (1:20, BD Bioscience, 550278), Ly-6G (1:20, BD Bioscience, 550291), BM8 (antibody to F4/80, 1:20, Invitrogen, MF48000), FITC-labeled antibody to BrdU (BD Bioscience, 347583), rat antibody to CD45 (1:50, BD Bioscience, 550539) for mouse samples, mouse antibody to CD45 (1:50, Abcam, Ab781) for human samples, chicken antibody to TGF-β1 (1:50, R&D, AF-101-NA), rabbit antibody to CtBP1 (1:100, Millipore, 07-306), rabbit antibody to NF-κB p50 (1:200, Santa Cruz Biotechnology, SC-7178), rabbit antibody to PCNA (1:200, Santa Cruz Biotechnology, SC-7907), rabbit antibody to pSmad2 (1:100, Cell Signaling Technology, 3101), and mouse antibody to V5 (1:500, Invitrogen, 460705). For IF, secondary antibodies to different species IgG were Alexa Fluor® 594 (red) or 488 (green) conjugated (1:200 for all, Invitrogen). For IHC, secondary biotinylated antibodies to different species IgG (1:300, Vector Labs) were used and were developed using Vectastain ABC kit (Vector Labs). A Terminal deoxynucleotidyl transferase uridine nick end-labeling (TUNEL, G3250) kit (Promega) was used on formalin fixed tissue sections to detect apoptotic cells. BrdU labeling was performed *in vivo* by i.p. injection of 0.125 mg $g^{-1}$ BrdU 1 hour prior to euthanization. PCNA or BrdU were quantified as cells $mm^{-1}$ epithelial length including all epithelial cells, TUNEL or CD45-positive cells as cells $mm^{-1}$ epithelial length including all epithelial layers and stroma above the muscle layer, nuclear pSmad2 or NF-κB p50 positive cells as the number of positive cells/existing total remaining epithelial cells (*i.e.*, excluding sloughed epithelial cells induced by irradiation). Consecutive fields of slides were used to count BrdU-labeled cells using MetaMorph software.

Biotechnology, SC1616). Gray-scale images were obtained using the ODYSSEY® v.1.2 software (LI-COR Biosciences).

**[0257]** *Rac1 activation assay.* Active GTP-bound Rac1 was examined using a BIOCHEM™ Kit for Rac1 activation (Cytoskeleton Inc, BK035). Wild-type and Smad7 transgenic keratinocytes were cultured in 15 cm diameter tissue culture plates and prepared protein lysates using the provided lysis buffer. To assay Rac1 activity, 1 mg of cell lysate was used. To examine total Rac1 and Smad7 proteins, 50 μg of lysate was used. To measure GTP-bound Rac1 in mouse tongues, half of the tongue was ground to a powder in liquid nitrogen and lysed with lysis buffer to extract protein, GTP-bound Rac1 was assayed in 2 mg of protein lysate per sample and 50 μg of protein lysate was loaded for total Rac1 protein western blot.

**[0258]** *ChIP assays.* ChIP assays were performed using the ChIP-IT express kit (Active Motive, 53009) as previously described (Hoot, K.E., *et al.* HGF upregulation contributes to angiogenesis in mice with keratinocyte-specific Smad2 deletion. *J Clin Invest* **120**, 3606-3616 (2010); Hoot, K.E., *et al.*. Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. Owens, *et al.*, *J.Clin.Invest* 118, 2722-2732 (2008). Smad4-dependent desmoglein-4 expression contributes to hair follicle integrity. Owens, *et al.*, *Dev. Biol.* 322:156-166 (2008). DNA-protein complex was isolated from primary mouse keratinocytes. For ChIP, 6.3 μg sheared chromatin was incubated with protein-G magnetic beads and 2 μg each of rabbit antibodies to Smad2 (Cell Signaling Technology, 3122), Smad3 (Cell Signaling Technology, 9523), Smad4 (Cell Signaling Technology, 9515), Smad7 antibody (Santa Cruz Biotechnology, SC-11392), CtBP1 (Millipore) or a negative control rabbit IgG (Santa Cruz Biotechnology, SC-2027). Eluted DNA from the protein-DNA complex was used for PCR analyses, and CtBP1 binding to the *Rac1* promoter was compared in wild-type and Smad7 transgenic keratinocytes by ChIP band intensities on gel images or by quantitative PCR using Power SYBR Green Master Mix (Applied Biosystems). Primers used to amplify the *Rac1* SBE-1.5 Kb promoter regions:

5'-TGGAATTCCTGGTCTGGTTT-3' (sense) (SEQ ID NO: 13)

5'-GCCAAGCTGCTCTTCCAGTA-3' (antisense) (SEQ ID NO: 14)

5'-TCTCAGGGGGCCAAAGGTGTT-3' (sense) (SEQ ID NO: 15)

5'-TCCCAGCACCTGAATCACATGG-3' (antisense) (SEQ ID NO: 16)

**[0259]** *Rac1 promoter luciferase reporter construct, site-directed mutagenesis and luciferase assay.* The 883 bp fragment of -1671 bp to -789 bp of the *Rac1* promoter, encompassing the SBE-1.5 Kb site, was amplified from wild-type mouse DNA using 5' XhoI and 3' HindIII tagged primers, and this *Rac1* promoter fragment was cloned into pGL4.26 vector (Promega) to make the *Rac1* promoter-pGL4.26 luciferase reporter (*Rac1*-Luc) construct. For site-directed mutagenesis, the SBE sequence 5'-TGTCTGTGCT-3' (SEQ ID NO: 17) was mutated to 5'-TGATAGAGCT-3' (SEQ ID NO: 18). *Rac1*-Luc and pGL4.74 (1:20) were co-transfected with Smad7 siRNA, CtBP1 siRNA or scrambled siRNA using Lipofectamine 2000 (Invitrogen) to primary mouse keratinocytes, or primary mouse keratinocytes with Tat-Smad7 treatment (1 μg $mL^{-1}$). Cell lysates were collected and luciferase assays were performed 48 hours after transfection or Tat-Smad7 treatment, using the DUAL-LUCIFERASE® Reporter Assay kit (Promega) following manufacturer's instructions. Rac1-luciferase activity was measured with the Glomax machine (Promega) and expressed by the ratio of firefly activity to Renilla activity. Primers used for amplification of *Rac1* promoter sequence were:

5'-ATCCTCGAG-TATCCTCCAGGTCTGGG-3' (SEQ ID NO: 19)

5'-GCCAAGCTT-AGCGTCCAGCGTTAACCTG-3' (SEQ ID NO: 20)

**[0260]** *Statistical analysis.* Statistical differences in molecular analyses and oral mucositis ulcer size were analyzed using the Student's *t*-test and all data was presented by mean ± s.d. except ulcer size, which was presented by mean ± s.e.m. Oral mucositis incidences were analyzed by Fisher's exact test.

Example 6: Codon optimization for Smad7 protein production in *E. coli* or yeast

**[0261]** Although many mammalian proteins can be produced in bacteria without nucleotide sequence modification, the analysis indicated that the Smad7 nucleotide sequence would need to be modified to allow protein expression in bacteria.

**[0262]** Analysis of Smad7 cDNA mammalian codon use revealed nine arginine amino acids coded for by the following nucleotides: 7-9, 43-45, 169-171, 403-405, 490-492, 526-528, 526-528, 823-825, 1057-1059 are a rare codon (AGG, codon utilization 1.7%). Since these codons are rare codons in bacteria, it is expected that they could halt or reduce protein translation and/or production in bacteria. The amino acids coded for by rare

arginine codons are indicated by bold capitals below in the illustrated human Smad7 protein, including arginines at positions 3, 15, 57, 135, 164, 169, 176, 275, and 353. Additionally, the following arginine codons also have low frequency usages. CGA (3.5% codon utilization): nucleotides 16-18, 136-138, 199-201, 598-600, which code for arginine at positions 6, 46, 67, 200; CGG (5.4% codon utilization): nucleotides 31-33, 112-114, 316-318, 772-774, 940-942, 973-975, 1135-1137, 1276-1278, which code for arginine at positions 11, 38, 106, 258, 314, 325, 379, 426; AGA (2.8% codon utilization): nucleotides 637-639, 814-816, which code for arginine at positions 213, 272. These arginine residues are highlighted in bold upper case R below and they are changed to CGC in at least one of the codon-optimized nucleic acid sequences (20.6% codon utilization):

1 mf**R**tk**R**salv **R**rlw**R**srapg gedeeegagg ggggge**R**ge gatds**R**ahga
51 ggggpg**R**agc clgkavrgak ghhhphppaa gagaaggaea dlkalthsvl
101 kklke**R**qlel llqavesrgg trtaclllpg rldc**R**lgpga pagaqpaqpp
151 ssyslpllle kvf**R**wpdl**R**h ssevk**R**lccc esygkinpel vccnphhls**R**
201 lcelespppp ys**R**ypmdflk ptadcpdavp ssaetggtny lapgglsdsq
251 lllepgd**R**sh wcvvayweek t**R**vg**R**lycvq epsldifydl pqgngfclgq
301 lnsdnksqlv qkv**R**skigcg iqlt**R**evdgv wvynrssypi fiksatldnp
351 ds**R**tllvhkv fpgfsikafd yekayslq**R**p ndhefmqqpw tgftvqisfv
401 kgwgqcytrq fisscpcwle vifns**R** (SEQ ID NO: 12)

**[0263]** Based on this analysis, it was decided to optimize the Smad7 nucleotide sequence to codons that were believed to allow increased Tat-Smad7 protein production in *E. coli* or yeast. Provided below is the optimized nucleic acid codon sequence made by Genscript. Briefly, the sequence has the following composition: nucleotides 1-6 include the restriction recognition site for BamHI; nucleotides 7-36 include the Tat sequence; nucleotides 37-1314 include codon-optimized human Smad7 cDNA; nucleotides 1342-1383 include the V5 epitope; nucleotides 1384-1386 are the stop codon; and nucleotides 1387-1392 including the restriction recognition site for SalI. In this sequence, ATG is removed to be used with GST. The entire designed sequence was converted to *E. coli* codons based on "Codon-Usage Database." The initial optimized Smad7 sequence (SEQ ID NO: ~~26~~23) is shown below:

1 ggatccggcc gtaaaaaacg ccgtcaacgc cgccgtggtt tccgtacgaa acgctcggcc

61 ctggtccgtc gcctgtggcg ctcccgtgct ccgggtggtg aagatgaaga agaaggtgct

1381 acgtgagtcg ac (SEQ ID NO: 23)

**[0264]** A nucleotide sequence comparison between Tat-Smad7-V5 (SEQ ID NO: 23) and human Smad7 (SEQ ID NO: 22) cDNA is provided below. Human Smad7 and codon-optimized Tat-Smad7-V5 share 68% codon homology. Human Smad7 and codon-optimized Tat-Smad7 share 71% codon homology. Human Smad7 and codon-optimized Smad7 share 73% codon homology.

aaagcgttcgattacgagaaagcctactcgctgcagcgcccgaacgaccatgaattcatg

K A F D Y E K A Y S L Q R P N D H E F M

cagcaaccgtggacgggttttactgtgcagatctctttcgttaaaggctggggtcaatgc

Q Q P W T G F T V Q I S F V K G W G Q C

tacacccgtcagtttatctcgtcctgtccgtgctggctggaagtgattttcaatagccgc

Y T R Q F I S S C P C W L E V I F N S R

aagggcgagctcaattcgaagcttgaaggtaagcctatccctaaccctctcctcggtctc

K G E L N S K L E G K P I P N P L L G L

gattctacgtgagtcgac (SEQ ID NO: 24)

D S T - (SEQ ID NO: 25)

**[0270]** In the nucleic acid sequence, nucleotides 1-6 include the restriction recognition site for BamHI; nucleotides 7-36 include the Tat PTD sequence; nucleotides 37-810 include codon-optimized for the N terminal amino acids 1-258 of human Smad7; nucleotides 811-852 include the V5 epitope sequence (corresponding amino acid sequence in bold); nucleotides 853-855 include the stop sequence; and nucleotides 856-861 include the restriction recognition site for SalI. ATG is removed to allow for fusion with GST:

ggatccggccgtaaaaaacgccgtcaacgccgccgtggtttccgtacgaaacgctcggcc

G S G R K K R R Q R R R G F R T K R S A

ctggtccgtcgcctgtggcgctcccgtgctccgggtggtgaagatgaagaagaaggtgct

L V R R L W R S R A P G G E D E E E G A

ggcggcggtggcggtggcggtgaactgcgtggcgagggtgcaaccgatagtcgtgcacac

G G G G G G G E L R G E G A T D S R A H

ggtgcaggcggtggcggtccgggtcgtgctggttgctgtctgggtaaagctgtgcgcggc

ctcgattctacgtgagtcgac_ (SEQ ID NO: 26)

**L D S T -** (SEQ ID NO: 27)

Example 8: Testing of truncated Smad7 proteins

**[0271]** Activity of truncated Smad7 proteins is tested using the *in vitro* and *in vivo* assays used to test full-length Smad7 described above, among other assays. Such assays include, but are not limited to, the ability to block phosphorylation of Smad2 and/or nuclear translocation of the NF-κB p50 subunit, increase cell proliferation, reduce apoptosis and/or radiation-induced DNA damage, reduce inflammation and/or angiogenesis, promote healing in oral mucositis, surgical wounds, diabetes wounds, and/or wounds associated with chronic inflammation in mice.

**[0272]** In a wound healing assay, 6-mm punch biopsies were performed in wild-type mice followed by daily topical application of C-terminal or N-terminal Tat-Smad7. By measuring gross wound closure, both truncated Smad7 proteins described above (*e.g.*, Tat-Smad7 C-terminal and N-terminal protein) were found to have promoted wound healing similar to full length Tat-Smad7.

Example 9: Truncated Smad7 proteins accelerate wound healing

**[0273]** The ability of truncated Smad7 proteins to accelerate wound healing was examined in wild-type mice. **FIG. 16A** demonstrates the effect of C-terminally truncated (259-426aa) Tat-C-Smad7 on a mouse wound healing model. Wild-type C57BL/6 mice were anaesthetized, dorsally wounded by a 6-mm dermal punch biopsy, and treated every other day with the topical application of PBS (control, 3 mice, 4 wounds/mouse), full-length Tat-Smad7 (0.4μg/10μL PBS/wound, 3 mice, 4 wounds/mouse) or Tat-C-Smad7 (0.4μg/10μL/wound PBS, 3 mice, 4 wounds/mouse, total of 12 wounds were treated). Wound area was photographed with Canon digital camera and measured by imaging analysis using the Image J software with a 6-mm circle inside of the photo for normalization at 1, 2, 4 and 5 days after wounding, and the average percent wound area remaining was calculated for each treatment group. The ability of Tat-C-Smad7 to accelerate wound healing was similar to that of full-length Smad7 (**FIG. 16A**).

**[0274]** **FIG. 16B** illustrates the effect of N-terminally truncated (1-258aa) Tat-N-Smad7 on wound healing. Wild-type C57BL/6 mice were anaesthetized, dorsally wounded by a

**[0285]** The comparison between original codon-optimized Tat-Smad7-V5 and further changes is provided below. Top strand: Tat-Smad7-V5 (SEQ ID NO: 23); bottom strand: after optimized Ser,

```
Alignment:   Local DNA homologies.
Parameters:  Both strands.  Method:  FastScan - Max Score

   Mol 1 20120323113102B12-S54366-164160-1-PGEX-5.seq (1-1463)     Mol 2  TatSmad7 Ser-His optim
   Number of sequences to align:  2
   Settings:  Similarity significance value cutoff:  >= 60%

Homology Block:  Percent Matches 94   Score 1227   Length 1392

201203231131     30 ggatccggccgtaaaaaacgccgtcaacgccgccgtggtttccgtacgaaacgctcggcc
TatSmad7 Ser      1 ..................................................agc...

201203231131     90 ctggtccgtcgcctgtggcgctcccgtgctccgggtggtgaagatgaagaagaaggtgct
TatSmad7 Ser     61 ....................ag..........................................

201203231131    150 ggcggcggtggcggtggcggtgaactgcgtggcgagggtgcaaccgatagtcgtgcacac
TatSmad7 Ser    121 ..................................................c.....t

201203231131    210 ggtgcaggcggtggcggtccgggtcgtgctggttgctgtctgggtaaagctgtgcgcggc
TatSmad7 Ser    181 ..........................................................

201203231131    270 gcgaaaggtcatcaccatccgcacccgccggcagcaggtgcaggtgcagctggcggtgcg
TatSmad7 Ser    241 ............t.....t.....................................

201203231131    330 gaagccgatctgaaagccctgacccatagtgtcctgaaaaaactgaaagaacgtcagctg
TatSmad7 Ser    301 ............................c.............................

201203231131    390 gagctgctgctgcaagcagtagaatcccgtggcggtacccgtacggcttgtctgctgctg
TatSmad7 Ser    361 ........................ag..................................

201203231131    450 ccgggtcgtctggattgccgtctgggtccgggtgcaccggctggtgcgcagccggcacaa
TatSmad7 Ser    421 ..........................................................

201203231131    510 ccgccgagctcttacagcctgccgctgctgctgtgtaaagtgtttcgttggccggacctg
TatSmad7 Ser    481 .........agc..............................................

201203231131    570 cgccacagttccgaagttaaacgcctgtgctgttgcgagagctatggcaaaattaacccg
TatSmad7 Ser    541 .....t..cag..............................................

201203231131    630 gaactggtttgttgcaatccgcaccatctgtctcgtctgtgtgaactggagagcccgccg
TatSmad7 Ser    601 ........................t......agc.........................

201203231131    690 ccgccgtattctcgttacccgatggatttcctgaaaccgactgcagattgcccggacgca
TatSmad7 Ser    661 .........agc.........c..................................

201203231131    750 gtcccgtcatcggctgagaccggcggcaccaactatctggcaccgggcggtctgagtgat
TatSmad7 Ser    721 ......agcagc.....................................................c...

201203231131    810 tcccagctgctgctggaaccgggcgaccgttcacattggtgtgtggttgcctattgggaa
TatSmad7 Ser    781 ag.........................agc.....................................

201203231131    870 gagaaaacgcgtgtcggtcgcctgtactgcgtacaggaaccgtcgctggatatcttttat
TatSmad7 Ser    841 ..........................................agc.....................

201203231131    930 gacctgccgcagggcaatggtttctgtctgggccaactgaactcagataataaatcgcag
TatSmad7 Ser    901 ..........................................agc.........agc...

201203231131    990 ctggtgcaaaaagttcgctcaaaaattggctgcggtatccagctgacccgtgaagttgac
TatSmad7 Ser    961 ..................agc.....................................

201203231131   1050 ggtgtctgggtatataaccgcagctcttacccgatttttatcaaaagtgccaccctggat
TatSmad7 Ser   1021 ..........................agc.....................c.........

201203231131   1110 aatccggactcccgtacgctgctggtccacaaagtatttccgggcttctcaatcaaagcg

201203231131   1170 ttcgattacgagaaagcctactcgctgcagcgcccgaacgaccatgaattcatgcagcaa
TatSmad7 Ser   1141 ......................agc.....................................

201203231131   1230 ccgtggacgggttttactgtgcagatctctttcgttaaaggctggggtcaatgctacacc
TatSmad7 Ser   1201 ............................agc.............................

201203231131   1290 cgtcagtttatctcgtcctgtccgtgctggctggaagtgattttcaatagccgcaagggc
TatSmad7 Ser   1261 ............agcag.....................................

201203231131   1350 gagctcaattcgaagcttgaaggtaagcctatccctaaccctctcctcggtctcgattct
TatSmad7 Ser   1321 .........agc.....................................agc

201203231131   1410 acgtgagtcgac
TatSmad7 Ser   1381 ............
```

His and M216L mutation (SEQ ID NO: 30).

F D Y E K A Y S L Q R P N D H E F M Q Q

ccgtggacgggttttactgtgcagatcagcttcgttaaaggctggggtcaatgctacacc

P W T G F T V Q I S F V K G W G Q C Y T

cgtcagtttatcagcagctgtccgtgctggctggaagtgattttcaatagccgcaagggc

R Q F I S S C P C W L E V I F N S R *K G*

gagctcaatagcaagcttgaaggtaagcctatccctaaccctctcctcggtctcgatagc

*E L N S K L E* **G K P I P N P L L G L D S**

acgtgagtcgac (SEQ ID NO: 30)

**T** - (SEQ ID NO: 31)

**[0287]** The optimized nucleotide and amino acid sequences will also be used to make a variety of N-terminal and C-terminal Tat-Smad7 fragments. Representative examples are provided below.

**[0288]** The Tat-N-Smad7–V5 most optimized nucleotide and amino acid sequences are provided. The protein sequence includes the V5 epitope, which is indicated by bold capital letters.

ggatccggccgtaaaaaacgccgtcaacgccgccgtggtttccgtacgaaacgcagcgcc

G S G R K K R R Q R R R G F R T K R S A

ctggtccgtcgcctgtggcgcagccgtgctccgggtggtgaagatgaagaagaaggtgct

L V R R L W R S R A P G G E D E E E G A

ggcggcggtggcggtggcggtgaactgcgtggcgagggtgcaaccgatagccgtgcacat

G G G G G G G E L R G E G A T D S R A H

ggtgcaggcggtggcggtccgggtcgtgctggttgctgtctgggtaaagctgtgcgcggc

G A G G G G P G R A G C C L G K A V R G

gcgaaaggtcatcatccgcatccgccggcagcaggtgcaggtgcagctggcggtgcg

A K G H H H P H P P A A G A G A A G G A

gaagccgatctgaaagccctgacccatagcgtcctgaaaaaactgaaagaacgtcagctg

E A D L K A L T H S V L K K L K E R Q L

gagctgctgctgcaagcagtagaaagccgtggcggtacccgtacggcttgtctgctgctg

E L L L Q A V E S R G G T R T A C L L L

ccgggtcgtctggattgccgtctgggtccgggtgcaccggctggtgcgcagccggcacaa

P G R L D C R L G P G A P A G A Q P A Q

ccgccgagcagctacagcctgccgctgctgctgtgtaaagtgtttcgttggccggacctg

P P S S Y S L P L L L C K V F R W P D L

cgccatagcagcgaagttaaacgcctgtgctgttgcgagagctatggcaaaattaacccg

R H S S E V K R L C C C E S Y G K I N P

gaactggtttgttgcaatccgcatcatctgagccgtctgtgtgaactggagagcccgccg

E L V C C N P H H L S R L C E L E S P P

ccgccgtatagccgttacccgatggatttcctgaaaccgactgcagattgcccggacgca

P P Y S R Y P M D F L K P T A D C P D A

gtcccgagcagcgctgagaccggcggcaccaactatctggcaccgggcggtctgagcgat

V P S S A E T G G T N Y L A P G G L S D

agccagctgctgctggaaccgggcgaccgtggtaagcctatccctaaccctctcctcggt

S Q L L L E P G D R **G K P I P N P L L G**

ctcgattctacgtgagtcgac (SEQ ID NO: 32)

**L D S T -** (SEQ ID NO: 27)

**[0289]** Tat-C-Smad7–V5 most optimized nucleotide and amino acid sequences are provided. The protein sequence includes the V5 epitope (indicated by bold capital letters), and the pET101-Topo backbone (indicated by underlined italics).

ggatccggccgtaaaaaacgccgtcaacgccgccgttcacattggtgtgtggttgcctat

G S G R K K R R Q R R R S H W C V V A Y

tgggaagagaaaacgcgtgtcggtcgcctgtactgcgtacaggaaccgagcctggatatc

W E E K T R V G R L Y C V Q E P S L D I

ttttatgacctgccgcagggcaatggtttctgtctgggccaactgaacagcgataataaa

F Y D L P Q G N G F C L G Q L N S D N K

agccagctggtgcaaaaagttcgcagcaaaattggctgcggtatccagctgacccgtgaa

S Q L V Q K V R S K I G C G I Q L T R E

gttgacggtgtctgggtatataaccgcagcagctacccgatttttatcaaaagcgccacc

V D G V W V Y N R S S Y P I F I K S A T

ctggataatccggacagccgtacgctgctggtccataaagtatttccgggcttcagcatc

L D N P D S R T L L V H K V F P G F S I

aaagcgttcgattacgagaaagcctacagcctgcagcgcccgaacgaccatgaattcatg

K A F D Y E K A Y S L Q R P N D H E F M

cagcaaccgtggacgggttttactgtgcagatcagcttcgttaaaggctggggtcaatgc

Q Q P W T G F T V Q I S F V K G W G Q C

tacacccgtcagtttatcagcagctgtccgtgctggctggaagtgattttcaatagccgc

Y T R Q F I S S C P C W L E V I F N S R

aagggcgagctcaatagcaagcttgaaggtaagcctatccctaaccctctcctcggtctc

*K G E L N S K L E* **G K P I P N P L L G L**

gatagcacgtgagtcgac (SEQ ID NO: 34)

**D S T** - (SEQ ID NO: 25)

**[0290]** The comparisons before and after the above optimizations are provided below. C-terminal optimization (top strand) (alignment discloses SEQ ID NOS 34 and 24, respectively, in order of appearance):

```
Alignment:   Local DNA homologies.
Parameters:  Both strands.  Method:  FastScan - Max Score

   Mol 1 Tat-C-Smad7-ser-his optimized (1-618)     Mol 2  Tat-C-termal Smad7-V5 (1-618)
   Number of sequences to align:  2
   Settings:  Similarity significance value cutoff:  >= 60%

Homology Block:  Percent Matches 93   Score 541   Length 618

Tat-C-Smad7-      1 ggatccggccgtaaaaaacgccgtcaacgccgccgttcacattggtgtgtggttgcctat
Tat-C-termal      1 ............................................................

Tat-C-Smad7-     61 tgggaagagaaaacgcgtgtcggtcgcctgtactgcgtacaggaaccgagcctggatatc
Tat-C-termal     61 ....................................................tcg.....

Tat-C-Smad7-    121 ttttatgacctgccgcagggcaatggtttctgtctgggccaactgaacagcgataataaa
Tat-C-termal    121 ................................................tca.........

Tat-C-Smad7-    181 agccagctggtgcaaaaagttcgcagcaaaattggctgcggtatccagctgacccgtgaa
Tat-C-termal    181 tcg.....................tca.....................................

Tat-C-Smad7-    241 gttgacggtgtctgggtatataaccgcagcagctacccgatttttatcaaaagcgccacc
Tat-C-termal    241 ..............................tct...................t......

Tat-C-Smad7-    301 ctggataatccggacagccgtacgctgctggtccataaagtatttccgggcttcagcatc
Tat-C-termal    301 ..............tc.................c.................tca...

Tat-C-Smad7-    361 aaagcgttcgattacgagaaagcctacagcctgcagcgcccgaacgaccatgaattcatg
Tat-C-termal    361 ............................tcg.............................

Tat-C-Smad7-    421 cagcaaccgtggacgggttttactgtgcagatcagcttcgttaaaggctggggtcaatgc
Tat-C-termal    421 ................................tct.........................

Tat-C-Smad7-    481 tacacccgtcagtttatcagcagctgtccgtgctggctggaagtgattttcaatagccgc
Tat-C-termal    481 ..................tcgtc.....................................

Tat-C-Smad7-    541 aagggcgagctcaatagcaagcttgaaggtaagcctatccctaaccctctcctcggtctc
Tat-C-termal    541 ..............tcg.........................................

Tat-C-Smad7-    601 gatagcacgtgagtcgac
Tat-C-termal    601 ...tct............
```

N-terminal optimization (top strand) (alignment discloses SEQ ID NOS 32 and 26 respectively, in order of appearance):

```
Alignment:   Local DNA homologies.
Parameters:  Both strands.  Method:  FastScan - Max Score

   Mol 1 Tat-N-Smad7-V5-Ser-His optimized (1-861)     Mol 2  Tat-N-Smad7-V5 (1-861)
   Number of sequences to align:  2
   Settings:  Similarity significance value cutoff:  >= 60%

Homology Block:  Percent Matches 95   Score 781   Length 861

Tat-N-Smad7-      1 ggatccggccgtaaaaaacgccgtcaacgccgccgtggtttccgtacgaaacgcagcgcc
Tat-N-Smad7-      1 ...................................................tcg...

Tat-N-Smad7-     61 ctggtccgtcgcctgtggcgcagccgtgctccgggtggtgaagatgaagaagaaggtgct
Tat-N-Smad7-     61 ......................tc..................................

Tat-N-Smad7-    121 ggcggcggtggcggtggcggtgaactgcgtggcgagggtgcaaccgatagccgtgcacat
Tat-N-Smad7-    121 ...................................................t........c

Tat-N-Smad7-    181 ggtgcaggcggtggcggtccgggtcgtgctggttgctgtctgggtaaagctgtgcgcggc
Tat-N-Smad7-    181 ............................................................

Tat-N-Smad7-    241 gcgaaaggtcatcatcatccgcatccgccggcagcaggtgcaggtgcagctggcggtgcg
Tat-N-Smad7-    241 ..............c........c....................................

Tat-N-Smad7-    301 gaagccgatctgaaagccctgacccatagcgtcctgaaaaaactgaaagaacgtcagctg
Tat-N-Smad7-    301 ............................t...............................

Tat-N-Smad7-    361 gagctgctgctgcaagcagtagaaagccgtggcggtacccgtacggcttgtctgctgctg
Tat-N-Smad7-    361 ........................tc..................................

Tat-N-Smad7-    421 ccgggtcgtctggattgccgtctgggtccgggtgcaccggctggtgcgcagccggcacaa
Tat-N-Smad7-    421 ............................................................

Tat-N-Smad7-    481 ccgccgagcagctacagcctgccgctgctgctgtgtaaagtgtttcgttggccggacctg
Tat-N-Smad7-    481 .........tct.....................................................

Tat-N-Smad7-    541 cgccatagcagcgaagttaaacgcctgtgctgttgcgagagctatggcaaaattaacccg
Tat-N-Smad7-    541 .....c..ttc.....................................................

Tat-N-Smad7-    601 gaactggtttgttgcaatccgcatcatctgagccgtctgtgtgaactggagagcccgccg
Tat-N-Smad7-    601 ......................c......tct.....................................

Tat-N-Smad7-    661 ccgccgtatagccgttacccgatggatttcctgaaaccgactgcagattgcccggacgca
Tat-N-Smad7-    661 .........tct.....................................................

Tat-N-Smad7-    721 gtcccgagcagcgctgagaccggcggcaccaactatctggcaccgggcggtctgagcgat
Tat-N-Smad7-    721 ......tcatcg.....................................................t...

Tat-N-Smad7-    781 agccagctgctgctggaaccgggcgaccgtggtaagcctatccctaaccctctcctcggt
Tat-N-Smad7-    781 tc..............................................................

Tat-N-Smad7-    841 ctcgattctacgtgagtcgac
Tat-N-Smad7-    841 ....................
```

**[0291]** In addition, other codon-optimized nucleic acids will also be assessed for their ability to produce Smad7 protein in one or more expression systems. Provided below is another example of such a sequence.

**[0292]** Tat-Smad7$^{M7216L}$-V5 optimized by Optimizer program:

ggatccggtcgtaaaaaacgtcgtcagcgtcgtcgtggtttccgtaccaaacgttctgcg

G S G R K K R R Q R R R G F R T K R S A

ccgatcccgaacccgctgctgggtctggactctacctgagtcgac (SEQ ID NO: 36)

P I P N P L L G L D S T - - (SEQ ID NO: 37)

**[0293]** Nucleotide sequence:

1-6: BamHI; 7-36: Tat; 37-1314: codon-optimized human Smad7; 1315-1356: V5; 137-1359: stop; 1360-1365 SalI

ATG is removed to be used with GST; 682 ATG to CTG (M216 to L)

1 ggatccggtc gtaaaaaacg tcgtcagcgt cgtcgtggtt tccgtaccaa acgttctgcg

61 ctggttcgtc gtctgtggcg ttctcgtgcg ccgggtggtg aagacgaaga agaaggtgcg

121 ggtggtggtg gtggtggtgg tgaactgcgt ggtgaaggtg cgaccgactc tcgtgcgcac

181 ggtgcgggtg gtggtggtcc gggtcgtgcg ggttgctgcc tgggtaaagc ggttcgtggt

241 gcgaaaggtc accaccaccc gcacccgccg gcggcgggtg cgggtgcggc gggtggtgcg

301 gaagcggacc tgaaagcgct gacccactct gttctgaaaa aactgaaaga acgtcagctg

361 gaactgctgc tgcaggcggt tgaatctcgt ggtggtaccc gtaccgcgtg cctgctgctg

421 ccgggtcgtc tggactgccg tctgggtccg ggtgcgccgg cgggtgcgca gccggcgcag

481 ccgccgtctt cttactctct gccgctgctg ctgtgcaaag ttttccgttg gccggacctg

541 cgtcactctt ctgaagttaa acgtctgtgc tgctgcgaat cttacggtaa aatcaacccg

601 gaactggttt gctgcaaccc gcaccacctg tctcgtctgt gcgaactgga atctccgccg

661 ccgccgtact ctcgttaccc gctggacttc ctgaaaccga ccgcggactg cccggacgcg

721 gttccgtctt ctgcggaaac cggtggtacc aactacctgg cgccgggtgg tctgtctgac

781 tctcagctgc tgctggaacc gggtgaccgt tctcactggt gcgttgttgc gtactgggaa

841 gaaaaaaccc gtgttggtcg tctgtactgc gttcaggaac cgtctctgga catcttctac

901 gacctgccgc agggtaacgg tttctgcctg ggtcagctga actctgacaa caaatctcag

961 ctggttcaga aagttcgttc taaaatcggt tgcggtatcc agctgacccg tgaagttgac

1021 ggtgtttggg tttacaaccg ttcttcttac ccgatcttca tcaaatctgc gaccctggac

1081 aacccggact ctcgtaccct gctggttcac aaagttttcc cgggtttctc tatcaaagcg

1141 ttcgactacg aaaaagcgta ctctctgcag cgtccgaacg accacgaatt catgcagcag

1201 ccgtggaccg gtttcaccgt tcagatctct ttcgttaaag gttggggtca gtgctacacc

1261 cgtcagttca tctcttcttg cccgtgctgg ctggaagtta tcttcaactc tcgtggtaaa

1321 ccgatcccga acccgctgct gggtctggac tctacctgag tcgac (SEQ ID NO: 36)

**[0294]** Sequence comparison with Tat-Smad7$^{M7216L}$-V5 described in Example 4 (alignment discloses SEQ ID NOS 36 and 30, respectively, in order of appearance):

1681 acaggggaat ggattttgtc tgggccagct gaactctgac aataagagtc agctggtgca

1741 gaaagtccgg agcaagattg gctgcggcat ccagctgacc agggaggtgg acggcgtgtg

1801 ggtctacaac cgcagttcat atccaatctt catcaagagc gccactctgg acaatcccga

1861 ttcccgcacc ctgctggtgc ataaggtctt ccccggcttc agcatcaagg ccttcgacta

1921 cgagaaggct tatagtctcc agcggcccaa cgatcacgag ttcatgcagc agccttggac

1981 aggcttcact gtgcagatca gcttcgtcaa gggatggggc cagtgctaca caaggcagtt

2041 catctcaagc tgtccctgtt ggctggaagt cattttcaat agtaggacgc gtac**gcggcc**

2101 **gc**tcgagcag aaactcatct cagaagagga tctggcagca aatgatatcc tggattacaa

2161 ggatgacgac gataaggttt aa (SEQ ID NO: 87)

**[0297]** The entire sequence (up to the NotI site) is codon optimized for mammalian expression, using the OPTIMUMGENE™ program from GenScript.

1790-1792: changes GAT to GAC, (kept Asp) to avoid alternative ORF (highlight)

1-82: cloning sites+kozac sequence in pCMV6-Entry

83-777: GST+ precision enzyme sites; 776-805: Tat;

806-2086: full length human Smad7

2087-2107: MluI, NotI, XhoI

2108-2182: myc-flag from pCMV6-Entry

Bold underline: unique NotI site

**[0298]** The above nucleotide sequence is 79% homologous to native human Smad7.

```
Mol 1 human Smad7 mRNA (1-3103)     Mol 2  optimized GST-Tat-Smad7-myc-flag
(1-2182)

Number of sequences to align:  2

Settings:  Sort results list by Score


human Smad7     500 tcaccaccatccccacccgccagccgcgggcgccggcgcggccgggggcg

optimized GS   1027 .....c.....-.....----..t.....a..a..a.....c..a.


human Smad7     550 ccgaggcggatctgaaggcgctcacgcactcggtgctcaagaaactgaag

optimized GS   1071 .t.....a..c.....a..c..g..t...agt.....g...........
```

```
optimized GS   1621 a........a.....g..t..c...........t..c.....c.......

human Smad7    1150 atgatctacctcaggggaatggcttttgcctcggacagctcaattcggac
optimized GS   1671 .c.....g..a...........a.....t..g..c.....g..c..t...

human Smad7    1200 aacaagagtcagctggtgcagaaggtgcggagcaaaatcggctgcggcat
optimized GS   1721 ..t.....................a..c........g..t..........

human Smad7    1250 ccagctgacgcgggaggtggatggtgtgtgggtgtacaaccgcagcagtt
optimized GS   1771 .........ca..........c..c........c...........ttca.

human Smad7    1300 accccatcttcatcaagtccgccacactggacaacccggactccaggacg
optimized GS   1821 .t..a............ag......t........t..c..t...c.c..c

human Smad7    1350 ctgttggtacacaaggtgttccccggtttctccatcaaggctttcgacta
optimized GS   1871 ...c....g..t.....c........c...ag.........c........

human Smad7    1400 cgagaaggcgtacagcctgcagcggcccaatgaccacgagtttatgcagc
optimized GS   1921 .........t..t..t..c...........c..t........c.......

human Smad7    1450 agccgtggacgggctttaccgtgcagatcagctttgtgaagggctggggt
optimized GS   1971 ....t.....a.....c..t.............c..c.....a.....c

human Smad7    1500 cagtgctacacccgccagttcatcagcagctgcccgtgctggctagaggt
optimized GS   2021 ...........aa.g.........tca.....t..c..t.....g..a..

human Smad7    1550 catcttcaa (SEQ ID NO: 88)
optimized GS   2071 ...t..... (SEQ ID NO: 89)
```

**[0299]** Optimized GST-Tat-Smad7-myc-flag protein sequence from the above nucleotide sequence:

```
  1 mspilgywki kglvqptrll leyleekyee hlyerdegdk wrnkkfelgl efpnlpyyid
 61 gdvkltqsma iiryiadkhn mlggcpkera eismlegavl dirygvsria yskdfetlkv
```

```
121 dflsklpeml kmfedrlchk tylngdhvth pdfmlydald vvlymdpmcl dafpklvcfk
181 krieaipqid kylksskyia wplqgwqatf gggdhppksd levlfqgplg sgrkkrrqrr
241 rsrfrtkrsa lvrrlwrsra pggedeeega ggggggggelr gegatdsrah gaggggpgra
301 gcclgkavrg akghhhphpp aagagaagga eadlkalths vlkklkerql elllqavesr
361 ggtrtaclll pgrldcrlgp gapagaqpaq ppssyslpll lckvfrwpdl rhssevkrlc
421 ccesygkinp elvccnphhl srlcelespp ppysrypmdf lkptadcpda vpssaetggt
481 nylapgglsd sqlllepgdr shwcvvaywe ektrvgrlyc vqepsldify dlpqgngfcl
541 gqlnsdnksq lvqkvrskig cgiqltrevd gvwvynrssy pifiksatld npdsrtllvh
601 kvfpgfsika fdyekayslq rpndhefmqq pwtgftvqis fvkgwgqcyt rqfisscpcw
661 levifnsrtr trpleqklis eedlaandil dykdddddkv (SEQ ID NO: 90)
```

1-229: GST+precision sites

232-668 Tat-Smad7

671-699: restriction sites+myc-Flag

**[0300]** Optimized GST-Tat-N-Smad7-myc-flag nucleotide sequence, cloned in pCMV6-Entry plasmid:

```
  1 gaattcgtcg actggatccg gtaccgagga gatctgccgc cgcgatcgcc atgtccccca
 61 ttctgggcta ctggaagatt aagggcctgg tgcagcctac tagactgctg ctggaatacc
121 tggaggaaaa atatgaagag catctgtatg aaagagacga gggggataaa tggaggaaca
181 agaaattcga actgggactg gagtttccta atctgccata ctatattgac ggcgatgtga
241 agctgactca gtctatggct atcattagat acatcgcaga caaacacaac atgctgggcg
301 ggtgtcctaa ggaaagggca gagattagta tgctggaggg agccgtgctg gatattagat
361 acggcgtctc acgcatcgcc tatagcaaag acttcgaaac cctgaaggtg gattttctga
421 gcaaactgcc tgaaatgctg aagatgttcg aggacagact gtgccacaaa acctacctga
481 atggcgacca tgtcacacac ccagatttta tgctgtacga cgccctggat gtggtcctgt
541 atatggaccc catgtgtctg gatgctttcc ctaagctggt gtgctttaag aaaaggatcg
601 aggcaattcc ccagatcgat aagtacctga aaagctccaa gtatatcgct tggcctctcc
661 agggctggca ggcaacattc ggaggcgggg accatccccc taaaagcgac ctggaggtgc
721 tgtttcaggg accactgggc agcggccgga agaagcggcg gcagaggcgc cgaagtaggt
781 tccgcactaa gcggtcagca ctggtgcgga gactgtggcg atctcgggct cctggaggag
841 aggacgagga agagggagca ggcggcggcg gaggaggagg agaactgcgc ggggagggag
901 ctacagatag ccgagcccac ggagctggag gaggaggacc agggcgagcc ggatgctgtc
```

961 tgggcaaagc agtgagaggc gccaaggggc accatcaccc ccatccaccc gccgctggcg

1021 caggagcagc cggcggagct gaggcagacc tgaaagccct gactcacagt gtgctgaaga

1081 aactgaagga aagacagctg gagctgctgc tccaggcagt cgaatcacgc ggaggcaccc

1141 gaacagcttg tctgctgctg cccggccggc tggactgccg gctgggaccc ggcgcccctg

1201 ctggggcaca gccagcccag cctccatcta gttatagcct gcccctgctg ctgtgtaagg

1261 tgttccgatg gcctgatctg cggcattcaa gcgaagtcaa aaggctgtgc tgttgcgagt

1321 cctacggcaa gattaaccca gaactggtgt gttgcaatcc ccatcacctg tctcgactgt

1381 gtgaactgga gtccccccct ccaccctact ctaggtatcc tatggacttt ctgaagccaa

1441 ccgctgactg cccagatgca gtgccctcct ctgccgagac tgggggaacc aactacctgg

1501 ctcctggcgg actgagcgac tcccagctgc tgctggaacc aggggatcg**g** **cggccgc**tcg

1561 agcagaaact catctcagaa gaggatctgg cagcaaatga tatcctggat tacaaggatg

1621 acgacgataa ggtttaa (SEQ ID NO: 91)

**[0301]** The entire sequence (up to the NotI site) is codon optimized for mammalian expression, using the OPTIMUMGENE™ program from GenScript.

1-50: cloning sites+kozac sequence in pCMV6-Entry

51-743: GST+ precision enzyme sites

744-773-: Tat

774-1549-:human Smad7 encoding N-terminal 1-258aa

1550-1563: NotI, XhoI, Bold underline: unique NotI site

1564-1637: myc-flag from pCMV6-Entry

**[0302]** Optimized GST-Tat-N-Smad7-myc-flag protein sequence from the above nucleotide sequence:

1 mspilgywki kglvqptrll leyleekyee hlyerdegdk wrnkkfelgl efpnlpyyid

61 gdvkltqsma iiryiadkhn mlggcpkera eismlegavl dirygvsria yskdfetlkv

121 dflsklpeml kmfedrlchk tylngdhvth pdfmlydald vvlymdpmcl dafpklvcfk

181 krieaipqid kylksskyia wplqgwqatf gggdhppksd levlfqgplg sgrkkrrqrr

241 rsrfrtkrsa lvrrlwrsra pggedeeega gggggggelr gegatdsrah gaggggpgra

301 gcclgkavrg akghhhphpp aagagaagga eadlkalths vlkklkerql elllqavesr

361 ggtrtaclll pgrldcrlgp gapagaqpaq ppssyslpll lckvfrwpdl rhssevkrlc

421 ccesygkinp elvccnphhl srlcelespp ppysrypmdf lkptadcpda vpssaetggt

```
481  nylapgglsd sqlllepgdr rpleqklise edlaandild ykddddkv (SEQ ID NO: 92)
```

1-241: GST-Tat

244-500: N-Smad7 2-258

501-528: restriction sites+myc-Flag

**[0303]** Optimized GST-Tat-C-Smad7-myc-flag nucleotide sequence, cloned in pCMV6-Entry plasmid:

```
   1  gaattcgtcg actggatccg gtaccgagga gatctgccgc cgcgatcgcc atgtccccca
  61  ttctgggcta ctggaagatt aagggcctgg tgcagcctac tagactgctg ctggaatacc
 121  tggaggaaaa atatgaagag catctgtatg aaagagacga gggggataaa tggaggaaca
 181  agaaattcga actgggactg gagtttccta atctgccata ctatattgac ggcgatgtga
 241  agctgactca gtctatggct atcattagat acatcgcaga caaacacaac atgctgggcg
 301  ggtgtcctaa ggaaagggca gagattagta tgctggaggg agccgtgctg gatattagat
 361  acggcgtctc acgcatcgcc tatagcaaag acttcgaaac cctgaaggtg gattttctga
 421  gcaaactgcc tgaaatgctg aagatgttcg aggacagact gtgccacaaa acctacctga
 481  atggcgacca tgtcacacac ccagatttta tgctgtacga cgccctggat gtggtcctgt
 541  atatggaccc catgtgtctg gatgctttcc ctaagctggt gtgctttaag aaaaggatcg
 601  aggcaattcc ccagatcgat aagtacctga aaagctccaa gtatatcgct tggcctctcc
 661  agggctggca ggcaacattc ggaggcgggg accatccccc taaaagcgac ctggaggtgc
 721  tgtttcaggg accactgggc agcggccgga agaagcggcg gcagaggcgc agccactggt
 781  gtgtggtcgc ctactgggaa gagaagacaa gagtgggaag gctgtattgc gtccaggagc
 841  cttccctgga catcttctac gatctgccac aggggaatgg attttgtctg ggccagctga
 901  actctgacaa taagagtcag ctggtgcaga aagtccggag caagattggc tgcggcatcc
 961  agctgaccag ggaggtggac ggcgtgtggg tctacaaccg cagttcatat ccaatcttca
1021  tcaagagcgc cactctggac aatcccgatt cccgcaccct gctggtgcat aaggtcttcc
1081  ccggcttcag catcaaggcc ttcgactacg agaaggctta tagtctccag cggcccaacg
1141  atcacgagtt catgcagcag ccttggacag gcttcactgt gcagatcagc ttcgtcaagg
1201  gatggggcca gtgctacaca aggcagttca tctcaagctg tccctgttgg ctggaagtca
1261  ttttcaatag taggacgcgt acgcggccgc tcgagcagaa actcatctca gaagaggatc
1321  tggcagcaaa tgatatcctg gattacaagg atgacgacga taaggtttaa (SEQ ID NO: 93)
```

**[0304]** The entire sequence (up to the NotI site) is codon optimized for mammalian expression, using the OPTIMUMGENE™ program from GenScript.

1-50: cloning sites+kozac sequence in pCMV6-Entry

51-743: GST+ precision enzyme sites

744-773: Tat

774-1274: c-terminal Smad7

1275: Mlu

1283: NotI

1275-1370: mluI, NotI, XhoI, myc+flag

**[0305]** Optimized GST-Tat-C-Smad7-myc-flag protein sequence from the above nucleotide sequence:

```
  1 mspilgywki kglvqptrll leyleekyee hlyerdegdk wrnkkfelgl efpnlpyyid
 61 gdvkltqsma iiryiadkhn mlggcpkera eismlegavl dirygvsria yskdfetlkv
121 dflsklpeml kmfedrlchk tylngdhvth pdfmlydald vvlymdpmcl dafpklvcfk
181 krieaipqid kylksskyia wplqgwqatf gggdhppksd levlfqgplg sgrkkrrqrr
241 rshwcvvayw eektrvgrly cvqepsldif ydlpqgngfc lgqlnsdnks qlvqkvrski
301 gcgiqltrev dgvwvynrss ypifiksatl dnpdsrtllv hkvfpgfsik afdyekaysl
361 qrpndhefmq qpwtgftvqi sfvkgwgqcy trqfisscpc wlevifnsrt rtrpleqkli
421 seedlaandi ldykddddkv  (SEQ ID NO: 94)
```

1-241: GST+precision+Tat

242-409: c-Smad7 (259-426aa)

410-440: Mlu+Not+myc+flag: restriction sites+myc-Flag

**[0306]** Based on the Smad7 structure, it is anticipated that the linker (203-258aa), which contains the PY domain (203-217aa), will have therapeutic effects by blocking TGF-β-induced inflammation or growth inhibition (**FIG. 15**).

**[0307]** The amino acid sequence for the Tat-Smad7-linker peptide (203-258aa) is:

GRKKRRQRRR-
ELESPPPPYSRYPMDFLKPTADCPDAVPSSAETGGTNYLAPGGLSDSQLLLEPGDR
(SEQ ID NO: 95)

1-10: Tat

**[0308]** The codon optimized nucleotide sequence for the above peptide in bacterial production is:

```
  1 ggccgtaaaa aacgccgtca acgccgccgt gaactggaga gcccgccgcc gccgtatagc
 61 cgttacccga tggatttcct gaaaccgact gcagattgcc cggacgcagt cccgagcagc
121 gctgagaccg gcggcaccaa ctatctggca ccgggcggtc tgagcgatag ccagctgctg
181 ctggaaccgg gcgaccgtgg taagcctatc cctaaccctc tcctcggtct cgattctacg
241 tgagtcgac (SEQ ID NO: 96)
```

1-30: Tat

31-198: Smad7 linker

199-249: V5

**[0309]** The codon optimized nucleotide sequence for the above peptide in mammalian cell production is:

```
  1 ggccggaaga agcggcggca gaggcgccga gaactggagt ccccccctcc accctactct
 61 aggtatccta tggactttct gaagccaacc gctgactgcc cagatgcagt gccctcctct
121 gccgagactg ggggaaccaa ctacctggct cctggcggac tgagcgactc ccagctgctg
181 ctggaaccag gggatcggcg gccgctcgag cagaaactca tctcagaaga ggatctggca
241 gcaaatgata tcctggatta caaggatgac gacgataagg tttaa (SEQ ID NO: 97)
```

1-30: mammalian codon optimized Tat

31-198: Smad7 linker

199-285: NotI, Myc tag, Flag tag

**[0310]** The amino acid sequence for the Tat-Smad7-PY peptide (203-217aa) is:

GRKKRRQRRR-ELESPPPPYSRYPMD (SEQ ID NO: 98)

1-10: Tat

**[0311]** The codon optimized nucleotide sequence for the above peptide in bacterial production is:

```
  1  ggccgtaaaa aacgccgtca acgccgccgt gaactggaga gcccgccgcc gccgtatagc
 61  cgttacccga tggatggtaa gcctatccct aaccctctcc tcggtctcga ttctacgtga
121  gtcgac (SEQ ID NO: 99)
```

1-30: Tat

31-75: Smad7-PY

76-126: V5

**[0312]** The codon optimized nucleotide sequence for the above peptide in mammalian cell production is:

```
  1  ggccggaaga agcggcggca gaggcgccga gaactggagt ccccccctcc accctactct
 61  aggtatccta tggaccggcc gctcgagcag aaactcatct cagaagagga tctggcagca
121  aatgatatcc tggattacaa ggatgacgac gataaggttt aa (SEQ ID NO: 100)
```

1-30: mammalian codon optimized Tat

31-75: Smad7 PY

76-162: NotI, Myc tag, Flag tag

**[0313]** The foregoing discussion of the present technology has been presented for purposes of illustration and description. The foregoing is not intended to limit the present technology to the form or forms disclosed herein. Although the description of the present technology has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the present technology, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

## CLAIMS

What is claimed is:

1. A nucleic acid molecule comprising a codon-optimized human Smad7 cDNA nucleotide sequence.

2. The nucleic acid molecule of claim 1, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codon for arginine optimized for expression in one or more of bacteria or yeast.

3. The nucleic acid molecule of either one of claims 1 or 2, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codon for serine optimized for expression in one or more of bacteria or yeast.

4. The nucleic acid molecule of any one of claims 1 to 3, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codon for histidine optimized for expression in one or more of bacteria or yeast.

5. The nucleic acid molecule of any one of claims 1 to 4, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codons for serine, one or more codons for histidine, and one or more codons for arginine optimized for expression in one or more of bacteria or yeast.

6. The nucleic acid molecule of claim 5, wherein the codon-optimized human Smad7 nucleotide sequence includes 28 serine codons, 6 histidine codons, and 9 arginine codons optimized for expression in one or more of bacteria or yeast.

7. The nucleic acid molecule of claim 1, wherein the codon-optimized human Smad7 nucleotide sequence is selected from the group consisting of SEQ ID NOS: 9-11, 21, 23-41).

8. The nucleic acid molecule of claim 1, wherein the codon-optimized human Smad7 nucleotide sequence has about 65 to 75 percent homology to human Smad7 cDNA.

9. The nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises a nucleotide sequence encoding an N-terminal fragment SMAD7.

10. The nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises a nucleotide sequence encoding a C-terminal fragment of SMAD7.

11. The nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises nucleotides encoding amino acids 2-258 of the human Smad7 protein.

12. The nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises nucleotides encoding amino acids 259-426 of the human Smad7 protein.

13. The nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises nucleotides encoding amino acids 204-258 of the human Smad7 protein.

14. The nucleic acid molecule of any one of claims 1 to 14, wherein the nucleotide sequence further comprises a nucleotide sequence encoding a protein transduction domain.

15. The nucleic acid molecule of claim 14, wherein the protein transduction domain is Tat.

16. The nucleic acid molecule of any one of claims 1 to 15, wherein the nucleotide sequence further comprises a nucleotide sequence encoding one or more of an epitope tag or a purification tag.

17. The nucleic acid of claim 16, wherein the epitope tag is V5.

18. The nucleic acid of claim 16, wherein the purification tag is one or more of glutathione-S-transferase or 6-Histidine.

19. The nucleic acid molecule of any one of claims 1-18, wherein the nucleic acid molecule is one or more of isolated or purified.

20. The nucleic acid of any one of claims 1-19, wherein the nucleic acid molecule encodes a polypeptide having one or more biological activity selected from the group consisting of reducing or eliminating phosphorylation of Smad2, reducing or eliminating nuclear translocation of the NFκB p50 subunit, increasing cell proliferation, reducing apoptosis, reducing radiation-induced DNA damage, reducing inflammation, reducing angiogenesis, promoting healing in oral mucositis, promoting wound healing, and treating auto-immune disease.

21. A pharmaceutical composition comprising the nucleic acid molecule of any one of claims 1-20, and one or more pharmaceutically acceptable excipients.

22. An expression vector comprising the nucleic acid molecule of any one of claims 1 to 20 operably linked to a promoter.

23. A host cell comprising the expression vector of claim 22.

24. A pharmaceutical composition comprising the expression vector of claim 22 and one or more pharmaceutically acceptable excipients.

25. A protein molecule comprising a human Smad7 protein having leucine at position 216.

26. The protein molecule of claim 25, wherein the human Smad7 protein is truncated at the C-terminal.

27. The protein molecule of claim 25, wherein the human Smad7 protein is truncated at the N-terminal.

28. The protein molecule of any one of claims 26 to 27, wherein the truncated human Smad7 protein includes about 50% of the full-length Smad7 sequence.

29. The protein molecule of any one of claims 26 to 27, wherein the truncated human Smad7 protein includes about 13% of the full-length Smad7 sequence.

30. A protein molecule comprising a human Smad7 protein having amino acids 2-258 of the human Smad7 protein.

31. The protein molecule of claim 30, wherein the Smad7 protein comprises amino acids 204-258.

32. A protein molecule comprising a human Smad7 protein having amino acids 259-426 of the human Smad7 protein.

33. The protein molecule of any one of claims 25 to 32, wherein the protein molecule has one or more biological activity selected from the group consisting of reducing or eliminating phosphorylation of Smad2, reducing or eliminating nuclear translocation of the NFκB p50 subunit, increasing cell proliferation, reducing apoptosis, reducing

radiation-induced DNA damage, reducing inflammation, reducing angiogenesis, promoting healing in oral mucositis, promoting wound healing, and treating auto-immune disease.

34. The protein molecule of any one of claims 25 to 33, wherein the amino acid sequence further comprises a protein transduction domain.

35. The protein molecule of claim 34, wherein the protein transduction domain is Tat.

36. The protein molecule of any one of claims 25 to 35, wherein the amino acid sequence further comprises one or more of an epitope tag or a purification tag.

37. The protein molecule of claim 36, wherein the epitope tag is v5.

38. The protein molecule of claim 36, wherein the purification tag is one or more of glutathione-S-transferase or 6-histidine.

39. A pharmaceutical composition comprising the protein molecule of any one of claims 25 to 38, and one or more pharmaceutically acceptable excipient.

40. A method for treating or preventing an inflammatory condition in a subject comprising providing to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 21, 24 or 39.

41. The method of claim 40, wherein the inflammatory condition is one or more of a chronic wound, skin inflammation, psoriasis, or an autoimmune disease.

42. The method of claim 40, wherein the composition reduces inflammation through inhibition of TGFβ and NFκB signaling.

43. A method for preventing or treating a disease or disorder in a subject comprising one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage in the subject comprising providing to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 21, 24 or 39, wherein one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage is useful in preventing or treating the disease or disorder.

44. The method of claim 43, wherein the disease or disorder includes one or more of chronic wounds, acute wounds, or mucositis.

45. The method of claim 44, wherein the chronic wounds include one or more of diabetic ulcers, pressure ulcers, venous ulcers, or oral ulcers.

46. The method of claim 44, wherein the acute wounds include one or more of trauma-induced wounds, surgical wounds, or scarring.

47. The method of claim 44, wherein the mucositis includes one or more of radiation-induced mucositis or chemotherapy-induced mucositis.

48. The method of claim 44, wherein the mucositis includes one or more of oral mucositis or gut mucositis.

## CLAIMS

1. A nucleic acid molecule comprising a codon-optimized human Smad7 cDNA nucleotide sequence, wherein the codon-optimized human Smad7 nucleotide sequence includes one or more codons for serine, one or more codons for histidine, and one or more codons for arginine optimized for expression in one or more of bacteria, yeast, or mammalian cells.

2. The nucleic acid molecule of claim 1, wherein the codon-optimized human Smad7 nucleotide sequence is selected from the group consisting of SEQ ID NOs: 9, 21, 23, 24, 26, 28, 30, 32, 33, 34, 36, 38, 39, 87, 89, 91, 93, 96, 97, nucleotides 1-75 of SEQ ID NO: 99, and nucleotides 1-75 of SEQ ID NO: 100.

3. The nucleic acid molecule of either one of claim 1 or 2, wherein the nucleotide sequence comprises nucleotides encoding amino acids 2-258 of the human Smad7 protein.

4. The nucleic acid molecule of either one of claim 1 or 2, wherein the nucleotide sequence comprises nucleotides encoding amino acids 259-426 of the human Smad7 protein.

5. The nucleic acid molecule of either one of claim 1 or 2, wherein the nucleotide sequence comprises nucleotides encoding amino acids 203-217 of the human Smad7 protein.

6. The nucleic acid molecule of any one of the preceding claims, wherein the nucleotide sequence further comprises a nucleotide sequence encoding a protein transduction domain.

7. The nucleic acid molecule of claim 6, wherein the protein transduction domain is Tat.

8. The nucleic acid of any one of the preceding claims, wherein the nucleic acid molecule encodes a polypeptide having one or more biological activity selected from the group consisting of promoting healing in oral mucositis, promoting wound healing, reducing scarring, reducing fibrosis, and treating auto-immune disease.

9. A pharmaceutical composition comprising the nucleic acid molecule of any one of the preceding claims, and one or more pharmaceutically acceptable excipients.

10. A protein molecule comprising a human Smad7 protein having leucine at position 216.

11. A protein molecule comprising a human Smad7 protein having amino acids 2-258 of the human Smad7 protein.

12. The protein molecule of claim 11, wherein the Smad7 protein comprises amino acids 203-217.

13. A protein molecule comprising a human Smad7 protein having amino acids 259-426 of the human Smad7 protein.

14. The protein molecule of any one of claims 10-13, wherein the protein molecule has one or more biological activity selected from the group consisting of promoting healing in oral mucositis, promoting wound healing, reducing scarring, reducing fibrosis, and treating auto-immune disease.

15. The protein molecule of any one of claims 10-14, wherein the amino acid sequence further comprises a protein transduction domain.

16. The protein molecule of claim 15, wherein the protein transduction domain is Tat.

17. A pharmaceutical composition comprising the protein molecule of any one of claims 10-16, and one or more pharmaceutically acceptable excipients.

18. Use of the pharmaceutical composition of either one of claims 9 or 17 in the manufacture of a medicament for the treatment or prevention of an inflammatory condition in a subject.

19. The use of claim 18, wherein the inflammatory condition is one or more of a chronic wound, skin inflammation, psoriasis, or an autoimmune disease.

20. Use of the pharmaceutical composition of either one of claims 9 or 17 in the manufacture of a medicament for the prevention or treatment of a disease or disorder in a subject comprising one or more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage in the subject, wherein one or

more of increasing one or more of cell proliferation or cell migration, or preventing one or more of apoptosis or DNA damage is useful in preventing or treating the disease or disorder.

21. The use of claim 20, wherein the disease or disorder includes one or more of chronic wounds, acute wounds, hypertrophic scarring, fibrosis, or oral mucositis.