Abstract

Disclosed are compositions and methods for maintaining the integrity of smooth muscle, particularly vascular smooth muscle. Vascular diseases are characterized by an excessive build-up of vascular smooth muscle cells, resulting in an occlusion of a blood vessel, and/or by loss of elasticity in the blood vessels. Causes of blood vessel occlusion include smooth muscle cell proliferation and inflammatory responses. Inhibition of the proliferation of smooth muscle cells or inflammatory responses represents an effective treatment for vascular disorders, such as atherosclerosis and restenosis. Treatment may include administration of a morphogenic protein. The protein itself may be delivered to the site of vascular occlusion or the protein may be delivered by a vector, such as an adenoviral vector containing a DNA insert encoding a morphogenic protein. Such compositions and methods may also inhibit the responses of smooth muscle cells to various traumas, such as exposure to toxic agents. All of these treatments operate to preserve the cell phenotype by inhibiting an increase in extracellular matrix proteins, such as collagen, or by maintaining the normal balance of extracellular matrix proteins, such as Types I and III collagen.
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MAINTENANCE OF SMOOTH MUSCLE INTEGRITY
BY MORPHOGENIC PROTEINS

Field of the Invention

The invention relates to the maintenance of vascular integrity using morphogenic proteins or nucleic acids.

Background of the Invention

Following trauma, characteristic changes occur in vascular smooth muscle. These changes may result in cell death, increased collagen synthesis (and a concomitant reduction in cellular elasticity), inflammatory responses, and a general cellular hypertrophy. Stimuli of cellular trauma include exposure to toxic agents, certain diseases, mechanical stress, and the like. Vascular trauma may result in the synthesis of extracellular matrix proteins, such as collagen. This, coupled with an inflammatory response and cellular proliferation, may result in conditions, such as atherosclerosis, in which the blood vessel intima thickens and loses its elasticity. A resulting loss of blood flow may underlie clinical manifestations of cardiovascular disease.

Atherosclerosis is a common form of cardiovascular disease. Typically, atherosclerotic conditions lead to insufficient blood supply to critical organs, resulting in heart attack, stroke, and kidney failure. Additionally, atherosclerosis is a complicating factor in hypertension and diabetes. Vascular smooth muscle cells typically become abnormally proliferative in atherosclerosis. Smooth muscle cell proliferation may reduce blood flow and make vessels susceptible to clotting.

endarterectomy, heart transplantation, balloon angioplasty, atherectomy, laser ablation, or endovascular stenting. It is responsible for recurrence of symptoms (or death), often requiring repeated revascularization surgery.

One method of treating restenosis, or other smooth muscle cell proliferative disorders, is to administer a protein that inhibits cell proliferation. For example, TGF-β1 is known to regulate vascular smooth muscle cell proliferation in vitro. Majack, et al., J. Cell Biol., 105:465-471 (1987); Battegay, et al., Cell, 63:515-524 (1990). Whether TGF-β1 stimulates or inhibits growth is, however, dependent on many factors, such as the conditions of the cell culture, the presence of other growth-regulatory molecules, the sequence of their addition, and the concentration of TGF-β1.

Other incidences of cell trauma may require treatment directed at inhibiting the effects of toxic or inflammatory agents. Exposure of cells to toxic agents, such as mercuric chloride or Antimycin-A, for example, often results in cell death. Compositions and methods of inhibiting or preventing the effects of such agents on smooth muscle cells may protect the cells from destruction. Likewise, inflammatory responses to various traumas can trigger the release of cellular components, such as inflammatory cytokines, which may lead to the production of intercellular adhesion molecules. In vascular smooth muscle cells, the clumping of intercellular adhesion molecules decreases the diameter of the vascular lumen, thereby restricting blood flow.

As discussed above, some treatments for maintaining vascular integrity result in the loss of cell phenotype by, for example, increasing synthesis of extracellular matrix protein. Such treatments may upset the normal balance of extracellular matrix proteins, such as Type I and Type III collagen. These disturbances in extracellular matrix protein synthesis or balance of extracellular matrix proteins may result in a loss of cellular elasticity. In vascular smooth muscles, such a loss in elasticity may result in a decreased ability to transport blood through the blood vessels.

Accordingly, there is a need in the art for compositions and methods for maintaining smooth muscle integrity. More specifically, there is a need in the art for compositions and methods that are capable of reducing the intimal thickening of vascular smooth muscle cells caused by smooth muscle cell proliferation, extracellular matrix protein synthesis, and
inflammatory responses. Furthermore, there exists a need in the art for treatment methods that can also maintain the smooth muscle cell phenotype and thereby preserve the cellular elasticity. The present invention addresses these needs.

Summary of the Invention

Broadly, the invention provides compositions and methods for maintaining vascular integrity, especially vascular smooth muscle integrity. In a preferred embodiment, the invention provides compositions and methods for inhibition of smooth muscle cell proliferation. In another embodiment, the invention provides compositions and methods for counteracting various cellular responses to trauma. For example, compositions and methods of the invention inhibit collagen synthesis and maintain cellular elasticity of smooth muscle cells, inhibit cellular inflammatory response, protect cells against cytotoxic injury, and maintain the normal balance of Type I and Type III collagen produced by these cells, thereby maintaining cell phenotype.

In a preferred embodiment, the invention relates to localized delivery of a protein or nucleic acid composition that inhibits smooth muscle cell proliferation and other responses to vascular trauma. A protein or nucleic acid composition preferably comprises a morphogenic protein, or nucleic acid encoding a morphogenic protein, in an acceptable vehicle. In a preferred embodiment, the vehicle is a vector that causes expression of a morphogen in mammalian cells. The morphogen preferably has an amino acid sequence with at least 70% sequence homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of human OP-1, corresponding to residues 326-431 or 330-431 of SEQ ID NO: 1.

The morphogenic protein may, therefore, comprise OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof. In preferred embodiments of the invention, the morphogenic protein is OP-1 or BMP-2.

In some preferred embodiments, an expression vector for delivery of morphogen-encoding nucleic acid may comprise a viral vector, most preferably an adenoviral vector. The vector preferably contains a nucleic acid encoding a morphogen protein in operative association with a promoter, such as the chicken β-actin promoter. In preferred embodiments, the vector further
comprises an enhancer element, such as a CMV-IE enhancer element, in association with the promoter. Alternatively, nucleic acids operatively encoding a morphogen of the invention may be delivered in liposomal or other lipid formulations which enhance delivery to target cells.

Administration of a nucleic acid and/or protein composition also may be accomplished by numerous other means known in the art. For example, a morphogen-encoding nucleic acid may be introduced as "naked DNA" (i.e., DNA not associated with proteins or with a vector). Naked DNA may be introduced via intravascular, intraperitoneal, intradermal or transdermal injection, by inhalation, or by other means known in the art. Naked DNA may comprise a linear or circular, single or double-stranded nucleic acid molecule. Thus, for example, naked DNA may comprise a plasmid or viral genome, or may be a linear expression sequence excised from a plasmid or viral genome, or amplified in vitro. Naked DNA may be administered substantially free from association with transfection-facilitating proteins, viral particles, liposomal formulations, charged lipids and calcium phosphate precipitating agents as is known in the art (see, e.g., U.S. Pat. No. 5,580,859, incorporated by reference herein). Naked DNA typically expresses its gene product transiently, and does not integrate into the host genome. Morphogen-encoding naked DNA may, therefore, be used to provide protective effects normally associated with morphogens. In the context of the present invention, such DNA provides protective effects against intima thickening, smooth muscle cell proliferation, and inflammatory imbalances caused by damage to blood vessels. Morphogen-encoding naked DNA, in addition to being injected prophylactically, may be administered topically to the vasculature (e.g., by application on a balloon catheter as described herein) at a site of injury.

Methods of the invention provide for the administration of a morphogen-encoding nucleic acid for treatment or prevention of vascular occlusion. Administration of a nucleic acid encoding a morphogen or administration of a morphogen itself is useful in treating vascular proliferative disorders, such as restenosis and atherosclerosis, by, for example, inhibiting smooth muscle cell proliferation. Methods of the invention are also useful to preserve the integrity of vascular tissue, by, for example, protecting cells from cytotoxicity, reducing the occurrence of inflammation of vascular tissue epithelium, and maintaining the balance of extracellular matrix proteins, such as, for example, Type I and/or Type III collagen. These effects maintain vascular integrity and cell phenotype.
The invention provides for the treatment of smooth muscle cell proliferative disorders and other disorders caused by vascular trauma. The invention provides an effective treatment for vascular proliferative disorders, such as atherosclerosis and restenosis, and for prevention of the loss of smooth muscle integrity caused by decreased cellular elasticity and inflammatory responses, through the administration of a morphogenic protein or a nucleic acid encoding a morphogenic protein. The invention will be understood further upon consideration of the following drawings, description, and claims.

Description of the Drawings

Figure 1A shows $^3$H-thymidine incorporation into the DNA of rat aortic smooth muscle cells treated with either BMP-2 or TGF-β1. The closed circles indicate cells that were treated with BMP-2, the open triangles indicate cells that were treated with TGFβ-1, and the open circles indicate cells that were left untreated.

Figure 1B shows $^3$H-thymidine incorporation into the DNA of rat aortic smooth muscle cells treated with either BMP-2 or TGF-β1 at various concentrations. The closed circles indicate cells that were treated with BMP-2, and the closed triangles indicate cells treated with TGF-β1.

Figure 1C shows $^3$H-thymidine incorporation into the DNA of rat aortic smooth muscle cells treated with either BMP-2 at 0.3 pM or TGF-β1 at 100 pM.

Figure 1D shows the number of cells grown in plates treated with either BMP-2 or TGF-β1. The closed circles indicate cells treated with BMP-2, the open triangles indicate cells treated with TGF-β1, and the open circles indicated cells that were untreated.

Figure 1E shows the percentage of $^3$H-thymidine incorporation into the DNA of rat aortic smooth muscle cells that are either untreated or treated with OP-1 at concentrations of 0.1 ng/ml, 1 ng/ml, 10 ng/ml or 40 ng/ml.

Figure 1F shows the amount of collagen synthesis by rat aortic smooth muscle cells treated with either BMP-2 or TGF-β1. The closed circles indicate cells treated with BMP-2, and the open circles indicate cells treated with TGF-β1.
Figure 2 is an illustration of the DNA of an adenoviral vector containing a DNA insert encoding BMP-2.

Figure 3 is an illustration of the DNA of an adenoviral vector containing a DNA insert encoding β-galactosidase.

Figure 4A shows a Western blot analysis of media from cells infected with an adenovirus containing a DNA insert encoding BMP-2. Lane 1 shows BMP-2, lane 2 shows cell culture media from cells infected with an adenoviral vector containing a DNA insert for β-galactosidase, and lane 3 shows cell culture media from cells infected with an adenoviral vector containing a DNA insert for BMP-2.

Figure 4B shows $^3$H-thymidine incorporation into the DNA of rat aortic smooth muscle cells treated with an adenovirus containing a DNA insert encoding either BMP-2 or β-galactosidase. The closed circles indicate treatment with the adenovirus containing a DNA insert encoding BMP-2, and the open circles indicate treatment with the adenovirus containing a DNA insert encoding β-galactosidase.

Figure 5A shows a cross-section of an Elastica von Gieson-stained rat carotid artery after undergoing balloon injury.

Figure 5B shows a cross-section of an Elastica von Gieson-stained rat carotid artery after undergoing balloon injury and subsequent treatment with an adenovirus containing a DNA insert encoding BMP-2.

Figure 5C shows a cross-section of an Elastica von Gieson-stained rat carotid artery after undergoing balloon injury and subsequent treatment with an adenovirus containing a DNA insert encoding β-galactosidase.

Figure 6A shows the intimal cross-sectional areas of rat carotid arteries after undergoing balloon injury and either no treatment or treatment with an adenovirus containing a DNA insert encoding either BMP-2 or β-galactosidase. Group 1 of the graph represents non-treated control, group 2 represents treatment with the adenoviral vector containing the DNA insert encoding
BMP-2, and group 3 represents treatment with the adenoviral vector containing the DNA insert encoding β-galactosidase.

Figure 6B shows the medial cross-sectional areas of rat carotid arteries after undergoing balloon injury and either no treatment or treatment with an adenovirus containing a DNA insert encoding either BMP-2 or β-galactosidase. Group 1 of the graph represents non-treated control, group 2 represents treatment with the adenoviral vector containing the DNA insert encoding BMP-2, and group 3 represents treatment with the adenoviral vector containing the DNA insert encoding β-galactosidase.

Figure 6C shows the intimal/medial ratio of cross-sectional areas of rat carotid arteries after undergoing balloon injury and either no treatment or treatment with an adenovirus containing a DNA insert encoding either BMP-2 or β-galactosidase. Group 1 of the graph represents non-treated control, group 2 represents treatment with the adenoviral vector containing the DNA insert encoding BMP-2, and group 3 represents treatment with the adenoviral vector containing the DNA insert encoding β-galactosidase.

Figure 7A shows the amount of α-actin synthesis in rat aortic smooth muscle cells that were either left untreated or were treated with 100 ng/ml OP-1 at 3, 4, 5 and 6 days post-confluence.

Figure 7B shows the amount of Type I collagen synthesis by rat aortic smooth muscle cells that were either left untreated or were treated with 100 ng/ml OP-1 at 3, 4, 5 and 6 days post-confluence.

Figure 7C shows the amount of Type III collagen synthesis by rat aortic smooth muscle cells that were either left untreated or were treated with 100 ng/ml OP-1 at 3, 4, 5 and 6 days post-confluence.

Figure 8A shows the effect of OP-1 treatment on the uptake of ^3H-thymidine by rat aortic smooth muscle cells that have undergone injury by mercuric chloride.

Figure 8B shows the effect of OP-1 treatment on the uptake of ^3H-thymidine by rat aortic smooth muscle cells that have undergone injury by Antimycin-A.
Figure 9 shows the effect of OP-1 on the production of I-CAM in rat aortic smooth muscle cells exposed to 1 ng/ml, 5 ng/ml or 10 ng/ml of interleukin-1 and either no OP-1 or 200 ng/ml of OP-1.

**Detailed Description**

The invention provides compositions and methods for maintenance of vascular integrity, especially vascular smooth muscle integrity. Vascular integrity is maintained, *inter alia*, by inhibition of smooth muscle cell proliferation, by maintaining expression of cell markers characteristic of smooth muscle cell phenotype, and by protection of smooth muscle cells against injury mediated by toxic agents and inflammatory cytokines. Compositions according to the invention comprise a morphogenic protein or nucleic acid. In a preferred embodiment, the invention comprises administering to a patient a composition including an adenoviral vector containing a DNA insert encoding a morphogenic protein. Other vectors may be used, including, for example, other viral vectors or liposomes. In methods of the invention, administration of a morphogenic protein, or a vector comprising a DNA encoding a morphogenic protein, inhibits the proliferation of smooth muscle cells, particularly vascular smooth muscle cells. Methods for maintaining smooth muscle cell phenotype and protecting the cells against injury include use of compositions comprising morphogenic proteins.

Example 1 compares the effect of TGF-β1 on the proliferation of vascular smooth muscle cells *in vitro* to the effect of BMP-2 or OP-1 on the same cells. Example 2 compares the amount of collagen synthesis resulting from the administration of either BMP-2 or TGF-β1 to rat aortic smooth muscle cells *in vitro*. Example 3 details the production of an adenoviral vector containing a DNA insert encoding BMP-2, and also details the production of a control adenoviral vector containing a DNA insert encoding β-galactosidase. Example 3 further describes the effects of administration of an adenoviral vector containing a DNA insert encoding either BMP-2 or β-galactosidase on the proliferation of vascular smooth muscle cells *in vitro*. Example 4 compares the effects of *in vivo* treatment with an adenoviral vector containing a DNA insert encoding BMP-2 with one containing β-galactosidase on vascular smooth muscle cells after undergoing a balloon angioplasty. Example 5 describes the effects of *in vivo* treatment with OP-1 delivered to the site of a vascular occlusion after performance of balloon angioplasty at that site. Example 6 describes the effect of OP-1 administration on cultured smooth muscle cells. Example 7 describes the effect
of OP-1 administration of smooth muscle cells that have been subjected to toxic insult. Finally, Example 8 describes the effect of OP-1 administration on smooth muscle cells treated with inflammatory cytokines.

Example 1

The effect of BMP-2 on the proliferation of rat aortic smooth muscle cells was compared to the effect of TGF-β1 in vitro. Rat aortic smooth muscle cells were prepared from 8 week old Wistar rats by the explant method of Campbell, et al., Physiol. Rev. 59:1-61 (1979), incorporated by reference herein. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Life Technologies, Inc., Tokyo Japan), 100 μg/ml streptomycin, and 100 U/ml penicillin.

The amount of cell proliferation was determined by the rate of DNA synthesis in the cells. This was estimated based on the incorporation of ³H-thymidine. The cells were seeded into a 24-well culture plate, grown to subconfluence, and serum-deprived by culturing in DMEM without FCS for 60 hours. The cells were then stimulated by addition of a 1% solution of FCS and pulse-labeled with 37 kBq/ml of [6-³H]thymidine in 500 μl of a 1% FCS solution for 2 hours.

The amount of ³H-thymidine incorporation into the cells was determined by a trichloroacetic acid precipitation method. See, Y. Takuwa, et al., Biochem. Biophys. Res. Commun. 174:96-101 (1991), incorporated by reference herein. The stimulated cells were either left untreated or were treated with either 0.3 pM of BMP-2 or 100 pM of TGF-β1. Cell proliferation was monitored for 48 hours. The results are shown in Fig. 1A. As shown in that figure, cells treated with BMP-2 had low cell proliferation at all times over the 48 hour test period. Untreated cells began to proliferate at approximately 14 hours after stimulation, reached peak proliferation at approximately 24 hours after stimulation, and then declined. The TGF-β1 treated cells showed 57% and 30% less cell proliferation at 19 and 24 hours, respectively. They reached peak proliferation at 32 hours. TGF-β1, therefore, only delayed the peak of cell proliferation.

Figure 1B shows the effect on DNA synthesis of varying concentrations of BMP-2 or TGF-β1. BMP-2 inhibited the growth of smooth muscle cells in a dose-dependent manner from
the threshold concentration of 0.03 pM to 0.3 pM. The effect of adding BMP-2 at varying times after serum stimulation is shown in Fig. 1C. As shown in that figure, BMP-2 was capable of inhibiting cell proliferation even when added after the cells had entered S phase (i.e., it inhibited cell growth even when added 20 hours after serum stimulation). However, TGF-β1 was unable to inhibit cell growth when added either directly before (i.e., 13 hours after serum stimulation) or after (i.e., 24 hours after serum stimulation) S phase entry. As shown in Fig. 1D, BMP-2 treatment also resulted in a 62% decrease in cell number compared to cells grown without treatment over a 72 hour period. The cells treated with TGF-β1 showed only a marginally lower incidence of cell growth as compared to untreated cells over the same 72 hour period. BMP-2 was, therefore, found to be superior in inhibiting smooth muscle cell proliferation in vitro as compared to TGF-β1.

The effect of OP-1 on the proliferation of aortic smooth muscle cells was also tested in vitro. Rat aortic smooth muscle cells were prepared as previously described. The cells were plated (4x10^4 cells per well of a 24 well plate) in growth medium containing 5% FCS. After standing overnight, the medium was replaced with medium containing no serum in order to initiate cell starvation and to synchronize subsequent cell growth. After 60 hours of starvation, cell growth was stimulated by addition of 1% FCS. OP-1 was also added at concentrations of 0.1 ng/ml, 1 ng/ml, 10 ng/ml and 40 ng/ml, and the cells were incubated for 24 hours.

The amount of cell proliferation was determined by the rate of DNA synthesis in the cells, which was estimated based on the amount of ^3H-thymidine incorporated. Twenty-two hours after the induction of cell growth, the cells were pulse-labeled with [6-^3H]thymidine for 2 hours. The amount of ^3H-thymidine incorporation into the cells was determined as previously described. The results of OP-1 treatment on the cells is shown in Figure 1E. Similarly to BMP-2, OP-1 inhibited the proliferation of smooth muscle cells in a dose-dependent manner.

**Example 2**

The effect of BMP-2 on collagen synthesis was compared to that of TGF-β1. An increase in extracellular matrix proteins, such as collagen and proteoglycans, is often seen in atherosclerotic lesions and in restenotic lesions following balloon angioplasty. Rat aortic smooth muscle cells were prepared as described in Example 1. These cells were tested for collagen
synthesis after stimulation and treatment with either BMP-2 or TGF-β1. Confluent rat aortic smooth muscle cells were seeded into a 12-well plate and serum-deprived by maintenance in minimum essential medium (α-MEM) for 24 hours. The cells were then treated with either BMP-2 or TGF-β1 in α-MEM containing 50 μg ascorbic acid and 50 μg β-aminopropionitrile for 24 hours. The cells were then labeled with 185 kBq/ml of l-[2,3-3H]proline in 1 ml of fresh α-MEM for 3 hours.

As shown in Fig. 1F, no collagen synthesis was observed in cells treated with BMP-2 at concentrations up to 1 nM, far above the concentration necessary to inhibit smooth muscle cell proliferation (see Example 1 above). Cells treated with TGF-β1 showed greater increases in collagen synthesis at increasing concentrations. As shown in Fig. 1F, TGF-β1 treated cells showed a 17% increase in collagen synthesis upon the addition of 10 pM TGF-β1, and a 40% increase in collagen synthesis upon addition of 100 pM TGF-β1. BMP-2, therefore, not only reduces the degree of smooth muscle cell proliferation as compared to TGF-β1, but also results in less extracellular matrix protein synthesis than TGF-β1. It is expected that administration of OP-1 will also result in less extracellular matrix protein synthesis than TGF-β1. Maintaining the level of collagen produced by these cells allows them to maintain their elasticity.

Example 3

The effects of a vector containing a DNA insert encoding BMP-2 on the proliferation of smooth muscle cells in vitro was next assessed. An adenoviral vector containing a DNA insert encoding BMP-2 was constructed. A control adenoviral vector containing a DNA insert encoding β-galactosidase was also constructed.

The adenovirus AxCABMP2 containing a BMP-2 insert useful in transfecting smooth muscle cells was created by inserting a cDNA encoding BMP-2 into plasmid pCAGGS. Plasmid pCAGGS is constructed by introducing CAG promoter and rabbit β-globin gene sequences, including a polyadenylation signal and a SV40 ori sequence, into vector pUC13. That vector is reported in Messing, Methods Enzymol., 101: 20-78 (1983), incorporated by reference herein. First, the EcoO109 site of pUC13 is changed to a XhoI site by insertion of a XhoI linker. The EcoO109-EcoRI region of pUC13 is then excised and the XhoI-EcoRI segment of pAGS-3, which includes the AG promoter, is inserted in its place. The EcoRI-SalI region of the plasmid is then
replaced by the EcoRI-XhoI fragment from pKCR-3 (See O’Hare, et al., Proc. Natl. Acad. Sci USA, 78:1527-1531 (1981), incorporated by reference herein), which includes a polyadenylation signal and the 3’-flanking sequence of the rabbit β-globin gene. A BamHI linker is then used to insert the BamHI fragment from pAGS-lacZ, which includes SV40 ori, into the PvuII site of the plasmid. Next, a SalI CMV-IE enhancer fragment is inserted in place of the AatII-XhoI region, after the AatII site has been changed to a SalI site by addition of a SalI linker. Finally, a XhoI site is made at the EcoRI site by addition of a XhoI linker. This resulted in plasmid pCAGGS. See Niwa, et al., Gene 108:193-199 (1991), incorporated by reference herein.

After insertion of cDNA encoding BMP-2 into the unique XhoI site in pCAGGS, the resulting vector contained CMV-IE enhancer, chicken β-actin promoter, an intron, the coding region of BMP-2 and rabbit β-globin poly-A. This vector was then blunt-end ligated into the Swa I site of the cosmid pAx-cw. The resulting vector was renamed pAxBMP2 and was then co-transfected into the 293 embryonic cell line with an Eco T221 digested DNA-TPC (from Ad5dlx) to generate AxCABMP2. The adenovirus thus obtained was isolated, screened for the BMP-2 insert, propagated, purified and titrated as described in Kanegaye, et al., Jpn. J. Med. Sci. Biol, 47:157-166 (1994), incorporated by reference herein. A schematic illustration of the BMP-2 containing adenoviral vector AxCABMP2 is shown in Fig. 2. An adenoviral vector containing a DNA insert encoding β-galactosidase (AxCAlacZ) was constructed in the same manner as described above, except that DNA encoding β-galactosidase was inserted into the XhoI site of pCAGGS. A schematic illustration of AxCAlacZ is shown in Fig. 3.

The adenoviral vector AxCABMP2 was tested for its ability to cause expression of BMP-2 in cultured smooth muscle cells. Smooth muscle cells transfected with either the AxCABMP2 adenoviral vector or the AxCAlacZ adenoviral vector were maintained in DMEM without FCS for 96 hrs. Because the BMP-2 gene possesses a signal peptide sequence, it is expected to be secreted into the extracellular space. Accordingly, the medium from each cell culture was harvested and separated on a 15% SDS-PAGE before being subjected to Western blotting analysis with anti-human BMP-2 antibody. Western blotting was performed by the avidin-biotin complex method using an ABC kit (Vector Laboratories, Burlingame, CA) and developed using Konica Immunostaining HRP-1000 (Konica, Inc., Japan).
A Western blot analysis of these conditioned media is shown in Fig. 4A. Lane 1 of the blot in Fig. 4A contains 5 ng of recombinant BMP-2, purified from a Chinese Hamster Ovary cell line (control). Lane 2 of the blot in Fig. 4A contains medium from the AxCALacZ transfected cells, and Lane 3 contains medium from the AxCABMP2 transfected cells. The conditioned media from cells transfected with AxCABMP2 were found to contain the BMP-2 protein. Conditioned media from cells transfected with AxCALacZ were not found to contain detectable amounts of BMP-2. The adenoviral vector AxCABMP2 was, therefore, able to induce BMP-2 expression in smooth muscle cells in vitro.

The ability of the adenoviral vector AxCABMP2 was then tested for its ability to inhibit the proliferation of serum-stimulated rat aortic smooth muscle cells in vitro. Subconfluent smooth muscle cells seeded onto a 24-well plate were incubated with 200 μl of DMEM containing 5% FCS for 2 hours and were then serum deprived for 60 hours. The cells were then stimulated with a 1% FCS solution, and the degree of cell proliferation was determined by measuring the amount of 3H-thymidine incorporated into the cells 24 hrs. after the stimulation. As shown in Fig. 4B, smooth muscle cells transfected with the adenoviral vector AxCABMP2 showed less cell proliferation (i.e., showed less 3H-thymidine incorporation into their DNA) than those cells transfected with the control adenoviral vector AxCALacZ, which did not show any decrease in the amount of cell proliferation. The AxCABMP2 and AxCALacZ adenoviral vectors were added at the same range of viral titer. The adenoviral vector AxCABMP2 was, therefore, shown to inhibit smooth muscle cell proliferation in vitro.

Example 4

The ability of the adenoviral vector AxCABMP2 to inhibit smooth muscle cell proliferation in vivo was tested next. The left common carotid arteries of 10 week-old SPF Wistar rats were injured by embolectomy catheter. General anesthesia consisting of 90 mg/kg of ketamine introduced intraperitoneally and 15 mg/kg of xylazine introduced intramuscularly was administered. After intravenous administration of 75 U/kg of heparin, the external and internal carotid arteries were cross-clipped using a 2v-clip microclip (S & T, Inc., Germany). A 2F Fogarty embolectomy catheter (Baxter, Irvine, CA) was introduced into the left common carotid artery through an approximately 3 mm longitudinal arteriotomy made in the external carotid artery. The left common carotid arteries were injured by six passes of the embolectomy catheter
inflated with 0.2 ml of air. After placement of a microclip on the proximal portion of the left common carotid artery, the arteriotomy was closed by suturing with 10-0 nylon. After suture, blood flow was resumed by removal of the clips.

Either AxCABMP2 or AxCALacZ was administered into the artery on the fifth day after the balloon injury. Through an incision made in the external carotid artery, 50 μl of virus fluid at 1 x 10^{10} plaque forming units (pfu)/ml of either AxCABMP2 or AxCALacZ virus was administered into a 1.5 cm length of the left common carotid artery. The AxCABMP2 vector was administered to one group of rats, and the AxCALacZ vector was administered to another group. The portion of the external carotid artery proximal to the incision was then threaded with 7-0 nylon. A group of untreated rats was used as a control. The vector was allowed to incubate for 40 minutes, and the cross-clipping was then released.

Fourteen days after the balloon injury, the cross-sectional areas of the intima and the media in each artery were measured to determine the ratio of intimal to medial (I/M) area. The arteries were harvested and the specimens fixed in phosphate-buffered saline solution containing 4% paraformaldehyde for 6-12 hrs. at 4°C. The specimens were then paraffin-embedded and analyzed by Elastica von Gieson staining. Figure 5A is a schematic illustration of an Elastica von Gieson-stained carotid artery after undergoing balloon injury, as described above, and receiving no further treatment. Figure 5B is a schematic illustration of an Elastica von Gieson-stained carotid artery after undergoing balloon injury, as described above, and then being transfected with AxCABMP2 five days after balloon injury. Figure 5C is a schematic illustration of an Elastica von Gieson-stained carotid artery after undergoing balloon injury, as described above, and then being transfected with AxCALacZ five days after balloon injury. The illustrations shown in Fig. 5A-5C are all shown at approximately x25 magnification.

Administration of AxCABMP2 was found to decrease the I/M ratio. For each 1 cm of artery specimens, five round cross-sections were stained with Hematoxylin and Eosin and photographed. The cross-sectional areas of intimal and medial regions were analyzed using image analyzing software (NIH image). Administration of AxCABMP2 was found to reduce the intimal mass of injured arteries by 41% as compared to administration of AxCALacZ. Figure 6A shows the mean intimal mass areas for untreated, AxCABMP2 transfected, and AxCALacZ transfected specimens. The medial mass was similar between all three groups. Figure 6B shows the mean
medial mass areas for untreated, AxCABMP2 transfected and AxCALacZ transfected specimens. As can be seen in Fig. 6C, the I/M ratio was, therefore, substantially reduced in rats transfected with AxCABMP2.

Example 5

Administration of a morphogenic protein itself to a vascular site will also inhibit the proliferation of smooth muscle cells in vivo. The left common carotid arteries of 10 week-old SPF Wistar rats are injured by embolectomy catheter. General anesthesia consisting of 90 mg/kg of ketamine introduced intraperitoneally and 15 mg/kg of xylazine introduced intramuscularly is administered. After intravenous administration of 75 U/kg of heparin, the external and internal carotid arteries are cross-clipped using a 2v-clip microclip (S & T, Inc., Germany). A 2F Fogarty embolectomy catheter (Baxter, Irvine, CA) coated with a biologically compatible composition containing OP-1 is introduced into the left common carotid artery of one group of the rats through an approximately 3 mm longitudinal arteriotomy made in the external carotid artery. In the remaining rats, the embolectomy catheter is not coated with protein. The left common carotid arteries are injured by six passes of the embolectomy catheter inflated with 0.2 ml of air. After placement of a microclip on the proximal portion of the left common carotid artery, the arteriotomy is closed by suturing with 10-0 nylon. After suture, blood flow is resumed by removal of the clips.

It is anticipated that the rats that undergo balloon angioplasty using a catheter coated with OP-1 will exhibit a decrease in the I/M ratio of their arteries as compared to untreated controls. For each 1 cm of artery specimens, five round cross-sections are stained with Hematoxylin and Eosin and photographed. The cross-sectional areas of intimal and medial regions are analyzed using image analyzing software (NIH image). It is anticipated that, as in Example 4, the intimal areas of rats undergoing balloon angioplasty with an OP-1 coated catheter will be less than the intimal areas of untreated controls. This will result in a lower I/M ratio for those rats undergoing balloon angioplasty with the OP-1 coated catheter.

Example 6

The ability of OP-1 to maintain the characteristics of the smooth muscle cell phenotype was tested in vitro. Rat aortic smooth muscle cells were prepared as previously described.
cells were plated (4x10^5/100 mm dish) and were grown in medium containing 5% FCS overnight. The next day, the plates were divided into two sets. To one set, 100 ng/ml of OP-1 was added every day for six days. No OP-1 was added to the second set. One plate from each set was harvested on each of days 3, 4, 5 and 6 post-confluence and the RNA extracted. RT-PCR was performed on the extracted RNA, and a Northern blot was performed to identify the amount of Type I collagen, Type III collagen, and α-actin RNA produced by the control cells (no OP-1 added) and the cells that received OP-1. The results are shown in Figures 7A-7C.

Figure 7A illustrates the effect of OP-1 on the expression of α-actin in the cultured cells. Lanes 1-4 illustrate the amount of α-actin RNA synthesis in the control cells on each of days 3, 4, 5, and 6 post-confluence. Lanes 5-8 illustrate the amount of α-actin RNA synthesis in the cells treated with OP-1 on days 3, 4, 5, and 6 post-confluence. As is seen by the figure, the cells treated with OP-1 were able to maintain or increase their level of α-actin RNA production in the prolonged culture.

Similar results for Type I and Type III collagen can be seen in Figures 7B and 7C, respectively. As shown in Figure 7B, the level of Type I collagen RNA is maintained in cells treated with OP-1 as compared to the control cells. Likewise, as shown in Figure 7C, the level of Type III collagen RNA is maintained or increased in cells treated with OP-1 as compared to the control cells, which showed a decrease in Type III collagen synthesis. Maintaining the normal balance of Type I and Type III collagen produced by these cells allows them to maintain their elasticity. The OP-1 treated cells were, therefore, able to maintain their characteristic phenotypic markers as compared to cells not treated with OP-1.

Example 7

The ability of OP-1 to protect smooth muscle cells against toxic insult (e.g., exposure to toxic substances such as mercuric chloride (HgCl₂) or Antimycin-A) was tested in vitro. Smooth muscle cells treated with OP-1, either before or after undergoing toxic insult, were able to survive as compared to cells left untreated.

The ability of OP-1 to protect against cell damage caused by HgCl₂ was first tested. Rat aortic smooth muscle cells were prepared as previously described. The cells were plated (4x10^4)
and treated with 0.1 mM HgCl₂ for three hours in growth medium containing 5% FCS. The plates were then washed exhaustively, and the cells were treated with serum-free growth medium and either no OP-1 or OP-1 at concentrations of 0.1 ng/ml, 1 ng/ml, 10 ng/ml, 40 ng/ml or 200 ng/ml. Forty-eight hours after treatment with OP-1, the cells were pulsed with [6-³H]thymidine to determine the amount of cell proliferation. The amount of ³H-thymidine incorporation into the cells was determined as previously described. Figure 8A shows the results. As can be seen by Figure 8A, the addition of OP-1 at concentrations of 10 ng/ml or more increased the survival rate of cells exposed to HgCl₂ in a dose-dependent manner as compared to cells left untreated or treated with only small amounts of OP-1.

The ability of OP-1 to protect smooth muscle cells against toxic insult by Antimycin-A was next tested. Rat aortic smooth muscle cells were prepared as described above. The cells were plated (4x10⁴) and treated with either 5 μM, 10 μM, or 20 μM Antimycin-A solution for three hours in growth medium containing 5% FCS. After washing exhaustively, the cells were treated with serum-free medium and OP-1 at concentrations of either 0.1 ng/ml, 1 ng/ml, 10 ng/ml, 40 ng/ml or 200 ng/ml. After 48 hours, the cells were pulse-labeled with [6-²H]thymidine to determine the amount of cell proliferation. The amount of ²H-thymidine incorporation into the cells was determined as previously described. Figure 8B shows the results. As can be seen by Figure 8B, the addition of OP-1 at concentrations of 10 ng/ml or more increased the survival rate of cells exposed to Antimycin-A as compared to cells left untreated or treated with only small amounts of OP-1. The ability of OP-1 to protect against cell death, however, reaches a plateau at approximately 40 ng/ml.

Example 8

The ability of OP-1 to inhibit the effects of inflammatory cytokines on smooth muscle cells was tested in vitro. The effect of OP-1 on the production of intercellular adhesion molecules (I-CAMs) in rat aortic smooth muscle cells treated with interleukin-1 (IL-1) was determined. IL-1 is released upon the occurrence of cellular trauma. The release of IL-1 in the cells results in increased production of I-CAMs, which clump together and can block the lumen of a blood vessel. OP-1 was shown to inhibit I-CAM production upon exposure of cells to IL-1 in vitro.
Rat aortic smooth muscle cells were prepared as previously described. The cells were then plated (4x10^4) in growth medium containing 5% FCS overnight. The growth medium was then replaced by a medium containing 0.5% FCS. IL-1 was added to the cells at concentrations of either 0 ng/ml, 1 ng/ml, 5 ng/ml, or 10 ng/ml. For each concentration of IL-1, the cells were also treated with 200 ng/ml of OP-1 or left untreated (control). After incubation for five days, the amount of I-CAM produced by the OP-1 treated and control cells at the various IL-1 concentrations was determined from cell extracts using I-CAM ELISA. The results are shown in Figure 9. As can be seen by Figure 9, cells treated with OP-1 showed less I-CAM production than the control cells. OP-1 therefore protects smooth muscle cells against the effects of inflammatory cytokines such as IL-1.

Morphogenic protein can therefore be used to preserve smooth muscle integrity following various traumas. Morphogenic protein is particularly useful in the treatment of vascular disorders, such as atherosclerosis and restenosis, because administration of morphogenic proteins not only reduces intimal thickening of blood vessels caused by excess collagen synthesis, inflammatory responses and cellular proliferation, but also maintains the phenotype of the cells.

Treatment of smooth muscle cells with morphogenic protein in vitro results in inhibition of the proliferation of the cells. Smooth muscle cell proliferation inhibition in vivo can also be accomplished by transfection of a cell with a vector containing a DNA insert encoding a morphogenic protein or administration of the protein itself directly to the site of smooth muscle cell proliferation. As will be clear to the skilled artisan, other vectors containing DNA inserts encoding a morphogenic protein of the invention may be used to transfect cells either in vitro or in vivo. Thus, the above examples describing an adenoviral vector capable of transfecting a mammalian cell and carrying a BMP-2 DNA insert is provided herein only for the purposes of illustrating a preferred embodiment of the invention.

Furthermore, treatment of smooth muscle cells with morphogenic protein following toxic insult or stimulation of the cells' inflammatory response inhibits the effects of such cellular trauma. The above examples describing the use of OP-1 to counteract the effects of cytotoxic agents and IL-1 stimulation of I-CAM production are provided herein for the purposes of illustrating a preferred embodiment of the invention. Accordingly, the scope of the invention is limited only by the scope of the appended claims.
Claims

What is claimed is:

1. A vector for expression of a morphogen in a mammalian cell, the vector comprising DNA encoding a morphogen, said morphogen comprising an amino acid sequence with at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

2. The vector of claim 1, further comprising a promoter that directs expression of said DNA, said promoter being in operative association with said DNA.

3. The vector of claim 2, wherein said promoter is a chicken β-actin promoter.

4. The vector of claim 2, further comprising a CMV-IE enhancer element in operative association with said promoter.

5. The vector of claim 1, wherein said vector is a viral vector.

6. The vector of claim 5, wherein said vector is an adenoviral vector.

7. The vector of claim 1, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

8. The vector of claim 1, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

9. The vector of claim 1, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative substitution variants thereof.

10. A method of inhibiting proliferation of smooth muscle cells, comprising the step of: administering to a mammal a composition comprising an expression vector, wherein said vector comprises DNA encoding a morphogen.
A method of inhibiting proliferation of smooth muscle cells, comprising the step of:

administering to a mammal a composition comprising a morphogen.

The method of claim 10 or 11, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

The method of claim 12, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

The method of claim 12, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

The method of claim 10 or 11, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

The method of claim 10, wherein said vector is a viral vector.

The method of claim 16, wherein said vector is an adenoviral vector.

The method of claim 10, wherein said vector further comprises expression control elements in operative association with said DNA.

The method of claim 10 or 11, wherein said smooth muscle is vascular smooth muscle.

A method of preserving the integrity of vascular tissue, comprising the step of:

administering to a mammal a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.

A method of preserving the integrity of a vascular tissue, comprising the step of:

administering to a mammal a composition comprising a morphogen.
22. The method of claim 20 or 21, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

23. The method of claim 20 or 21, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

24. The method of claim 20 or 21, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

25. The method of claim 20 or 21, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

26. The method of claim 20, wherein said vector further comprises expression control elements in operative association with said DNA.

27. The method of claim 20, wherein said vector is a viral vector.

28. The method of claim 27, wherein said vector is an adenoviral vector.

29. A method of protecting smooth muscle from cytotoxicity, comprising the step of:

administering to a mammal a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.

30. A method of protecting smooth muscle from cytotoxicity, comprising the step of:

administering to a mammal a composition comprising a morphogen.

31. The method of claim 29 or 30, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).
32. The method of claim 29 or 30, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

33. The method of claim 29 or 30, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

34. The method of claim 29 or 30, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

35. The method of claim 29, wherein said vector further comprises expression control elements in operative association with said DNA.

36. The method of claim 29, wherein said vector is a viral vector.

37. The method of claim 36, wherein said vector is an adenoviral vector.

38. A method for reducing inflammation of a vascular epithelium, comprising the step of: administering to a mammal a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.


40. The method of claim 38 or 39, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

41. The method of claim 38 or 39, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

42. The method of claim 38 or 39, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.
43. The method of claim 38 or 39, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

44. The method of claim 38, wherein said vector further comprises expression control elements in operative association with said DNA.

45. The method of claim 38, wherein said vector is a viral vector.

46. The method of claim 45, wherein said vector is an adenoviral vector.

47. A method of maintaining normal balance of Type I and Type III collagen in a smooth muscle tissue, comprising the step of:

administering to a mammal a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.

48. A method of maintaining normal balance of Type I and Type III collagen in a smooth muscle tissue, comprising the step of:

administering to a mammal a composition comprising a morphogen.

49. The method of claim 47 or 48, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

50. The method of claim 47 or 48, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

51. The method of claim 47 or 48, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

52. The method of claim 47 or 48, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1,
GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

53. The method of claim 47, wherein said vector further comprises expression control elements in operative association with said DNA.

54. The method of claim 47, wherein said vector is a viral vector.

55. The method of claim 54, wherein said vector is an adenoviral vector.

56. A method of treating a vascular proliferative disease, comprising the step of:

administering to a mammal a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.

57. A method of treating a vascular proliferative disease, comprising the step of:

administering to a mammal a composition comprising a morphogen.

58. The method of claim 56 or 57, wherein said vascular proliferative disease is atherosclerosis.

59. The method of claim 56 or 57, wherein said vascular proliferative disease is restenosis.

60. The method of claim 56 or 57, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

61. The method of claim 56 or 57, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

62. The method of claim 56 or 57, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

63. The method of claim 56 or 57, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1,
GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.

The method of claim 56, wherein said vector further comprises expression control elements in operative association with said DNA.

The method of claim 56, wherein said vector is a viral vector.

The method of claim 65, wherein said vector is an adenoviral vector.

A method of inhibiting restenosis after angioplasty, comprising the step of:

administering to a mammal which has undergone angioplasty a composition comprising an expression vector, said vector comprising DNA encoding a morphogen.

A method of inhibiting restenosis after angioplasty, comprising the step of:

administering to a mammal which has undergone angioplasty a composition comprising a morphogen.

The method of claim 67 or 68, wherein said morphogen has an amino acid sequence having at least 70% homology with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO: 1).

The method of claim 67 or 68, wherein said morphogen is OP-1, or a conservative substitution variant thereof.

The method of claim 67 or 68, wherein said morphogen is BMP-2, or a conservative substitution variant thereof.

The method of claim 67 or 68, wherein said morphogen is selected from the group consisting of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, BMP-10, BMP-11, BMP-12, BMP-15, BMP-16, DPP, Vgl, Vgr, 6A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, and conservative amino acid variants thereof.
73. The method of claim 67, wherein said vector further comprises expression control
elements in operative association with said DNA.

74. The method of claim 67, wherein said vector is a viral vector.

75. The method of claim 74, wherein said vector is an adenoviral vector.

76. The method of claim 67 or 68 wherein said composition is introduced within a lumen of an
artery which has undergone angioplasty.

77. The method of claim 67 or 68 wherein said composition is contacted with a site on a
luminal surface of an artery prior to angioplasty of said artery at said site.

78. The method of claim 67 or 68 wherein said composition is contacted with a site on a
luminal surface of an artery during angioplasty of said artery at said site.

79. The method of claim 67 or 68 wherein said composition is contacted with a site on a
luminal surface of an artery after angioplasty of said artery at said site.

80. The method of claim 67 or 68 wherein said composition is first adsorbed on a surface of
an angioplasty device and wherein said composition is contacted with a site on a luminal surface
of an artery using said device.

81. An angioplasty device having a surface which contacts a luminal surface of an artery
wherein

a composition is adsorbed to said surface, said composition selected from the group
consisting of (1) a vector comprising DNA encoding a morphogen, said morphogen
comprising an amino acid sequence with at least 70% homology with the C-terminal 102-
106 amino acids, including the conserved seven cysteine domain, of OP-1 (SEQ ID NO:
1), and (2) a morphogen comprising an amino acid sequence with at least 70% homology
with the C-terminal 102-106 amino acids, including the conserved seven cysteine domain,
of OP-1 (SEQ ID NO: 1).
Inhibitory Effect of OP-1 on AoSMC Cells

AoSMc cells were plated (4E4 cells per well of a 24 well plate) in complete growth medium containing 5% serum. Next day, media was replaced with one containing no serum to initiate serum starvation. 60 hours later, cells were stimulated with 1% serum plus various concentrations of OP-1 as indicated for a period of 24 hours. Cells were pulsed with 3H-Thymidine for the last 2 hours of this incubation.
Fig. 2
Fig. 3
MC cells were plated (4E5/100mm dish) in complete growth medium containing 5% serum. Day, the plates were divided into two sets. 100ng/ml mOP-1 was added to one set that day and day thereafter. One plate from each set was harvested on day 3, 4, 5 and 6 post-confluence.

CR was performed on the RNA extracted therefrom.

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Day 3 (with mOP-1)
4x10E4 AoSMC cells were treated with 0.1mM Mercuric Chloride for 3 hours in growth medium containing 5% serum. After exhaustive washing, cells were fed with serum free medium plus indicated concentrations of OP-1. 48 hours later cells were pulsed with 3H Thymidine. Data presented is an average of 4 samples.
Effect of OP-1 on Antimycin-A treated AoSMC Cells.

4x10E4 AoSMC cells were treated with 5, 10 or 20uM Antimycin-A for 3 hours in growth medium containing 5% serum. After exhaustive washing, cells were fed with serum free medium plus indicated concentrations of OP-1. 48 hours later, cells were pulsed with 3H Thymidine. Data presented is an average of 4 samples.
4x10E4 AoSMC cells were plated in growth medium containing 5% serum. Next day, growth medium was replaced by medium containing 0.5% serum. Indicated concentration of IL-1 with and without 200ng/ml OP-1 was added and subsequently incubated for a period of 5 days. I-CAM was measured in cell extract using a I-CAM-ELISA (R&D Systems). Data presented is an average of 4 samples.
### SEQUENCE LISTING

**SEQUENCE CHARACTERISTICS:**
- **LENGTH:** 1822 base pairs
- **TYPE:** nucleic acid
- **STRANDEDNESS:** single
- **TOPOLOGY:** linear

**MOLECULE TYPE:** cDNA

**FEATURE:**
- **NAME/KEY:** CDS
- **LOCATION:** 49..1341
- **OTHER INFORMATION:** /product= "HOP-1"

**SEQUENCE DESCRIPTION: SEQ ID NO:1:**

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