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(54) Title: BIOLOGICALLY ACTIVE PEPTIDE AND METHOD OF USING THE SAME

(57) Abstract: A peptide, a composition including the peptide, and a method for using the peptide and the composition including the peptide. The present invention may be used to regulate osteogenesis or other bodily process in a mammal, such as a human, for example. The present invention therefore may be used in the mammal to treat a bone disorder, such as osteoporosis or Paget's disease.

BIOLOGICALLY ACTIVE PEPTIDE AND METHOD OF USING THE SAME

CROSS REFERENCE TO RELATED APPLICATION

[001] The present application claims the priority benefit of US provisional patent application serial no. 60/956,421, filed August 17, 2007, entitled "BIOLOGICALLY ACTIVE PEPTIDE AND METHOD OF USING THE SAME" of the same named inventor. The entire contents of that prior application are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[002] The present invention relates to a peptide having a novel function. More particularly, the present invention relates to a peptide that is capable of regulating osteogenesis and a method for using the peptide to regulate osteogenesis. Even more particularly, the present invention relates to a peptide that is capable of promoting osteogenic cell proliferation and a method of using the peptide for promoting osteogenic cell proliferation. The peptide and method of the present invention therefore are useful for treating bone disorders, such as osteoporosis and Paget's disease, for example.

2. Description of the Prior Art

[003] Chronic bone disorders are prevalent and are often severely disabling to those who suffer from them. For example, the most common bone disorder, osteoporosis, which is a condition that weakens bone integrity, affects ten million people in the United States alone. As a group, these individuals suffer 1.5 million fractures annually, including approximately 300,000 hip fractures, 700,000 vertebral fractures, 250,000 wrist fractures, and more than 300,000 fractures at other sites. Further, another one million U.S. individuals are inflicted with Paget's disease, a malady which causes one or more regions of the skeleton to become enlarged or deformed, thereby leaving these regions susceptible to breakdown.

[004] Studies aimed at treating bone disorders are ongoing as existing treatments of these disorders are suboptimal. This is true because these existing treatments do not stimulate significant bone regeneration, do not reduce bone fracture risk, are not

compatible with certain individuals, and/or are prone to causing side effects. For example, many osteoporosis patients are currently being treated with parathyroid hormone (PTH), an agent which effects bone reformation. However, bone reformation in these patients can occur slowly. Further, PTH treatment is not recommended for several classes of individuals, such as children, pregnant women, and people who have had bone cancer, among others, and PTH has been shown to cause side effects such as headaches, nausea, dizziness, cramping, and hypercalcemia in a substantial number of patients.

[005] Alternative treatments for bone disorders clearly are needed. One class of therapeutic reagents that may be particularly effective for treating bone disorders are those that act at the molecular genetic level. Useful molecular reagents may include those that permit or enhance normal functioning and expression of Bone Morphogenetic Proteins (BMPs), which belong to a subgroup of the TGF-β superfamily of receptor ligands. In particular, reagents that regulate BMP function and expression might be potent for treating bone disorders because BMPs influence multipotent mesenchymal cells to differentiate into chondrocytes, osteoblasts, myocytes, and adipocytes, and because BMPs are crucial for skeletal development and homeostasis, as they play a role in osteoblast and osteoclast differentiation. It is therefore not surprising that abnormally functioning BMPs and reduced BMP levels are characteristic of several bone disorders, including osteoporosis and Paget's disease.

[006] Further, as an added benefit, identification of reagents that effect normal or enhanced functioning and expression of BMPs likely would be therapeutic for disorders other than those that affect bone. For example, because BMPs and other members of the TGF-β superfamily are needed for normal function of the heart, liver, kidney and other organs, a reagent that promotes functioning of BMPs may prevent failure or correct abnormalities of these organs and their processes.

[007] The initiation of BMP signal transduction involves binding of the cognate ligand to a combination of BMP receptors (BMPRs) on the plasma membrane. Recent research has indicated that not only do BMP type-I (BRIa) and type-II (BRII) receptors, which also are known as activin receptor-like kinases (ALKs), aggregate and cluster in specific domains on the cell surface, but that these receptors also must shuttle between

distinct membrane domains in order to transduce their signals. Interdomain shuttling influences BMP signaling and regulates the early steps of osteoblast differentiation.

[008] The present inventor identified casein kinase-2 (CK-2), which is a messenger-independent protein Ser/Thr kinase that is typically found in tetrameric complexes consisting of two catalytic (alpha and/or alpha') subunits and two regulatory beta subunits [Canton *et al.*, *Cell Signal* (2006), vol. 18, pp. 267-275], and that also has been shown to be involved in phosphorylation of tyrosine in *in vitro* studies [Canton *et al.*, *Cell Signal* (2006), vol. 18, pp. 267-275], as being a protein that binds to BMPRs and that is involved in BMPR re-shuttling and signal silencing on the cell surface. The present inventor has also determined that bone marrow stromal cell (BMSC) mineralization is enhanced when normal binding of CK-2 to BMPRs is altered. The present inventor further has determined that normal binding of CK-2 to BRIa can be altered by peptide. It is the alteration to normal binding of CK-2 to BMPRs that forms the basis of the present invention.

SUMMARY OF THE INVENTION

[009] The present invention is a peptide having a novel function, which optionally may be combined in a composition with one or more other peptides and/or with other organic or inorganic compounds, and a method of using the peptide and/or composition to regulate a bodily process in a mammal, including a human. The peptide of the present invention is capable of altering the normal interaction between CK-2 and BRIa. Therefore, the peptide may be used to regulate any bodily process that involves, but does not necessarily require, normal interaction between CK-2 and BRIa. In one aspect, the peptide of the present invention is capable of altering the normal interaction of CK-2 with BRIa, and therefore the peptide is capable of enhancing BMSC mineralization and osteogenic cell proliferation.

[010] In one example, the peptide of the present invention has an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 as described herein.

[011] In another example, the peptide has an amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 and an amino acid signal sequence

linked thereto the sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[012] In another example, the peptide is a conservative variant of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[013] In another example, the peptide is a conservative variant of the amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 and an amino acid signal sequence linked thereto the sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[014] The method of the present invention may be used to regulate a bodily process of a mammal, such as for the purpose of treating that mammal, for example. In one example, the method of the present invention is used to treat a human having a bone disorder, such as osteoporosis or Paget's disease, for example, and the method includes administering to the human one or more therapeutically effective doses of the peptide of the present invention. A variety of means for administering the therapeutically effective dose of the peptide are disclosed and claimed herein, but the invention is not limited thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[015] FIG. 1 is a table showing the alignment of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13.

[016] FIG. 2 is a graph showing the effects of three examples of the peptide (each one having sequence corresponding to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3) of the present invention on Fluorescence Resonance Energy Transfer (FRET) efficiency between BRIa and CK-2.

[017] FIG. 3 is a graph showing the effects that altering the normal interaction between CK-2 and BRIa by using three examples of the peptide (each one having sequence corresponding to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3) of the present invention has on BMP-2-independent signaling.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions and Usage of Terms

[018] The following terms are used herein or are otherwise known to those skilled in the art. Except where stated otherwise, the following definitions apply throughout the specification and claims.

[019] The "ALK portion" of the peptide of the present invention is any amino acid sequence that is homologous to a mammalian ALK protein amino acid sequence. For example, the ALK portions of the peptides represented by SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 are the amino acid sequences corresponding to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

[020] "Amino acid signal sequence" or, alternatively, "signal sequence" is any sequence of amino acids that allows a cell to take within its plasma membrane, including into its nucleus or cytoplasm, any peptide having that sequence that is outside the plasma membrane.

[021] A "conservative variant" is any peptide that is able to alter the interaction between CK-2 and BRIa and that has a single amino acid substitution, or a plurality of amino acid substitutions, such as, for example, two or three amino acid substitutions, to the ALK portion of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 as described herein. Further, a "conservative variant" includes any peptide that is able to alter the interaction between CK-2 and BRIa and that has an amino acid sequence having a small insertion or deletion of amino acids, such as an insertion or deletion of one-to-three amino acids, for example, to the ALK portion of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6. A conservative variant therefore may be a peptide having amino acid sequence corresponding to SEQ ID NO:14 through and including SEQ ID NO:25, as described herein, and no other amino acid sequence corresponding to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[022] A "therapeutically effective dose" is any amount of the peptide that is sufficient to effect regulation of a bodily process of a mammal for the purpose of aiding the treatment of that mammal.

[023] The present inventor has identified CK-2 as a protein that interacts with BMPRs, such as BRIa, for example, in mammal. Briefly, the present inventor has performed FRET assays (see the Example section included herein), immunoprecipitation assays, and Western assays, and, based on observations made in these studies, has determined that CK-2, including its α, α', and β isoforms, co-localizes and interacts with BRIa.

[024] The present inventor further has performed a motif search using seven human ALK proteins (the amino acid sequences of which are shown in FIG. 1) using publicly available human protein sequence (available at http://www.ncbi.nlm.nih.gov). Specifically, these seven human ALK proteins are ALK-1, ALK-2, ALK-3, ALK-6, ALK-4, ALK-5, and ALK-7, the amino acid sequences of which correspond to SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13, respectively. As a result of this search, the present inventor has identified a plurality of consensus CK-2 recognition sites responsible for CK-2 phosphorylation of BRIa (these sites are shown in the three boxed in regions of the ALK protein amino acid sequences of FIG. 1). Further, the present inventor has determined that the normal interaction between CK-2 and BRIa can be altered by peptide including one of these recognition sites (a specific example of a method for altering the interaction between CK-2 and BRIa by peptide is described in the Example section included herein).

[025] The present inventor even further has determined that alteration to the normal interaction between CK-2 and BRIa inhibits BMP-independent signaling and induces osteoblast differentiation in BMSCs (see the Example section included herein).

[026] The present invention includes one or more peptides capable of regulating a mammalian bodily process and a method for using these peptides to regulate a mammalian bodily process. The peptide of the invention includes one of the CK-2 recognition sites (shown within the boxed areas of FIG. 1) of seven human ALK proteins, represented by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13. The peptide of the present invention is capable of altering the normal interaction between CK-2 and BRIa for the purpose of regulating the mammalian bodily process. The present invention therefore is useful for regulating, for example, osteogenesis. Because the present invention is useful for

regulating osteogenesis, it is useful for treating bone disorders, such as osteoporosis and Paget's disease. However, it is to be understood that the peptide and method of the present invention are not limited to being used to regulate osteogenesis or to treat bone disorders.

[027] Generally, the peptide of the present invention includes at least four amino acid residues. Amino acid residues are abbreviated as follows: Alanine is A or Ala; Arginine is R or Arg; Asparagine is N or Asn; Aspartic Acid is D or Asp; Cysteine is C or Cys; Glutamic Acid is E or Glu; Glutamine is Q or Gln; Glycine is G or Gly; Histidine is H or His; Isoleucine is I or Ile; Leucine is L or Leu; Lysine is K or Lys; Methionine is M or Met; Phenylalanine is F or Phe; Proline is P or Pro; Serine is S or Ser; Threonine is T or Thr; Tryptophan is W or Trp; Tyrosine is Y or Tyr; and Valine is V or Val. X or Xaa represents any amino acid. Other relevant amino acids include, but are not limited to, 4-hydroxyproline and 5-hydroxylysine. The amino acid sequence of the peptide of the present invention is presented in conventional form in that the left-most amino acid residue of the sequence is the N-terminal residue and the right-most amino acid residue of the sequence is the carboxy-terminal residue.

[028] The peptide of the present invention may have one of the following three amino acid sequences, but is not limited to having one of the following three amino acid sequences corresponding to SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

[029] (SEQ ID NO:1)

[030] RQIKIWFQNRRMKWKKYHEMGSLYDYLQL;

[031] (SEQ ID NO:2)

[032] RQIKIWFQNRRMKWKKYVPNDPSFEDM; and

[033] (SEQ ID NO:3)

[034] RQIKIWFQNRRMKWKKIPVGESLKDLIDQ.

[035] Each one of the peptides having amino acid sequence corresponding to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 contains the Antennapedia (AP) homeodomain signal sequence, RQIKIWFQNRRMKWKK, or what is also known as the "penetratin sequence". This penetratin sequence allows the peptides having SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 to penetrate biological membranes, such as to allow uptake of the peptide including the penetratin sequence by a cell into its cytoplasm

or nucleus, for example. The amino acid sequence following the penetratin sequence in each of SEQ ID NO:1 and SEQ ID NO:2, YHEMGSLYDYLQL and YVPNDPSFEDM, respectively, corresponds to partial amino acid sequence from the human ALK-2 protein (SEQ ID NO:8), and amino acid sequence following the penetratin sequence in SEQ ID NO:3, which specifically is IPVGESLKDLIDQ, corresponds to partial amino acid sequence from the human ALK-3 protein (SEQ ID NO:9).

[036] Specifically, the ALK portions of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 include one of a plurality of consensus CK-2 recognition sites that were identified in motif searches for such sequences. As shown in the boxed in regions of FIG. 1, the consensus CK-2 recognition sequences from the seven human ALK proteins reviewed in the motif search are TMLGD (SEQ ID NO:14), STLAD (SEQ ID NO:15), SLKD (SEQ ID NO:16), SLRD (SEQ ID NO:17), TLQD (SEQ ID NO:18), TLKD (SEQ ID NO:19), SLYD (SEQ ID NO:20), SLFD (SEQ ID NO:21), SFED (SEQ ID NO:22), SYED (SEQ ID NO:23), SIEE (SEQ ID NO:24), and SVEE (SEQ ID NO:25). (Specifically, SEQ ID NO:14 corresponds to amino acid sequence from SEQ ID NO:7; SEQ ID NO:15 corresponds to amino acid sequence from SEQ ID NO:8; SEQ ID NO:16 corresponds to amino acid sequence from SEQ ID NO:9; SEQ ID NO:17 corresponds to amino acid sequence from SEQ ID NO:12; SEQ ID NO:18 corresponds to amino acid sequence from SEQ ID NO:10; SEQ ID NO:19 corresponds to amino acid sequence from SEQ ID NO:11 and SEQ ID NO:13; SEQ ID NO:20 corresponds to amino acid sequence from SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:12, and SEQ ID NO:13; SEQ ID NO:21 corresponds to amino acid sequence from SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:22 corresponds to amino acid sequence from SEQ ID NO:7 and SEQ ID NO:8; SEQ ID NO:23 corresponds to amino acid sequence from SEQ ID NO:9 and SEQ ID NO:12; SEQ ID NO:24 corresponds to amino acid sequence from SEQ ID NO:10 and SEQ ID NO:13; and SEQ ID NO:25 corresponds to amino acid sequence from SEQ ID NO:11.)

[037] It is to be understood, however, that the peptide of the present invention is not limited to having an amino acid sequence that is identical to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3. The peptide of the present invention therefore includes

other peptides having ALK amino acid sequence that are capable of altering the normal interaction between CK-2 and one or more BMPRs.

[038] In one example, the peptide may have only the amino acid sequence YHEMGSLYDYLQL (SEQ ID NO:4), YVPNDPSFEDM (SEQ ID NO:5), or IPVGESLKDLIDQ (SEQ ID NO:6). Further, the peptide may have the amino acid sequence corresponding to SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6 and an amino acid signal sequence other than the penetratin sequence linked thereto for allowing the peptide to cross a biological membrane. Those of ordinary skill in the art would recognize that a plurality of peptide signal sequences, such as TAT, transportan and polyarginine, for example, exist and would be suitable for allowing any peptide, including the peptide having SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, to penetrate a biological membrane. See, *e.g.*, Deshayes, *Cell Mol. Life Sci.* 62:1839-49 (2005); and Jones, *Br. J. Pharmacol.* 145:1093-1102 (2005); Saalik, *Bioconjug. Chem.* 15:1246-53 (2004), each of which is incorporated herein by reference in its entirety.

[039] The ALK portion of the peptide of the present invention also is not limited to including an amino acid sequence corresponding to the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:9. The amino acid sequence of the peptide, therefore, may correspond to an amino acid sequence from another ALK protein, such as those represented by SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13, for example.

[040] The ALK portion of the peptide also may be only the amino acid sequence corresponding to SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, which are the CK-2 recognition sites identified by the present inventor. The peptide also may include the amino acid sequence corresponding to SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, and additional amino acid that does not correspond to ALK sequence. This additional amino acid sequence may be, but is not limited to being, cellular uptake signal sequence, such as the pentratin sequence, for example.

[041] The skilled artisan would recognize that deviations to the ALK portion of the peptide may be made without deviating from the scope of the invention. Therefore, the amino acid sequence of the peptide may be a conservative variant of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[042] Further, the peptide having SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or a conservative variation thereof, may include additional amino acid sequence. This sequence may be very long, and therefore may include up to hundreds of amino acids, and may lie between the signal sequence or the ALK portion of the peptide, or it may immediately flank either the signal sequence or the ALK sequence or both. Therefore, the peptide of the present invention may contain more than or fewer than the number of amino acids present in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

[043] It is to be further understood that the ALK portion of the sequence of the peptide may be selected based on the species of mammal in which the bodily process is to be regulated. As an example, the peptide of the present invention may include all or part of the amino acid sequence of any of the mouse, rat, canine, feline, equine, bovine, chimpanzee, ape, or monkey homologs of the ALK protein sequences shown in FIG. 1. This would be useful, for example, where the peptide is to be used to alter the interaction of CK-2 and BRIa to regulate oseteogenesis in a pet mammal, such as a pet dog or cat, for example for the purpose of treating a bone disorder in that pet mammal. This also would be useful, for example, where the peptide is to be used to conduct studies in an experimental laboratory mammal, such as a mouse, rat, or chimpanzee, for example.

[044] The peptide of the present invention may be naturally occurring or artificially synthesized. Several methods for synthesizing peptides are presented in, *e.g.*, Pennington and Dunn, *Peptide Synthesis Protocols (Methods in Molecular Biology)*, Humana Press, Totowa, New Jersey (1994), which is entirely incorporated by reference herein. Specifically, the peptide may be artificially synthesized, for example, by using standard solid-phase peptide synthesis (SPPS) methods, such as those described by, e.g., Nelson et al., *Methods in Enzymology, Volume 289: Solid-Phase Peptide Synthesis*, Academic Press, New York (1997), which is entirely incorporated by reference herein.

Further, the peptide may be purified. For example, when the peptide is to be purified, the peptide may be purified to be at least 95% free of compounds that are not the peptide, and more preferably, at least 98% free of compounds that are not the peptide. Methods of purifying peptides are well understood to those of ordinary skill in the art and are described in Pennington and Dunn, *Peptide Synthesis Protocols (Methods in Molecular Biology)*, Humana Press, Totowa, New Jersey (1994) and in Nelson et al., *Methods in Enzymology, Volume 289: Solid-Phase Peptide Synthesis*, Academic Press, New York (1997).

[045] The peptide alternatively may be artificially synthesized by introducing DNA which encodes the peptide into a cell, such as a mammalian cell, for example, in a manner that enables the peptide to be expressed in the cell. After being expressed in engineered cells, the peptide may be isolated from the cells and purified. Methods for artificially engineering a cell to express a peptide and for isolating and purifying such expressed peptides are well understood to those of ordinary skill in the art. See, *e.g.*, S.C. Makrides, *Gene Transfer and Expression in Mammalian Cells (New Comprehensive Biochemistry)*, Elsevier Science, Saint Louis, Missouri (2004).

[046] It is to be further understood that the one or more peptides of the present invention may be part of a composition including one or more other compounds. For example, this composition may include the peptide of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or the peptide of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 which also includes a signal sequence that is not the penetratin sequence, and one or more other compounds. The one or more other compounds preferably are biologically benign, and therefore may be an inert solid carrier or polymer matrix material which is capable of helping to deliver and localize the peptide to bone, for example. Such a solid carrier or polymer matrix material may, for example, allow for the timed-release of the peptide.

[047] For example, U.S. Pat. No. 4,536,489, which is entirely incorporated herein by reference, describes a biodegradable organic polymer delivery system for bone morphogenetic protein which is capable of inducing formation of new bone in viable tissue. As a second example, U.S. Pat. No. 5,286,763, which is entirely incorporated herein by reference, describes bioerodible polymers for drug delivery in bone, including

polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, and copolymers thereof, that may be used for the delivery of bioactive agents, including antibiotics, chemotherapeutic agents, inhibitors of angiogenesis, and simulators of bone growth, directly into bone. The peptide of the present invention may be included as part of these systems, or systems similar thereto, for delivery of the peptide to bone.

[048] Other compounds which may be included as part of a matrix mixture for delivering the peptide to bone include celluloses, polysaccharides, starches, agarose, hyaluronic acid, hyaluron polyethylene glycol, fibrin, elastin, collagen, gelatin, vitronectin, fibronectin, laminin, dextrans, alginates, chitin, chitosan, and glycosaminoglycans.

[049] The peptide of the present invention also may be included in a composition having one or more other compounds that have therapeutic properties. Therefore, the peptide may be included in a composition further containing a growth factor, such as, but not being limited to, transforming growth factor-α, transforming growth factor-β, interleukin-1, and/or nerve growth factor, for example. The peptide may also be included in a composition that also includes antibacterial and/or antifungal agents.

[050] The peptide of the present invention also may be included in a composition that has one or more inorganic compounds. Such inorganic compounds include, but are not limited to, those that may be used as part of implantable grafts, such as ceramic materials, titanium mesh, and hydroxyapatite, for example.

[051] Further, the composition may include two or more peptides, such as those having SEQ ID NO:1 and SEQ ID NO:3, for example, and one or more compounds as described above, or it may consist of only two or more peptides, such as those having SEQ ID NO:1 and SEQ ID NO:3, and no other compound.

[052] The method of the present invention includes using the peptide or the composition of the present invention to regulate a bodily process in either a whole mammal, such as a human, for example, or a living sample obtained therefrom, such as a human cell line, for example. The bodily process may be, for example, osteogenesis, but it is to be understood that it is not limited thereto. The bodily process therefore may be any bodily process that is capable of being regulated, partially or wholly, by alteration of normal interaction between CK-2 and one or more BMPRs, such as BRIa, for example,

by using the peptide of the present invention. Further, because the bodily process is not limited to being used to regulate a bodily process associated with any particular bone or the skeletal system as a whole, the bodily process regulated by the method of the present invention may be associated with another organ system or region of a mammal's body. For example, when a human is subjected to the method, the bodily process may be one which occurs in, and/or is associated with the brain, pituitary, thyroid, heart, liver, lung, pancreas, colon, adrenal glands, kidney, muscle, blood vessel, skin, testis and/or ovary. The bodily process may also be an abnormal bodily process, such as cancer, for example. Therefore, the method may be used to alter interaction of CK-2 with one or more BMPRs, such as BRIa, for example, in all types of cancerous cells *in vivo* or *in vitro*. It is to be understood, however, that these are meant only to be examples, and that the present invention is not limited to these examples.

[053] In a specific example, the method is used to regulate osteogenesis in a mammal and includes the step of administering one or more therapeutically effective doses of the peptide or composition having the peptide of the present invention to the mammal.

[054] The mammal may be, for example, a human patient having a bone disorder, such as osteoporosis or Paget's disease, for example. The step of introducing the peptide into the mammal is variable. For example, the peptide may be artificially purified *in vitro* and then the therapeutically effective dose may be, for example, subcutaneously injected into a patient at or near the area in need of treatment.

[055] As another example, the therapeutically effective dose may be administered to a human patient by adding the peptide to an inert solid carrier or polymer matrix material which would help localize the peptide to bone in need of repair. For example, this might be achieved by using the peptide in conjunction with the delivery systems described in U.S. Patent Nos. 4,536,489 and 5,286,763. Further, the solid carrier or polymer matrix material, for example, may allow for the timed release of the peptide.

[056] Another means for administering the therapeutically effective dose to the human patient is to include the peptide as part of an implantable graft and then implanting the graft into the human patient at or near the area that is in need of treatment, such as a weakened bone, for example. As a specific example, the graft including the

peptide may be wrapped around bones of the spine after those bones have been subjected to a spinal fusion procedure.

[057] Alternatively, the peptide further may be synthesized within the mammal that is to be treated by the peptide. For example, the peptide may be encoded for by a DNA construct and the DNA construct may be expressed in a human cell line. The human cell line, such as an osteoblast cell line, for example, then may be administered to a human patient having a bone disorder, such as osteoporosis, for example. Accordingly, the cell line administered to the human synthesizes the peptide in an amount sufficient to provide the therapeutically effective dose.

[058] The present invention is more specifically described with reference to three Examples; however, it is not to be construed as being limited thereto.

EXAMPLE ONE

[059] In Example One, observation of normal interaction between BRIa and CK-2 and the experimental alteration of said interaction is described.

[060] Synthesis and Purification of Peptides. A motif search was performed of the ALK proteins of the TGF-β superfamily using the search tool PROSITE (available at: http://www.expasy.org/prosite/) and, as a result of this search, a plurality of CK-2 recognition sites were identified. Three peptides, having amino acid sequences corresponding to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3. each one of which contains one of these recognition sites, were designed to alter the interaction between CK-2 and BRIa. (These peptides were synthesized and purified by the GenScript Corporation of Piscataway, NJ.)

[061] Cell Culture. Cells used in this Example were subcloned from a myoblast line established from normal adult C3H mouse leg muscle referred to as C2C12 cells. These cells were cultured in Dulbecco's modified Eagle medium (DMEM) with the addition of 10% fetal bovine serum (FBS) in a humidified 5% CO_2 incubator at 37°C. Antibiotics penicillin (50 IU/mL) and streptomycin (100 μg/mL) were added to the media to control growth of bacterial and fungal contamination. Cells were subcultured approximately twice a week when the confluency was at 60-70% (they were split at a 1:10 ratio at these times into 25 cm^2 tissue culture flasks). Prior to being used in the

described experiments, these cells were seeded into tissue culture dishes (Falcon 3001 35 x 10mm) containing a 25 x 25 mm glass coverslip (Fisher Scientific) in 2 ml culture medium.

[062] Introduction of Peptides into Cell Cytoplasm. Each one of the amino acids sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 includes the AP homeodomain signal sequence for cellular uptake. The AP homeodomain signal sequence is a 16-amino acid polypeptide that has been shown to be able to cross cellular membranes with attached biologically active compounds. The AP homeodomain signal sequence has direct interaction with the membrane phosopholipids without any specific binding location identified, making it ideal for transport into the cell cytoplasm. The AP homeodomain signal sequence is a functional vector that allows the direct transport of polypeptides across the membrane.

[063] Introduction of the peptides of this Example into the cell cytoplasm specifically was carried out as follows. C2C12 cells were grown on coverslips as described above. Peptide (10 μM) having amino acid sequence which corresponds to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 was added to the cells and said peptide was allowed to incubate with the cells for 2 hours. Following this incubation period, the cells were fixed and labeled with anti-BRIa antibody and anti-CK-2 antibody as described above. Cells not exposed to any exogenous peptide comprised the "control group".

[064] Fixing/labeling of Cells. Cells grown on coverslips in tissue culture dishes as described above were rinsed three times with 1X phosphate buffered saline (PBS) at 4°C. The coverslips were removed from the dishes and placed in a glass rack holder and incubated in methanol at -20°C for 5 minutes and then with acetone for 2 minutes (also at -20°C). After this fixation, these coverslips were rinsed with PBS (1X) and air dried. The dried coverslips were used immediately thereafter for immunofluorescence labeling. Specifically, the fixed cells were blocked with 3% bovine serum albumin (BSA) in PBS, pH 7.2 for 60 minutes to avoid non-specific binding of protein. For immunolabeling of BRIa, a goat monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), at a concentration of 50 μg/ml was used in conjunction with donkey anti-goat AF568 (Molecular Probes, Inc., Eugene, OR) at a

concentration of 5 μg/ml. Immunostaining of CK-2 was achieved using a rabbit monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), at a concentration of 50 μg/ml, in conjunction with goat anti-rabbit AF488 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). All antibodies were exposed to their protein of interest for 30 minutes. This incubation was followed by incubation with secondary antibody for another 30 minutes. Following the labeling periods, the coverslips were rinsed twice with PBS (1X), pH 7.2 and once with 3% BSA. The coverslips were then adhered to slides by using Airvol.

[065] Measurement of FRET Efficiency. FRET is a microscopy-based method which those of ordinary skill in the art would readily recognize as being useful for studying the interactions of protein in cells. A description of the FRET method is generally described in, *e.g.*, Periasamy et al., *Molecular Imaging: FRET Microscopy and Spectroscopy*, Academic Press (2006); Goldman et al., *Live Cell Imaging*, Cold Spring Harbor Laboratory Press (2005); Spector et al., *Basic Methods in Microscopy: Protocols And Concepts from Cells, a Laboratory Manual*, Cold Spring Harbor Laboratory Press (2005), each one of which is incorporated by reference herein in its entirety.

[066] FRET was conducted using a multi-line Argon (457nm, 488nm, 514nm) laser and Green Helium Neon (543nm) laser. All images were recorded using an Olympus Fluoview FV300 inverted laser scanning confocal microscope (Olympus, Center Valley, PA). Labeled cell imaging was performed using a 60X objective viewing cells. An FITC filter was used for visualization of the green and TRITC filter for red fluorescence. Images were recorded using Fluoview 5.0 software (Olympus, Center Valley, PA) and cropped and assembled with ImageJ software (available at: http://rsb.info.nih.gov/ij/download.html). For each experimental coverslip, 38 images were taken in total. Images of the labeled molecules were taken sequentially. After these images were captured, the power of the laser for the red was increased to 100% allowing the fluorescent molecules of the acceptor to be bleached out. Another set of simultaneous images of the labeled molecules then was taken. Background fluorescence was estimated from an area free of cells and subtracted. The images before and after bleaching were then compared and the increase in the fluorescence of the green molecules on the cell membrane was measured.

[067] The effect that each peptide of this Example had on FRET activity between CK-2-β and BRIa is shown in FIG. 2. Specifically, FRET activity was measured to be about 20% in cells belonging to the control group, which were cells that were untreated with any exogenous peptide. This indicates that CK-2-β interacts with BRIa in normal cells that have not been artificially molecularly manipulated. It was further observed that FRET activity between CK-2-β and BRIa was about 5% in cells belonging to the group treated with the peptide having SEQ ID NO:1, was about 15% in cells belonging to the group treated with the peptide having SEQ ID NO:2, and was about 5% in cells belonging to the group treated with the peptide having SEQ ID NO:3. Therefore, FRET activity was reduced by about 75% in cells treated with either the peptide having SEQ ID NO:1 or SEQ ID NO:3, and by about 25% in cells treated with the peptide having SEQ ID NO:2, as compared to the FRET activity measured in the control group. This indicates that each one of these three peptides is useful for altering the normal interaction between CK-2 and BRIa.

EXAMPLE TWO

[068] In Example Two, effects on BMP independent signaling caused by the alteration of the normal interaction between CK-2 and BRIa is described.

[069] **Cell Culture.** C2C12 cells were cultured in DMEM with 10% FBS in a humidified 5% CO2 incubator at 37°C. Antibiotics penicillin (50 IU/mL) and streptomycin (100 μg/mL) were added to the media to control growth of bacterial and fungal contamination. Cells were subcultured approximately twice a week when the confluency was at 60-70% (they were split at a 1:10 ratio at these times into 25 cm^2 tissue culture flasks). Prior to being used in the reporter gene assays described below, these cells were seeded into tissue culture dishes (Falcon 3001 35 x 10mm) containing a 25 x 25 mm glass coverslip (Fisher Scientific) in 2 ml culture medium.

[070] **Reporter Gene Assays.** C2C12 cells were transfected with the reporter gene pSBE according to protocols described in A. Nohe et al., *Journal of Cell Science* 116: 3277-3284 (2003), which is incorporated herein by reference in its entirety. Peptide having sequence corresponding to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 was added at a concentration of about 10 μM to separate populations of cells and allowed

to co-incubate with the cells overnight at 37°C. Cells were then stimulated or not stimulated with about 20 nM BMP-2 in DMEM according to protocols described in A. Nohe et al., *Journal of Cell Science* 116: 3277-3284 (2003). About 24 hours later, the cells were lysed and the luciferase activity was measured using the Dual-Luciferase® Reporter Assay System, which is commercially available (Promega Corporation, Madison, WI; catalog number E1910), according to manufacturer's protocol. To achieve and measure normalization, the cells were transfected with pRLuc, which is a constitutive active Renilla Luciferase construct. The results of these reporter gene assays is shown in Fig. 3. Specifically, Fig. 3 shows the amount of luciferase activity that was measured for the unstimulated or BMP-2 stimulated cell lines that either were not co-transfected with a peptide ("control") or were co-transfected with peptide having amino acid sequence corresponding to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3. These data indicate that CK-2 is needed for inhibiting BMP-independent signaling.

EXAMPLE THREE

[071] In Example Three, the induction of osteoblast differentiation of BSMCs by altering the normal interaction between CK-2 and BRIa is described.

[072] Isolation and van Kossa Staining of BSMCs. BSMCs were isolated from 8-week old C57BL/6 (B6) mice and cultured for seven days. A peptide having a sequence corresponding to either SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 was "exposed to" the BSMCs by adding the peptide at a concentration of about 10 μM to separate cultures of BSMCs and allowing the peptide and BMSCs to co-incubate for 48 hours at 37°C. (Throughout this 48-hour incubation period, individual BSMC cultures were either stimulated with BMP-2 or not stimulated with BMP-2 as described in Example Two herein.) The BSMCs were then stained using standard van Kossa staining protocols to identify calcium deposits and the modules that formed were counted. The results of this experiment are shown in Table 1.

TABLE 1

Alteration of Normal BRIa-CK-2 Interaction Enhances Mineralization in BMSCs

Cell treatment	Percent induction	Standard deviation
- BMP-2	0	0.050
+ BMP-2	2.5	0.087
+ peptide having SEQ ID NO:1	20	0.050
+ peptide having SEQ ID NO:1 + BMP-2	27.5	0.132
+ peptide having SEQ ID NO:2	3.3	0.029
+ peptide having SEQ ID NO:2 + BMP-2	5.8	0.076
+ peptide having SEQ ID NO:3	90	0.205
+ peptide having SEQ ID NO:3 + BMP-2	100	0.250

[073] As the results in Table 1 show, the peptide of the present invention is able to induce nodule formation in BMSCs within 48 hours. This indicates that the peptide of the present invention may be used to induce osteoblast differentiation of BSMCs by altering the normal interaction between CK-2 and BRIa.

[074] A number of examples to help illustrate the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention.

What is claimed is:

1. A peptide having an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

2. The peptide of Claim 1 wherein an amino acid signal sequence is linked to the amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

3. The peptide of Claim 1 wherein the amino acid sequence of the peptide is a conservative variant of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

4. A composition, the composition including a peptide having an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

5. The composition of Claim 4 wherein the peptide includes the amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6 and an amino acid signal sequence linked to the amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

6. The composition of Claim 4 wherein the amino acid sequence of the peptide is a conservative variant of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

7. A method for treating a mammal having a bone disorder, the method includes administering to the mammal one or more therapeutically effective doses of a peptide having an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

8. The method of Claim 7 wherein an amino acid signal sequence is linked to the amino acid sequence represented by SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

9. The method of Claim 7 wherein the peptide is included in a composition and the composition is administered to the mammal.

10. The method of Claim 8 wherein the peptide is exposed to mammalian cells in culture and the cells are administered to the mammal.

11. The method of Claim 8 wherein mammalian cells are engineered to synthesize the peptide and the cells are administered to the mammal.

12. The method of Claim 9 wherein the composition includes an inert solid carrier or polymer matrix material.

13. The method of Claim 9 wherein the composition is included as part of an implantable graft and the implantable graft is implanted into the mammal.

14. The method of Claim 7 wherein the amino acid sequence of the at least one peptide is a conservative variant of the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

15. The method of Claim 14 wherein the at least one peptide is included in a composition and the composition is administered to the mammal.

16. The method of Claim 14 wherein the at least one peptide is exposed to mammalian cells in culture and the cells are administered to the mammal.

17. The method of Claim 14 wherein mammalian cells are engineered to synthesize the at least one peptide and the cells are administered to the mammal.

18. The method of Claim 15 wherein the composition includes an inert solid carrier or polymer matrix material.

19. The method of Claim 15 wherein the composition is included as part of an implantable graft and the implantable graft is implanted into the mammal.

20. The method of Claim 8 wherein the bone disorder is osteoporosis or Paget's disease.

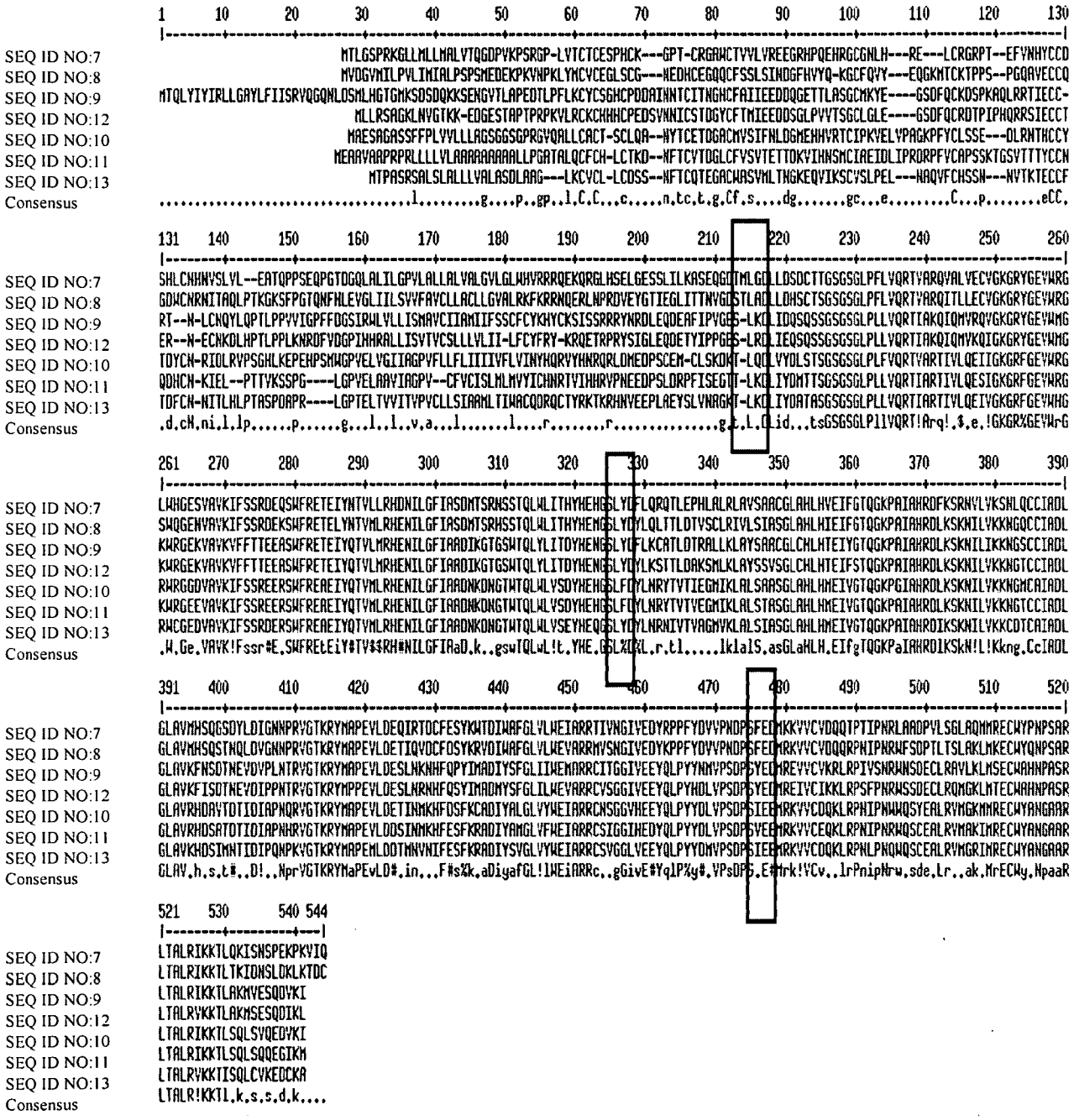
SEQ ID NO:7
SEQ ID NO:8
SEQ ID NO:9
SEQ ID NO:12
SEQ ID NO:10
SEQ ID NO:11
SEQ ID NO:13
Consensus

FIG. 1

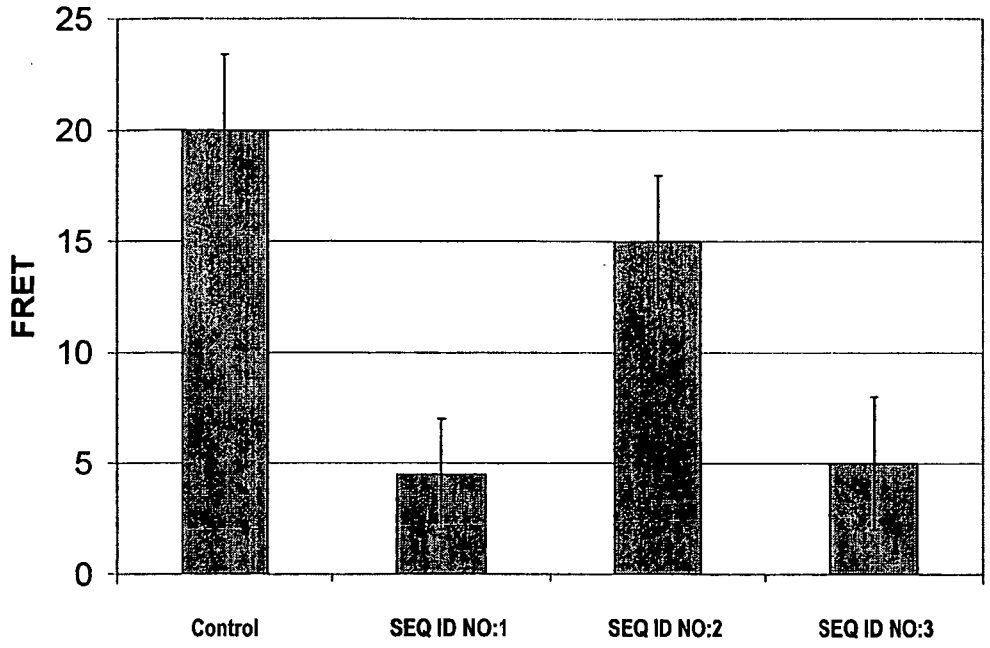

FIG. 2

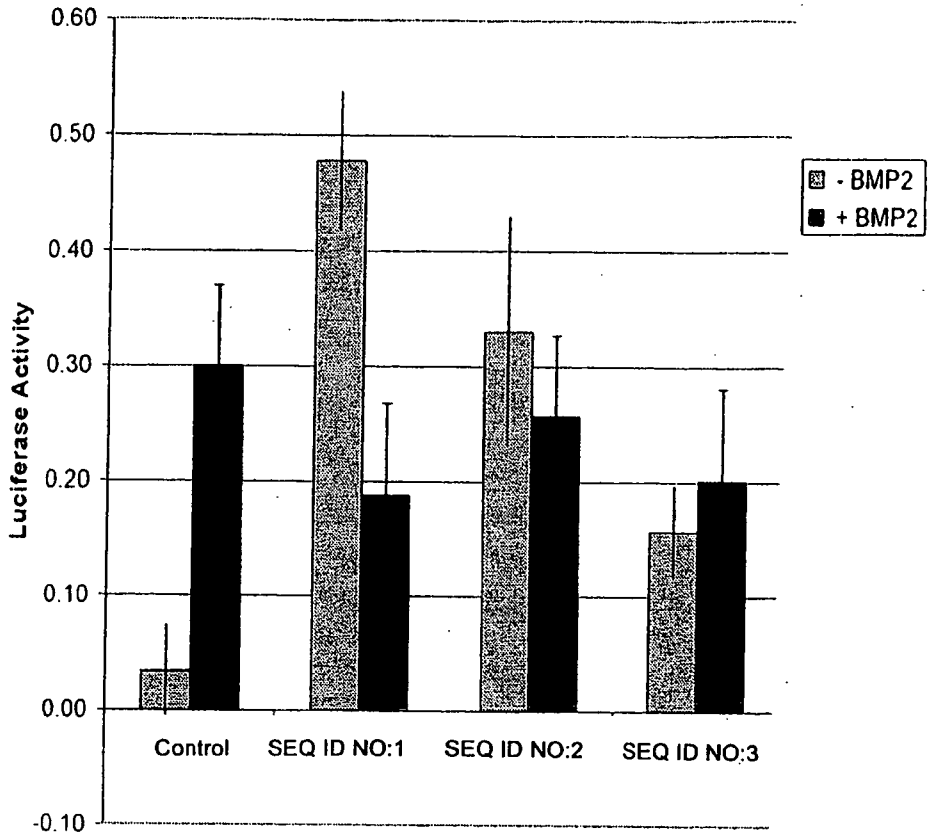
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
Luciferase Activity
- BMP2
+ BMP2
Control
SEQ ID NO:1
SEQ ID NO:2
SEQ ID NO:3

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/23020

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 38/06(.2006.01)**

USPC: 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0082233 A1 (LYONS et al.) 01 May 2003 (01.05.2003), SEQ ID NO: 2	1 and 4
X	US 6,723,830 B2 (BEN-SASSON) 20 April 2004 (20.04.2004), SEQ ID NO: 87	1, 3, 4 and 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 May 2008 (15.05.2008)	07 JUL 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Christina Marchetti Bradley Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/23020

Continuation of B. FIELDS SEARCHED Item 3:
STN (Registry, CAPlus)
peptide sequences SEQ ID NOs: 1-6 were searched in Registry and CAPlus