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(54) **PEPTIDE HAVING FIBROSIS INHIBITORY ACTIVITY AND COMPOSITION CONTAINING SAME**
PEPTID MIT FIBROSEHEMMENDER WIRKUNG UND ZUSAMMENSETZUNG DAMIT
PEPTIDE PRÉSENTANT UNE ACTIVITÉ INHIBITRICE CONTRE LA FIBROSE, ET COMPOSITION
LE CONTENANT

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- **BRUNSVIG ET AL.:** 'Telomerase peptide vaccination in NSCLC: a phase II trial in stage III patients vaccinated after chemoradiotherapy and an 8-year update on a phase I/II trial' **CLINICAL CANCER RESEARCH** vol. 17, no. 21, 2011, ISSN 1078-0432 pages 6847 - 6857, XP002678605
- **YI ET AL.:** 'Radiation-induced complications after breast cancer radiation therapy: a pictorial review of multimodality imaging findings' **KOREAN JOURNAL OF RADIOLOGY** vol. 10, no. 5, 2009, ISSN 1229-6929 pages 496 - 507, XP055230194

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Description

[Technical Field]

5 **[0001]** The present invention relates to a peptide having an anti-fibrosis effect and a composition including the same, and more particularly, to an anti-fibrosis composition which includes a telomerase-derived peptide and thus is effective in inhibiting the occurrence of fibrosis of various types of tissue cells.

[Background Art]

10 **[0002]** Fibrosis is a disease eliciting abnormal formation, accumulation and precipitation of an extracellular matrix, caused by fibroblasts, and refers to abnormal accumulation of a collagen matrix due to injury or inflammation that changes the structures and functions of various types of tissue. Regardless of where fibrosis arises, most aetiology of fibrosis includes excessive accumulation of a collagen matrix substituting normal tissue. Particularly, fibrosis occurring in the
15 kidney, liver, lung, heart, bone or bone marrow, and skin induces organ failure and leads to death at worst. The fibroblast serves to form fibrous tissue by producing an extracellular matrix precursor in a normal state. The extracellular matrix, which is a material between cells in connective tissue, exists in the form of a protein such as fibronectin, laminin, chondronectin or collagen.

20 **[0003]** Meanwhile, TGF- β plays various roles in the abnormal formation and accumulation of the extracellular matrix caused by fibroblasts, cell proliferation, inflammation, and cancer cell metastasis, and many cellular signaling pathways and targets have been identified. Accordingly, in many disease models, TGF- β has been studied, and fields in which the study on TGF- β and development of related drugs are most active may be fibrotic diseases and cancer.

25 **[0004]** In the TGF- β mechanism, generally, TGF- β binds to a TGF- β receptor to induce phosphorylation of Smad proteins in the cytoplasm and transfers signals through interactions with various Smad proteins. Here, Smad2 and Smad3 are usually involved, and Smad1 and Smad5 are involved in bone morphogenic protein (BMP) signaling. In addition, Smad4 is known as a common signaling substance involved in activin and BMP, as well as TGF- β (Heldin, CH et al. Nature, 1997, 390, 465-471; Massague J. Annu. Rev. Biochem. 1998, 67,753-791).

30 **[0005]** As inhibition of tumor invasion and epithelial-mesenchymal transition (EMT) are regulated by inhibition of the TGF- β signaling mechanism, excellent anticancer therapeutic effects can be expected. Moreover, considering that TGF- β is the critical cytokine in the regulation of cell proliferation and differentiation, it has also attracted attention as a therapeutic method for preventing fibrosis caused by radiation, which is mentioned as the main side effect of an anticancer treatment (Zhang et al. JRadiat Res. 2013, 54, 630-636).

35 **[0006]** Recently, Esbriet[®] (Pirfenidone) approved by the FDA (Oct. 15, 2014) as a therapeutic agent for idiopathic pulmonary fibrosis is a TGF- β inhibitor, which is known to have inhibition of TGF- β formation as a main action mechanism. Also, it has been reported that TGF- β is a cell proliferation regulatory factor, which induces or suppresses cell proliferation and thus plays an important role in pathogenesis of various diseases including cancer, cardiac diseases, and diabetes, and various physiological activities thereof have also been reported. For example, Esbriet[®] (Pirfenidone) acts as an inhibitor against TGF- β synthesis (inhibition of forming a cell proliferation regulatory factor), a TGF- β antagonist (distur-
40 bance of a TGF receptor, signaling disturbance), a PDGF (platelet-derived growth factor) antagonist (inhibition of angiogenesis inducing factor), a p38 MAP kinase inhibitor (inhibition of cell proliferation signaling enzyme), and an anti-inflammatory agent (inhibition of formation of TNF-alpha and MAPK). Therefore, if a new pharmaceutical composition can be developed that is effective in directly inhibiting TGF- β or blocking a TGF- β -involved signaling process and even has no side effect, such a pharmaceutical composition can prevent and treat various diseases and senescence, which are caused by fibrosis.

45 **[0007]** Particularly, in a cancer environment, there are various fibrosis-causing factors such as cancer tissue, anticancer agents, and radiation therapy, and therefore fibrosis inhibition has more significance in the cancer environment. Today's most popular anticancer treatments, radiation therapy and chemotherapy are known to considerably attenuate the quality of life of a patient and severely worsen prognosis of the anticancer treatment due to side effects caused by fibrosis and the like.

50 **[0008]** For this reason, there is a demand for developing a therapeutic agent for inhibiting the cancer-associated TGF- β signaling mechanism which will be useful for not only developing anticancer vaccines but also being new therapeutic agents and methods for maximizing effects of conventional anticancer treatment. For instance, in anticancer treatment, fibrosis caused by cancer tissue, an anticancer agent, or radiation exposure interferes with delivery and efficacy of a drug designed to exhibit an anticancer effect when an anticancer agent is delivered to a cancerous region and prevents
55 effective anticancer treatment by blocking transfer of anticancer immune cells to the cancerous region. Therefore, if a new pharmaceutical composition which has no side effect on a human body and is able to inhibit fibrosis caused by cancer tissue, a chemotherapeutic agent, or radiation exposure, anticancer treatment may be more effectively performed.

[Prior Art Documents]

[0009]

5 (Patent Document 0001) KR1019930001915 A
(Patent Document 0002) KR1020040107492 A
(Patent Document 0003) WO 2013/135266 A1

10 Brunsvig et al. Clinical Cancer Research vol. 17, no. 21, 2011, pages 6847-6857
WO 2013/135266 A1 and Brunsvig et al. (2011) report a clinical study in which GV1001 (SEQ ID NO:1) was used for vaccination of non-small cell lung cancer (NSCLC) patients which had undergone chemotherapy with docetaxel and radiotherapy within the last 4 weeks.

[Disclosure]

15 [Technical Problem]

[0010] With the background described above, the inventors attempted to develop an anti-fibrosis composition having excellent effects without a side effect and thus, completed the present invention.

20 **[0011]** An object of the present invention is to provide an effective anti-fibrosis composition.

[Technical Solution]

25 **[0012]** In one aspect, the present invention provides an anti-fibrosis composition for use in preventing or treating fibrosis, which includes at least one selected from the group consisting of a peptide comprising an amino acid sequence of SEQ. ID. NO: 1 (hereinafter, referred to as "PEP1") and a variant thereof in which one, two or three amino acids are modified by conservative amino acid substitution.

[0013] In the composition for use according to the present invention, the fibrosis may be fibrosis induced by at least one selected from the group consisting of cancer, administration of an anticancer agent, and exposure to radiation.

30 **[0014]** In the composition for use according to the present invention, the composition may inhibit fibrosis of cancer cell tissue selected from the group consisting of pancreatic cancer, colorectal cancer, stomach cancer, prostate cancer, non-small cell lung cancer, breast cancer, melanoma and ovarian cancer.

35 **[0015]** In the composition for use according to the present invention, the composition may be used in combination with a chemotherapeutic agent or a radiation therapy to inhibit fibrosis of cancer tissue. The chemotherapeutic agent may be at least one selected from deoxynucleoside analogs and fluoropyrimidines, wherein the deoxynucleoside analog may be gemcitabine, and the fluoropyrimidine may be 5-fluorouracil or capecitabine.

[0016] In the composition for use according to the present invention, the composition may be a pharmaceutical composition further including pharmaceutically acceptable excipients and additives.

40 **[0017]** In the composition for use according to the present invention, the composition may be an anti-fibrosis composition involved in a TGF- β signaling process and therefore inhibiting fibrosis of dermal tissue.

[0018] In the composition for use according to the present invention, the composition may further include acceptable excipients, lubricants and additives, and may be a cosmetic composition.

[Advantageous Effects]

45 **[0019]** According to the present invention, a peptide comprising an amino acid sequence of SEQ. ID. NO: 1, a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, and a composition including the same, have an excellent anti-fibrosis effect without side effects. Therefore, tissue fibrosis caused by a TGF- β signaling process, particularly, fibrosis caused by cancer tissue or fibrosis caused by anticancer agents or exposure to radiation accompanied by anticancer treatment, etc. can be effectively inhibited and is very useful in preventing and/or treating a fibrotic disease.

[Description of Drawings]

55 **[0020]**

FIG. 1 is a diagram illustrating an entire experiment process of preparing xenograft models, in which nude mice are inoculated with AsPCI cells and then PEP1 and gemcitabine are administered to the mice, starting 10 days later.

FIG. 2 is a graph showing a weight of each mouse of a control in which nude mice inoculated with AsPC1 cells are not treated with anything, of groups in which each of PEP1 and gemcitabine is administered to nude mice inoculated with AsPC1 cells starting 10 days after inoculation, and of a group in which a combination of PEP1 and gemcitabine is administered to nude mice inoculated with AsPC1 cells from 10 days after inoculation to 24 days after administration to detect *in vivo* anti-cancer effects of PEP1 and gemcitabine.

FIG. 3 shows cell images of the control in which the nude mice inoculated with AsPC1 cells are not treated with anything.

FIG. 4 shows cell images of the group in which PEP1 is subcutaneously administered to the nude mice inoculated with AsPC1 cells once a day, starting 10 days after inoculation.

FIG. 5 shows cell images of the group in which gemcitabine is intraperitoneally administered to the nude mice inoculated with AsPC1 cells once every third day, starting 10 days after inoculation.

FIG. 6 shows cell images of the group in which both of PEP1 (once a day, subcutaneously) and gemcitabine (once every third day, intraperitoneally) are administered to the nude mice inoculated with AsPC1 cells starting 10 days after inoculation.

FIG. 7 is a graph showing relative Sma2 expression, which is assessed by qRT-PCR, in a control in which a HepG2 cell line is not treated with anything and an experiment group in which a HepG2 cell line is not treated with anything after TGF- β 1 treatment, a positive control in which SB431542 is administered into a HepG2 cell line, and experiment groups in which PEP1 is administered into HepG2 cell lines at different concentrations (1, 5 and 10 μ M).

FIG. 8 is a graph showing Smad2 inhibition ratios (%) in each experiment group in which HepG2 cell lines are treated with TGF- β 1 and then treated with PEP1 at different concentrations (1, 5 and 10 μ M).

FIG. 9 is a graph showing relative Smad4 expression, which is assessed by qRT-PCR, in a control in which a HepG2 cell line is not treated with anything and an experiment group in which a HepG2 cell line is not treated with anything after TGF- β 1 treatment, a positive control in which SB431542 is administered into a HepG2 cell line, and experiment groups in which PEP1 is administered into HepG2 cell lines at different concentrations (1, 5 and 10 μ M).

FIG. 10 is a graph showing Smad4 inhibition ratios (%) in experiment groups in which PEP1 is administered into HepG2 cell lines at different concentrations (1, 5 and 10 μ M), after TGF- β 1 treatment.

FIG. 11 is a graph showing relative fibronectin expression, which is assessed by qRT-PCR, in a control in which a HepG2 cell line is not treated with anything and an experiment group in which a HepG2 cell line is not treated with anything after TGF- β 1 treatment, a positive control in which SB431542 is administered to a HepG2 cell line, and experiment groups in which PEP1 is administered to HepG2 cell lines at different concentrations (1, 5 and 10 μ M).

FIG. 12 is a graph showing fibronectin inhibition ratios (%) in a positive control in which HepG2 cell lines are treated with SB431542 and experiment groups in which PEP1 is administered to HepG2 cell lines at different concentrations (1, 5 and 10 μ M), after TGF- β 1 treatment.

FIG. 13 shows cell culture states in controls in which normal human epidermal keratinocytes (NHEKs) are treated with a placebo (sham) and experimental groups treated with 1 mM of PEP1 10 days after one in each group is exposed to radiation (6 Gy, ionizing radiation (IR)) and the other one in each group is not exposed.

FIG. 14 is electrophoresis images showing GRHL2, p63, N-Cad, and P-Akt expressions in cells of controls in which NHEKs are treated with a placebo (sham) and experiment groups treated with 1 mM of PEP1 one day and 10 days after one in each group is exposed to radiation (6 Gy, ionizing radiation (IR)) and the other one in each group is not exposed.

FIG. 15 is western blotting images showing p-Smad2/3, Smad4, FN, Snail and N-Cad expressions in cells of a control in which NHEKs are treated with a placebo (sham) and an experiment group treated with 1 mM of PEP 1 after being exposed to radiation (6 Gy, IR).

[Modes of the Invention]

[0021] Hereinafter, the present invention will be described in further detail with reference to exemplary embodiments. However, the present invention is not limited to specific disclosures. For explaining the present invention, the detailed descriptions on related technology known in the art will be omitted when it is determined that they might obscure the gist of the present invention.

[0022] Telomere, which is a repetitive genetic material located at each terminus of a chromosome, is known to prevent damage to a corresponding chromosome or coupling to a different chromosome. The telomere is gradually shortened with cell divisions, becoming very short after a certain number of cell divisions, and the cell eventually stops being divided and dies. On the other hand, the elongation of telomeres is known to extend the life span of a cell. As an example, it has been known that, in cancer cells, an enzyme called telomerase is secreted to prevent the shortening of telomeres, resulting in steady proliferation of the cancer cells, without death. The inventors identified that a peptide derived from telomerase is effective in inhibiting fibrosis and thus completed the present invention.

[0023] In one aspect of the present invention, a peptide of SEQ. ID. NO: 1 or a variant thereof in which one, two or

three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution includes telomerase, particularly, a peptide derived from *Homo sapiens* telomerase.

[0024] The following paragraph is not a part of the invention but it is disclosed in the specification that the peptide may include a peptide having 80% or higher, 85% or higher, 90% or higher, 95% or higher, 96% or higher, 97% or higher, 98% or higher, or 99% or higher sequence homology. Also, the peptide disclosed in the specification may include a peptide of SEQ. ID. NO: 1, a fragment thereof, and a peptide in which at least one, two, three, four, five, six, or seven amino acids are modified.

[0025] The following paragraph is not a part of the invention but it is disclosed in the specification that some amino acids in a peptide that makes physicochemical characteristics of the peptide of SEQ. ID. NO: 1 changed may be modified within the scope of the present invention. For example, amino acids may be modified to allow the peptide to have enhanced thermal stability, changed substrate specificity, and shifted optimal pH.

[0026] The term "amino acid" used herein includes not only the 22 standard amino acids that are naturally integrated into peptide but also the D-isomers and transformed amino acids. Therefore, in one aspect of the present invention, a peptide herein includes a peptide having D-amino acids. On the other hand, in another aspect of the present invention, a peptide may include non-standard amino acids such as those that have been post-translationally modified. Examples of post-translational modification include phosphorylation, glycosylation, acylation (including acetylation, myristoylation, and palmitoylation), alkylation, carboxylation, hydroxylation, glycation, biotinylation, ubiquitinylation, a transformation of chemical properties (e.g. β -removing deimination, deamidation), and a structural transformation (e.g. formation of a disulfide bridge). The post-translational modification also include changes of amino acids occurring due to chemical reactions when coupling with crosslinkers to form a peptide conjugate, for example, a change of an amino acid occurring at an amino group, a carboxyl group or a side chain.

[0027] The peptide disclosed herein may be a wild-type peptide identified and isolated from a natural source. Meanwhile, the peptide disclosed in the specification may be an artificial variant comprising an amino acid sequence in which one or more amino acids are substituted, deleted, and/or inserted, compared with the fragments of the peptide of SEQ. ID. NO: 1. The changing of amino acids in the wild-type polypeptide, as well as the artificial variant, is the substitution of conservative amino acids, which does not have a significant influence on folding and/or activity of a protein. The conservative substitution may be carried out in the range of the group consisting of basic amino acids (arginine, lysine and histidine), acidic amino acid (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine, valine and methionine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine and threonine). Generally, amino acid substitution that does not change a specific activity is known in the art. The most frequently-occurring exchange takes place between Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly, and vice versa. Other examples of the conservative substitution are shown in Table 1 below.

[Table 1]

Original amino acid	Exemplary residue substitution	Preferred residue substitution
Ala (A)	val; leu; ile	Val
Arg (R)	lys; gln; asn	Lys
Asn (N)	gln; his; asp, lys; arg	Gln
Asp (D)	glu; asn	Glu
Cys (C)	ser; ala	Ser
Gln (Q)	asn; glu	Asn
Glu (E)	asp; gln	Asp
Gly (G)	Ala	Ala
His (H)	asn; gln; lys; arg	Arg
Ile (I)	leu; val; met; ala; phe; norleucine	Leu
Leu (L)	norleucine; ile ; val; met; ala; phe	Ile
Lys (K)	arg; gln; asn	Arg
Met (M)	leu; phe; ile	Leu

(continued)

Original amino acid	Exemplary residue substitution	Preferred residue substitution
Phe (F)	leu; val; ile; ala; tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	thr	Thr
Thr (T)	Ser	Ser
Trp (W)	tyr; phe	Tyr
Tyr (Y)	trp; phe ; thr; ser	Phe
Val (V)	ile; leu; met; phe; ala; norleucine	Leu

[0028] In terms of biological properties of the peptide, a substantial modification is performed by selecting a substitution part which has a considerably different effect in (a) maintaining the backbone structure, for example, a sheet- or helix-like three-dimensional structure, of the polypeptide in a substituted region, (b) maintaining charge or hydrophobicity of the molecule at a target site, or (c) maintaining the bulk of a side chain. Natural residues are classified into the following groups, based on general properties of the side chain:

- (1) Hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) Neutral hydrophilic: cys, ser, thr;
- (3) Acidic: asp, glu;
- (4) Basic: asn, gln, his, lys, arg;
- (5) Residues affecting chain conformation: gly, pro; and
- (6) Aromatic: trp, tyr, phe.

[0029] Not according to the invention but disclosed herein are non-conservative substitutions performed by exchanging a member of one of the groups to that of another group. Any cysteine residue, which is not associated with maintaining the proper three-dimensional structure of the peptide, may typically be substituted into serine, thus increasing the oxidative stability of the molecule and preventing improper crosslinks, and, conversely, enhanced stability can be achieved by adding cysteine bond(s) to the peptide.

[0030] Not according to the invention but disclosed herein are a different type of amino acid variant of the peptide is made by changing a glycosylation pattern of an antibody. The term "change" used herein refers to deletion of one or more carbohydrate residues that are found on the peptide and/or addition of one or more glycosylation sites which do not exist in the peptide.

[0031] Glycosylation in peptides are typically N- or O-linked glycosylation. The term "N-linked glycosylation" used herein refers to attachment of carbohydrate residues to side chains of asparagine residues. As tripeptide sequences, asparagine-X-serine and asparagine-X-threonine (where the X is any amino acid, excluding proline) are recognition sequences for enzymatically attaching a carbohydrate residue to a side chain of an asparagine. Therefore, when one of these tripeptide sequences is present in a polypeptide, a potential glycosylation site is created. The "O-linked glycosylation" used herein refers to the attachment of one of the saccharides, for example, N-acetylgalactosamine, galactose, or xylose, to hydroxyamino acids and, most typically, to serine or threonine, but 5-hydroxyproline or 5-hydroxylysine may also be used.

[0032] Not according to the invention but disclosed herein are addition of a glycosylation site to the peptide conveniently performed by changing the amino acid sequence to contain the tripeptide sequence described above (for an N-linked glycosylation site). Such a change may be made by addition of one or more serine or threonine residues to the first antibody sequence or by substitution into one of these residues (for an O-linked glycosylation site).

[0033] Not according to the invention but disclosed herein is the finding that the peptide comprising the sequence of SEQ. ID. NO: 1 or a fragment thereof, or a peptide having 80% or higher sequence homology with the peptide sequence has low cytotoxicity and high *in vivo* stability. In the present invention, SEQ. ID. NO: 1 represents the telomerase-derived peptide, which consists of 16 amino acids as follows.

[0034] The peptide set forth in SEQ. ID. NO: 1 is shown in Table 2. The "name" in the Table 2 below is given to distinguish one peptide from another. In one aspect of the present invention, the peptide set forth in SEQ. ID. NO: 1 represents the whole peptide of human telomerase. In another aspect of the present invention, the peptide comprising the sequence of SEQ. ID. NO: 1 or a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, includes a "synthetic peptide" syn-

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thesized from a peptide present at a corresponding location that has been selected from the peptides included in telomerase. SEQ. ID. NO: 2 represents the full-length amino acid sequence of the telomerase.

[Table 2]

SEQ. ID. NO:	Name	Position on telomerase	Sequence	Length
1	pep1	[611-626]	EARPALLTSRLRFIPK	16 aa
2		[1-1132]	MPRAPRCRAVRSLLRSHYREVLPLATFVRRLLGPQGWR LVQRGDPAAFRALVAQCLVCPWDARPPPAAPFRQV SCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGP PEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDV LVHLLARCALFVLVAPSCAYQVCGPPLYQLGAATQAR PPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGAR RRGGSASRSLPLPKRPRR GAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPAR	1132 aa

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(continued)

SEQ. ID. NO:	Name	Position on telomerase	Sequence	Length
5			PAEEATSLEGALSGTRHSHPSVGRQHHAGPPSTSRPPR	
			PWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPSL	
10			TGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRP	
			LFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAR	
			EKPQGSVAAPEEEDTDPRLVQLLRQHSSPWQVYGFV	
15			RACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAK	
			LSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLRE	
20			EILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFY	
			RKSVWSKLSIGIRQHLKRVLRELEASEAEVRQHREARP	
			ALLTSRLRFIPKPDGLRPIVNDYVVGARTFRREKRAE	
25			RLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRA	
			WRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLT	
30			EVIASIIKPQNTYCVRRYAVVQKAAHGHVRKAFKSHV	
			STLTDLQPYMRQFVAHLQETSPLRDVVIEQSSSLNEA	
			SSGLFDVFLRFMCHHAVRIRGKSYVQCQGIPOGSILSTL	
35			LCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLT	
			HAKTFLRTLVRGVPEYGCVVNLKTKTVVNFVVEDEALG	
40			GTAFVQMPAHGLFPWCGLLLDTRTLEVQSDYSSYART	
			SIRASLTFNRGFKAGRNMRRKLFGLVRLKCHSLFLDLQ	
			VNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWK	
45			NPTFFLRVISDTASLCYSILKAKNAGMSLGAKGAAGPL	
			PSEAVQWLCHQAFLLKLTRHRVTYVPLLGLSLRTAQTQ	
50			LSRKLPGT TLTALEAAANPALPSDFKTILD	

[0035] In one aspect, the present invention provides a pharmaceutical composition, which includes a peptide with an anti-fibrosis effect, for example, a peptide comprising an amino acid sequence of SEQ. ID. NO: 1 or a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, as an active ingredient.

[0036] The anti-fibrosis pharmaceutical composition according to an aspect of the present invention may include a peptide comprising an amino acid sequence of SEQ. ID. NO: 1 or a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, at a content of 0.01 mg/kg to 0.1 mg/kg, 1 mg/kg, or 10 mg/kg, and when there is a difference in effect according to content,

the content may be suitably adjusted. When the composition includes the peptide in the above range or a smaller range, it is suitable for exhibiting an effect intended by the present invention and is able to satisfy both stability and safety requirements of the composition. Moreover, the above range may be appropriate in terms of cost-effectiveness.

5 [0037] In one aspect, the present invention provides a use of a peptide comprising an amino acid sequence of SEQ. ID. NO: 1 or a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, to prepare any one of the compositions for inhibiting fibrosis described above.

[0038] The composition according to an aspect of the present invention may be applied to all types of animals including humans, dogs, chickens, pigs, cows, sheep, guinea pigs, and monkeys.

10 [0039] In one aspect of the present invention, the composition may be a pharmaceutical composition including a peptide having an anti-fibrosis effect, wherein the peptide consists of the amino acid sequence of SEQ. ID. NO: 1 or is a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution. The pharmaceutical composition according to an aspect of the present invention may be for administration orally, rectally, percutaneously, intravenously, intramuscularly, intraperitoneally, intramedullarily, intrathecally or subcutaneously.

15 [0040] Dosage form for oral administration may be, but not limited to, tablets, pills, soft or hard capsules, granules, a powder, a solution, or an emulsion. Dosage form for parenteral administration may be, but not limited to, injections, drips, lotions, ointments, gels, creams, suspensions, emulsions, a suppository, a patch, or a spray.

[0041] The pharmaceutical composition according to one aspect of the present invention, as necessary, may include additives such as diluents, excipients, lubricants, binders, disintegrants, buffers, dispersants, surfactants, coloring agents, flavorings, or sweeteners. The pharmaceutical composition according to one aspect of the present invention may be prepared by a conventional method used in the art.

20 [0042] The active ingredient of the pharmaceutical composition according to one aspect of the present invention may vary according to the patient's age, sex, weight, pathological condition and severity, administration route, or a prescriber's judgment. Administration doses are determined based on these factors by one of ordinary skill in the art, and a daily dose of the pharmaceutical composition may be, for example, 0.1 $\mu\text{g}/\text{kg}/\text{day}$ to 1 $\text{g}/\text{kg}/\text{day}$, specifically, 1 $\mu\text{g}/\text{kg}/\text{day}$ to 100 $\text{mg}/\text{kg}/\text{day}$, more specifically, 10 $\mu\text{g}/\text{kg}/\text{day}$ to 10 $\text{mg}/\text{kg}/\text{day}$, and further more specifically, 50 $\mu\text{g}/\text{kg}/\text{day}$ to 1 $\text{mg}/\text{kg}/\text{day}$, but when there is a difference in effect according to dose, the dose may be suitably adjusted. The pharmaceutical composition according to one aspect of the present invention may be for administration once to three times a day, but the present invention is not limited thereto.

25 [0043] According to the present invention, the composition is an anti-fibrosis composition, which includes a peptide comprising an amino acid sequence of SEQ. ID. NO: 1 or a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution, as an active ingredient.

30 [0044] In one aspect of the present invention, the composition may be a composition for inhibiting fibrosis, which is caused by cancer tissue, particularly, pancreatic cancer, stomach cancer, renal cell carcinoma, prostate cancer, larynx cancer, esophageal cancer, thyroid cancer, lung cancer, breast cancer, large and small intestine cancer, uterine cancer, cervical cancer, cancer of uterine body, urinary bladder cancer, genitourinary cancer, bladder cancer, and skin cancer tissue.

35 [0045] The composition according to one aspect of the present invention may be prepared in the form of a tablet, a granule, a powder, a liquid, or a solid. For each form, ingredients, other than an active ingredient, that are conventionally used in the corresponding field, may be easily mixed depending on the form or the purpose of use by one of ordinary skill in the art and may have a synergistic effect when simultaneously applied with a different base material.

40 [0046] Administration doses of the active ingredient are determined by one of ordinary skill in the art, a daily dose of the pharmaceutical composition may be, for example, specifically 1 $\mu\text{g}/\text{kg}/\text{day}$ to 100 $\text{mg}/\text{kg}/\text{day}$, more specifically 10 $\mu\text{g}/\text{kg}/\text{day}$ to 10 $\text{mg}/\text{kg}/\text{day}$, and further more specifically 50 $\mu\text{g}/\text{kg}/\text{day}$ to 1 $\text{mg}/\text{kg}/\text{day}$, and, when there is a difference in effect according to a content, the dose may be suitably adjusted and may vary depending on various factors including a subject's age, health condition, complications, etc.

45 [0047] The terms used in the specification are used only to explain specific examples and not to limit the present invention. Nouns without a number in front thereof do not limit quantity and indicate that at least one article describe by the noun exists. The terms "include", "have" and "contain" are construed as open terms (that is, means that "includes but is not limited").

50 [0048] The mention of a value range is simply because it is an easy way to substitute an alternative to each value included in the range. Unless particularly disclosed otherwise, each value is incorporated herein as if it is individually referred to the specification. End values in all ranges are included in each of the ranges and can be independently combined. All of the methods mentioned herein may be performed in suitable order unless disclosed otherwise or clearly contradicted by the context. The use of any or all of the embodiments or exemplary language (e.g., "such as") is not only to describe better the present invention, but also to limit the scope of the present invention. No language in the

specification should be construed indicating that a non-claimed component is essential for implementation of the present invention. Unless defined otherwise, a technical or scientific term used herein has the same meaning as that usually understood by one of ordinary skill in the art.

[0049] Exemplary embodiments of the present invention include the best mode known to the inventors in order to implement the present invention. Variations of the exemplary embodiments may be clearly understood by those of ordinary skill in the art by reading the above descriptions. The inventors expect those of ordinary skill in the art to suitably utilize such variations, and further expect that the present invention is implemented in modes that are different from that described in the specification. Moreover, all combinations of the components mentioned in all possible variations are included in the present invention unless disclosed otherwise or is clearly contradictory to the context.

[0050] Hereinafter, the configuration and effect of the present invention will be described in further detail with reference to examples and experimental examples. While the examples and experimental examples below are merely provided to help in understanding the present invention, the category and scope of the present invention are not limited thereto.

[Modes of the Invention]

Example 1: Synthesis of peptides

1. Synthesis of peptides

[0051] A peptide of SEQ. ID. NO: 1 (hereinafter, referred to as "PEP1") was prepared according to conventionally known solid peptide synthesis. Specifically, peptides were synthesized by coupling amino acids one by one from the C-terminus through a Fmoc solid phase peptide synthesis (SPPS) using ASP48S (Peptron, Inc., Daejeon, Korea). Resins to which the first amino acid at the C-terminus of peptides is attached, which were used herein, are as follows:

NH₂-Lys(Boc)-2-chloro-Trityl Resin
 NH₂-Ala-2-chloro-Trityl Resin
 NH₂-Arg(Pbf)-2-chloro-Trityl Resin

[0052] In all amino acid components used in the peptide synthesis, the N-terminus was protected by Fmoc, and all residues were protected by Trt, Boc, t-butylester (t-Bu), or 2,2,4,6,7-pentamethyl dihydro-benzofuran-5-sulfonyl (Pbf), which were removed from acids. Such amino acid components are as follows:

Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Phe-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Met-OH, Fmoc-Asn(Trt)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ahx-OH, Trt-Mercaptoacetic acid.

[0053] As coupling reagents, 2-(1H-Benzotriazole-1-yl)-1,3,3-tetamethylaminium hexafluorophosphate (HBTU)/N-Hydroxybenzotriazole (HOBT)/4-Methylmorpholine (NMM) were used. For Fmoc deprotection, 20% piperidine in DMF was used. For separation from the synthesized peptide from the resin and removal of the protection group of the residue, a cleavage cocktail [TFA (trifluoroacetic acid)/TIS (triisopropylsilane)/EDT (ethanedithiol)/H₂O=92.5/2.5/2.5/2.5] was used.

[0054] Each peptide was synthesized by repeating a process of protecting corresponding amino acids with an amino acid protection group-coupled start amino acid coupled to a solid phase scaffold, washing with a solvent, and deprotection. After cutting off the synthesized peptide from the resin, the peptide was purified by HPLC, assessed by MS to verify the synthesis, and then lyophilized.

[0055] As assessed by high performance liquid chromatography, all peptides used in the example had a purity of 95% or higher.

[0056] Detailed procedures for preparing the peptide PEP1 were as follows.

1) Coupling

[0057] Protected amino acid (8 equivalents) and coupling agents HBTU (8 equivalents)/HOBT (8 equivalents)/NMM (16 equivalents) dissolved in DMF were added to the NH₂-Lys(Boc)-2-chloro-Trityl resin, and the reaction mixture was reacted at room temperature for 2 hours and then sequentially washed with DMF, MeOH, and DMF.

2) Fmoc deprotection

[0058] Following the addition of 20% piperidine in DMF, the reaction mixture was reacted at room temperature for 5 minutes two times, and then sequentially washed with DMF, MeOH, and DMF.

3) The reactions 1 and 2 were repeatedly performed to prepare the basic backbone of a peptide, for example, NH₂-E(OtBu)-A-R(Pbf)-P-A-L-L-T(tBu)-S(tBu)-R(Pbf)L-R(Pbf)-F-I-P-K(Boc)-2-chloro-trityl resin.

4) Cleavage: A cleavage cocktail was added to the previously synthesized peptide resin to separate a peptide from the resin.

5) Following the addition of cooling diethyl ether to the obtained mixture, the peptide obtained by centrifugation was precipitated.

6) Following purification by Prep-HPLC, the peptide was analyzed by LC/MS to check molecular weight and lyophilized to obtain a powder.

Example 2: Fibrosis inhibition by PEP1 in AsPC1 cell xenograft model

[0059] To verify *in vivo* anticancer effects of PEP1 and gemcitabine on pancreatic cancer, xenograft experiments were performed using AsPC1 cell lines. A xenografting method used herein is as follows.

Preparation of reagents and materials

[0060] Reagents and materials used for the experiment are as follows. After powdery PEP1 was dissolved in 0.2 μm filtered sterile water, aliquots were stored at -70 °C and then dissolved before use, and gemcitabine was dissolved in 100% saline. 5-fluorouracil was dissolved in DMSO.

Preparation of cell lines

[0061] AsPC1 cell lines (Cell #6 x 10⁵ cells/ 100 ml), which were cell lines used in the experiment, are human pancreatic cancer metastatic cells purchased from the American Type Cell Culture (ATCC, Rockville, MD). The cell lines were cultured at 37 °C to give a cell density of 1 to 2 x 10⁶/ml in Roswell Park Memorial Institute (RPMI 1640) medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin.

Experimental methods

[0062] AsPC1 cells were injected into each of nude mouse experiment groups ① to ④. The reagents and materials and method of culturing cell lines, which were used in the experiment, are the same as described in Example 1.

[0063] The cultured 5 x 10⁶ AsPC1 cells were subcutaneously injected into nude mice (BALB/c-nu/nu nude mice, purchased from Joongang Laboratory animal, Seoul, Korea). The injection was performed until cancer was grown to a size of 100 mm³ (about 10 days). Following cancer engraftment, the mice were divided into groups, each group having a similar average weight, and then either or both of gemcitabine and Pep1 were subcutaneously and intraperitoneally injected into the mice according to the conditions for each of the four experiment groups below (refer to FIG. 1). Cancer volume was measured with calipers and calculated by the following formula [width² x length x 0.52].

[0064] After grafting, either or both of Pep1 and gemcitabine were administered to each of the four experiment groups.

- ① AsPC1 grafting control
- ② AsPC1 grafting + PEP1 2 mg/kg (once a day, s.c) administered group
- ③ 2 mg/kg grafting + gemcitabine 125 mg/kg (once every third day, i.p) administered group
- ④ 2 mg/kg grafting + PEP1 2 mg/kg (once a day, s.c) + gemcitabine 125 mg/kg administered group (once every third day, i.p)

[0065] After then, weight of each mouse was quantified every time. Also, cancer tissue sample preparation and cell proliferation marker (PCNA)/apoptosis marker (TUNEL) staining were performed.

[0066] For analyses of experiment results, averages between several experiment groups was assessed by a student's t-test. The standard of statistical significance was set as p=0.0002.

Experimental results

[0067] According to the analyses of individual experiment groups, it can be seen that, when a combination of gemcitabine and PEP1, and gemcitabine were administered, the weight of a mouse was the smallest at day 20 after the administration (refer to FIG. 2).

[0068] FIGS. 3 to 6 show cells in each experiment group.

[0069] In the AsPC1 cell-inoculated control (group ①; refer to FIG. 3), it was shown that collagen parts stained in blue color (corresponding to the dark gray parts in FIG. 3) were increased between cancer cells stained in red color (corre-

sponding to the light gray parts in FIG. 3). That is, it can be seen that fibrosis is considerably developed due to cancer.

[0070] In group ② in which AsPC1 cells were treated with PEP1 (refer to FIG. 4), compared with FIG. 3, parts stained in blue color (corresponding to the dark gray parts in FIG. 4) were not shown. This indicates that collagen was reduced, and thus, fibrosis was considerably inhibited by PEP1.

[0071] Meanwhile, in group ③ in which AsPC1 cells were treated with gemcitabine (refer to FIG. 5), it can be seen that parts stained in red color (corresponding to black parts in FIG. 5) were considerably reduced, and the parts stained in blue color (corresponding to the light gray parts in FIG. 5) were increased. This indicates that cancer cells were killed by gemcitabine, but fibrosis was considerably developed.

[0072] Here, remaining red-colored cells may probably be an anticancer agent against CD133+ cancer stem cells. In pancreatic cancer, it was known that the CD133+ cancer stem cells have resistance to gemcitabine, which is an anticancer agent, and development of fibrosis around these cells demonstrates that drug delivery is not well performed.

[0073] However, compared with FIG. 5, in group ④ in which AsPC1 cells were treated with PEP1 and gemcitabine (refer to FIG. 6), it was observed that parts stained in blue color (corresponding to the light gray parts in FIG. 6) were reduced. It was seen that fibrosis was considerably inhibited by PEP1, and thus the inhibition of drug delivery due to fibrosis was considerably reduced, which indicates that intracellular delivery of gemcitabine, which is an anticancer agent, may more easily occur.

Example 3: TGF-β inhibitory effect of PEP1 in HepG2 cell lines

[0074] To verify the TGF-β inhibitory effect of PEP1, which is considered as the main cause of fibrosis, an experiment for confirming a TGF-β signaling inhibiting action in HepG2 cell lines was performed as follows.

Preparation of reagents and materials

[0075] Reagents and materials used in the experiment are as follows. After powdery PEP1 was dissolved in 0.2 μm filtered sterile water, aliquots were stored at -70 °C and then dissolved before use. As a cell line, HepG2 (ATCC HB-8065; American Type Culture Collection) was used, and recombinant human TGF-β1 was dissolved in 4 mM HCl, thereby preparing a 10 μg/mL stock. SB431542 (Sigma) used as a positive control was prepared in a 10 mM stock.

Preparation of cell lines

[0076] 2×10⁶HepG2 cells (ATCC HB-8065) were seeded into a 60 mm petri-dish and cultured in a CO₂ incubator for 16 hours. Afterward, media were transferred to serum-free media (SFM), and then the cells were further cultured for 24 hours. Subsequently, 10 ng/ml of TGF-β1 was added to the media, and then various concentrations of PEP1 (0.1, 1, 5, 10 μM) were treated to media, followed by culturing for 72 hours. Each treated group was further cultured in a CO₂ incubator for 1 hour at 37 °C.

Experiment for analyzing expression of TGF-β inhibition-related genes

[0077] RNA was extracted from peptide-treated cells using an RNeasy® Plus Mini Kit (Qiagen) according to the manufacture's protocol. The extracted RNA was quantified, and then cDNA was synthesized from 1 μg of total RNA using a reverse transcription system (Promega). Afterward, qRT-PCR was performed using a CFX96-real-time system (Bio-rad). As TGF-β-related biomarkers, mRNA expression of fibronectin (FN), Smad2 and Smad4 were analyzed, and as a reference gene, GAPDH was used. For PCR, primers of each gene were constructed as follows. The genes were amplified real time using the primers of Table 3 below with 40 cycles (95 °C, 15 sec, 57 °C, 30 sec, 72 °C, 30 sec). The nucleotide sequences of the primers are as follows.

[Table 3]

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
FN	CAGGATCACTTACGGAGAAACAG (SEQ. ID. NO: 3)	GCCAGTGACAGCATACACAGTG (SEQ. ID. NO: 4)
Smad2	ATCCTAACAGAACTTCCGCC (SEQ. ID. NO: 5)	CTCAGCAAAAACCTTCCCCAC (SEQ. ID. NO: 6)
Smad4	GCATCGACAGAGACATACAG (SEQ. ID. NO: 7)	CAACAGTAACAATAGGGCAG (SEQ. ID. NO: 8)
GAPDH	AGGGCTGCTTTTAACTCTGGT (SEQ. ID. NO: 9)	CCCCACTTGATTTTGGAGGGA (SEQ. ID. NO: 10)

Experiment results

[0078] The TGF- β inhibitory effects of PEP1 were evaluated by qRT-PCR. Following 48-hour culture of PEP1 in the presence of TGF- β , mRNA expression levels of TGF- β -related genes such as Smad2 and Smad4 were measured and compared with the reference gene GAPDH (refer to FIGS. 7, 8, 9 and 10). Compared with the non-treated control, Smad2 and Smad4 expression was increased in the TGF- β treated group, and in the positive control SB431542, compared with the TGF- β treated group, as the concentration of PEP1 was increased, the Smad2 and Smad4, as TGF- β biomarkers, were concentration-dependently inhibited.

[0079] Also, decreased expression of fibronectin, which is a TGF- β -related fibrosis gene, shown after 48-hour culture of PEP1 in the presence of TGF- β was assessed by comparing mRNA expression levels of fibronectin and GAPDH (refer to FIG. 11). Compared with the non-treated negative control, in the TGF- β treated group, fibronectin expression was increased. Meanwhile, in the positive control SB431542, compared with the TGF- β treated group, the PEP1 treated group showed fibronectin inhibition as the concentration of PEP1 was increased. The positive control (SB431542) showed an inhibition ratio of 60.7%, and PEP1 showed an inhibition ratio of 22.8% to 47.5% (refer to FIG. 12).

[0080] Such results can show that PEP1 has a direct effect of inhibiting the expression of Smad2 and Smad4, which are downstream genes involved in the TGF- β signaling mechanism, and inhibits the fibronectin expression induced by TGF- β and thus has a possibility as an agent for preventing and treating fibrosis.

Example 4: TGF- β inhibitory effect of PEP1 in radiation-exposed NHEK cell lines

[0081] To confirm PEP1 effects on fibrosis induced by radiation exposure for anticancer treatment and other tumor treatments, an experiment for confirming a TGF- β inhibitory effect of PEP1 in radiation-exposed NHEKs was performed.

Preparation of reagents and cell lines

[0082] PEP1 synthesized according to Example 1 and a placebo (sham) as a control were prepared. Normal human epidermal keratinocytes (NHEKs) were cultured in a monolayer to be used for a radiation exposure experiment. For the radiation exposure experiment, ionized radiation was used at an intensity of 6 Gy.

Experiment method

[0083] To examine the cell differentiation of NHEK cell lines according to radiation exposure, NHEKs were treated with each of the placebo and 1 mM of PEP1, and then divided into radiation-exposed groups and non-exposed groups, followed by culturing for 10 days (refer to FIG. 13).

[0084] Also, to examine the change in expression levels of biomarkers for the NHEK cell lines due to the radiation exposure, the NHEKs were treated with each of the placebo and 1 mM of PEP1 and divided into non-exposed groups (-) and radiation-exposed groups (+), and then expression levels of the biomarkers were examined at day 1 and day 10 after the radiation exposure (refer to FIG. 14). In measurement of the expression levels of the biomarkers, GAPDH was used as a reference control.

[0085] In addition, to exactly observe the inhibition of TGF- β signaling by PEP1 when the NHEKs were continuously cultured after the radiation exposure, the cells were divided into a group in which the radiation (6 Gy, IR)-exposed NHEKs were treated with a placebo (sham) and a 1 μ M PEP1-treated group and then cultured for 10 days. The expression levels of respective markers were observed by performing western blotting on the TGF- β and fibrosis-related biomarkers (refer to FIG. 15).

[0086] Western blotting procedures were as follows. Peptide-treated cells were washed with PBS twice, collected into a 1.5 mL centrifuge tube using a cell scraper, followed by centrifugation (1,000 rpm, 4 $^{\circ}$ C, 2 minutes). After removal of the obtained supernatant, 100 μ L of RIPA buffer was added to the NHEKs. The cells were cultured on ice for 40 minutes and vortexed every 10 minutes (micro-centrifuge was previously cooled at 4 $^{\circ}$ C), and then the sample was stirred using a 1 ml syringe 40 to 50 times. Finally, the sample was centrifuged at 13,000 rpm for 15 minutes to obtain a supernatant.

[0087] 30 μ g of protein was transferred to a PVDF membrane (Millipore) by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The PVDF membrane was blocked with 5% skim milk, and incubated with specific primary antibodies. The antibodies used in the experiment were as follows: fibronectin (285 kDa, 5% BSA 1:3000, ab2413, Abcam), N-Cadherin (140 kDa, 5% BSA 1:1000, #4061, Cell Signaling), Smad4 (70 kDa, 5% BSA 1:1000, #9515, Cell Signaling), Smad 2/3 (60, 52 kDa, 5% BSA 1:1000, #3102, Cell Signaling), P-Akt (60 kDa, 5% BSA 1:1000, #9271, Cell Signaling), pSmad 2/3 (Cell Signaling #3102), GRHL2 (Abcam #ab15532), GAPDH (37 kDa, 5% BSA 1:1000, #2118, Cell Signaling). Subsequently, the PVDF membrane was washed with tris-buffered saline containing 0.1% Tween-20 (TBST) and reacted with HRP-conjugated anti-rabbit antibodies (Jackson Immuno Research Laboratories, INC.). Afterward, ECL detection (Amersham Pharmacia Biotech) was performed, and obtained images were analyzed using

an image analyzer (GE Healthcare, ImageQuant LAS 4000).

Experimental results

5 **[0088]** In an experiment of cell differentiation in NHEK cell lines according to radiation exposure, after a radiation exposure, a control showed differentiation (fibrosis development), but the PEP1 treated group hardly showed differentiation even after the radiation exposure. These results show that the progression of fibrosis caused by the radiation exposure may be inhibited by PEP1 administration.

10 **[0089]** In an experiment for observing changes in expression levels of biomarkers in NHEK cell lines according to radiation exposure, levels of increased expression of biomarkers GRHL2, p63, and N-Cad 10 days after the radiation exposure were observed in each experiment group. This showed that the expression levels of the biomarkers GRHL2 and p63, which had TGF- β signaling inhibitory effects, were decreased in the placebo treated group and increased in the PEP1 treated group. Such results demonstrate that the expression of GRHL2 and p63, which have inhibitory effects on TGF- β signaling involved in fibrosis development after the radiation exposure, were increased by PEP1.

15 **[0090]** Meanwhile, the expression level of a biomarker N-Cad expressed in fibrotic tissue was increased in the placebo (sham) treated group and decreased in the PEP1 treated group. The expression level of P-Akt (phosphorylation of Akt (protein kinase B)), which is a biomarker indicating a degree of cell survival, was increased in the PEP1 treated group. These results demonstrate that not only can PEP1 inhibit fibrosis development by the radiation exposure, but it can also be involved in a cell survival mechanism.

20 **[0091]** From the results of the experiment, PEP1 raised the expression of GRHL2 and p63 to inhibit the TGF- β signaling process, and thus fibrosis was able to be inhibited by the TGF- β signaling caused by the radiation exposure. Therefore, it can be seen that PEP1 has a possibility as a drug for preventing or treating fibrosis caused by radiation exposure and fibrosis of tissue cells.

25 **Example 5: Verification of in vitro and in vivo radiation defensive effects and tissue fibrosis inhibitory effects of PEP1**

Preparation of reagents, cell lines and experimental animals

30 **[0092]** PEP1 synthesized according to Example 1 and a placebo (sham) used as a control were prepared. NHEKs were cultured in a monolayer to prepare an *in vitro* radiation exposure experiment. For *in vitro*, *in situ*, and *in vivo* radiation exposure experiments, ionized radiation was used at various intensities ranging from 0 to 10 Gy (0, 2, 4, 6, 8, and 10 Gy).

35 **[0093]** Meanwhile, experimental animals were divided into two animal models. The first model was an oral mucositis model, which was prepared by inducing an ulcer to the tongue of each C3H mouse by exposing the head and the neck to radiation (6 Gy, IR) for 5 straight days. The second model is a dermal fibrosis model, which was prepared by inducing local scleroderma by subcutaneously injecting 0.1 cc of a dilution of bleomycin which was known as the cause of pulmonary fibrosis in a 0.9% NaCl aqueous solution at a concentration of 0.5 mg/ml once a day for 24 to 28 days.

Experimental method and results

40 **[0094]** *In vitro* and *in situ* experiments were performed on NHEK cell lines for confirming a PEP1 defensive effect on radiation exposure damage and showed that, when NHEKs were divided into groups, each treated with a placebo or PEP1 and then exposed to radiation at various intensities ranging from 0 to 10 Gy (0, 2, 4, 6, 8, and 10 Gy), expression levels of a marker exhibiting radiation exposure damage represented by radiation-induced premature senescence (RIPS), a DNA damage response (DDR) protein, and markers for senescence-associated heterochromatin foci (SAHF) were confirmed. The expression level of each marker was measured using qRT-PCR, western blotting, and immunofluorescence staining. For the measurement of the levels, senescence-associated β -galactosidase (SA β -Gal), p16INK4A, p-p53Ser15, p-ATM, γ -H2AX, 53BP1, H3K9me3, HP1 γ , and HMGA2 were used as markers.

45 **[0095]** Meanwhile, to confirm a PEP1 inhibitory effect on tissue fibrosis induced by TGF- β or bleomycin treatment in NHEK cell lines through an *in vitro* experiment, NHEKs were treated with each of a placebo and PEP1 to give a final concentration of 10 μ g/ml, cultured, and treated with TGF- β and bleomycin every 48 hours, followed by measuring expression levels of fibronectin (FN) and collagen type 1 (Col 1 α 1) using qRT-PCR and western blotting. For the *in vivo* experiment, first, in the first oral mucositis model, C3H mice were divided into groups, each intraperitoneally treated with a placebo or PEP1 to give a final dose of 1 mg/kg and exposed to radiation (6 Gy, IR) for five consecutive days to induce a tongue ulcer corresponding to an oral mucositis. Ten days later, the mice were sacrificed to collect tongue tissue, which was stained with toluidine blue and by H&E staining, to perform biopsy. In addition, using a comparative control which was treated with rapamycin for 5 days at a dose of 5 mg/kg per day, an experiment for relatively examining PEP1 effects was performed.

[0096] Meanwhile, in the second dermal fibrosis model, C3H mice were divided into groups, each intraperitoneally treated with a placebo or PEP1 to give the final dose of 50 mg/kg, and subcutaneously treated with bleomycin at the concentrations mentioned in the preparation of the experimental animals for 24 to 28 days. 28 days later, the mice were sacrificed to detect scleroderma, followed by an experiment for examining fibrosis diffusion using Masson's trichrome staining.

[0097] According to the *in vitro* and *in vivo* animal model experiments, it can be seen that the PEP1 treatment exhibits a defensive effect on radiation exposure damage. Specifically, according to the *in vitro* experiment, it can be seen that the PEP1 has an RIPS inhibitory effect, which was indicated by inhibition of the marker expression, and according to the *in vivo* experiment, it can be seen that the PEP1 has defensive effects against exposure to radiation and induction of tissue fibrosis, which were indicated by biopsy.

[0098] According to the experiment examples, it can be seen that the PEP1 and the composition including the PEP1 according to the present invention have effects of preventing or treating senescence and diseases related to cellular fibrosis occurring in various regions due to various reasons including TGF-β signaling, tissue fibrosis caused by cancer, treatment with an anticancer agent, and radiation exposure. Therefore, it is concluded that a therapeutic agent for preventing or treating fibrosis-related senescence and diseases can be developed using PEP1 and a composition including the PEP1 according to the present invention.

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<120> A Peptide Possessing Fibrosis Inhibition Activity and the Composition Comprising the Same

<130> OF15P068PCT

<150> KR 10-2014-0043586

<151> 2014-04-11

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Claims

- 30 1. An anti-fibrosis composition for use in preventing or treating fibrosis, comprising:
at least one peptide effective in inhibiting fibrosis and selected from the group consisting of SEQ ID NO: 1 and a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution.
- 35 2. An anti-fibrosis composition for use according to claim 1, wherein the composition further comprises pharmaceutically acceptable excipients and additives, and is a pharmaceutical composition.
3. An anti-fibrosis composition for use according to claim 1, wherein the composition further comprises acceptable excipients, lubricants and additives, and is a cosmetic composition.
- 40 4. An anti-fibrosis composition for use according to any one of claims 1 to 3 in preventing or treating a fibrotic disease, comprising administering the anti-fibrosis composition to a subject requiring treatment.
- 45 5. An anti-fibrosis composition for use according to claim 4, wherein the fibrosis is induced by at least one selected from the group consisting of cancer, administration of an anticancer agent, and exposure to radiation.
- 50 6. An anti-fibrosis composition for use according to claim 5, wherein the composition inhibits fibrosis of tissue of a cancer cell selected from the group consisting of pancreatic cancer, colorectal cancer, stomach cancer, prostate cancer, non-small cell lung cancer, breast cancer, melanoma, and ovarian cancer.
- 55 7. An anti-fibrosis composition for use according to claim 5, wherein the composition is for use in combination with a chemotherapeutic agent or a radiation therapy to inhibit fibrosis of tissue of cancer cell.
8. An anti-fibrosis composition for use according to claim 7, wherein the chemotherapeutic agent is at least one selected from deoxynucleoside analogs and fluoropyrimidines.
9. An anti-fibrosis composition for use according to claim 8, wherein the deoxynucleoside analog is gemcitabine, and the fluoropyrimidine is 5-fluorouracil or capecitabine.

10. An anti-fibrosis composition for use according to claim 4, wherein the composition is involved in a TGF- β signaling process to inhibit fibrosis of dermal tissue.
11. A peptide for use according to any one of claims 1 to 10, wherein the peptide is effective in inhibiting fibrosis and is selected from the group consisting of SEQ ID NO: 1 and a variant thereof in which one, two or three amino acids are modified, wherein, in the variant, the amino acids are modified by conservative amino acid substitution.

Patentansprüche

1. Fibrosehemmende Zusammensetzung zur Verwendung bei der Vorbeugung oder Behandlung von Fibrose, umfassend:
mindestens ein Peptid, das bei der Hemmung von Fibrose wirksam ist, und ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO: 1, und eine Variante davon, bei der eine, zwei oder drei Aminosäuren modifiziert sind, wobei die Aminosäuren in der Variante durch konservative Aminosäuresubstitution modifiziert sind.
2. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 1, wobei die Zusammensetzung ferner pharmazeutisch unbedenkliche Exzipienten und Additive umfasst und eine pharmazeutische Zusammensetzung ist.
3. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 1, wobei die Zusammensetzung ferner unbedenkliche Exzipienten, Gleitmittel und Additive umfasst und eine kosmetische Zusammensetzung ist.
4. Fibrosehemmende Zusammensetzung zur Verwendung nach einem der Ansprüche 1 bis 3 bei der Vorbeugung oder Behandlung einer fibrotischen Erkrankung, umfassend das Verabreichen der fibrosehemmenden Zusammensetzung an ein Subjekt, das der Behandlung bedarf.
5. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 4, wobei die Fibrose durch mindestens eines ausgewählt aus der Gruppe bestehend aus Krebs, Verabreichung eines Antikrebsmittels und Bestrahlung induziert wird.
6. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 5, wobei die Zusammensetzung Fibrose von Gewebe einer Krebszelle hemmt, ausgewählt aus der Gruppe bestehend aus Bauchspeicheldrüsenkrebs, Kolorektalkrebs, Magenkrebs, Prostatakrebs, nichtkleinzelligem Lungenkrebs, Brustkrebs, Melanom und Eierstockkrebs.
7. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 5, wobei die Zusammensetzung zur Verwendung in Kombination mit einem chemotherapeutischen Mittel oder einer Strahlentherapie vorgesehen ist, um die Fibrose des Gewebes einer Krebszelle zu hemmen.
8. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 7, wobei das chemotherapeutische Mittel mindestens ausgewählt ist aus Desoxynukleosidanaloga und Fluoropyrimidinen.
9. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 8, wobei das Desoxynukleosidanalogen Gemcitabin ist und das Fluoropyrimidin 5-Fluorouracil oder Capecitabin ist.
10. Fibrosehemmende Zusammensetzung zur Verwendung nach Anspruch 4, wobei die Zusammensetzung an einem TGF- β -Signalisierungsprozess beteiligt ist, um die Fibrose von Hautgewebe zu hemmen.
11. Peptid zur Verwendung nach einem der Ansprüche 1 bis 10, wobei das Peptid bei der Hemmung von Fibrose wirksam ist, und ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO: 1, und eine Variante davon, bei der eine, zwei oder drei Aminosäuren modifiziert sind, wobei die Aminosäuren in der Variante durch konservative Aminosäuresubstitution modifiziert sind.

Revendications

1. Composition anti-fibrose pour son utilisation dans la prévention ou le traitement de la fibrose, comprenant :

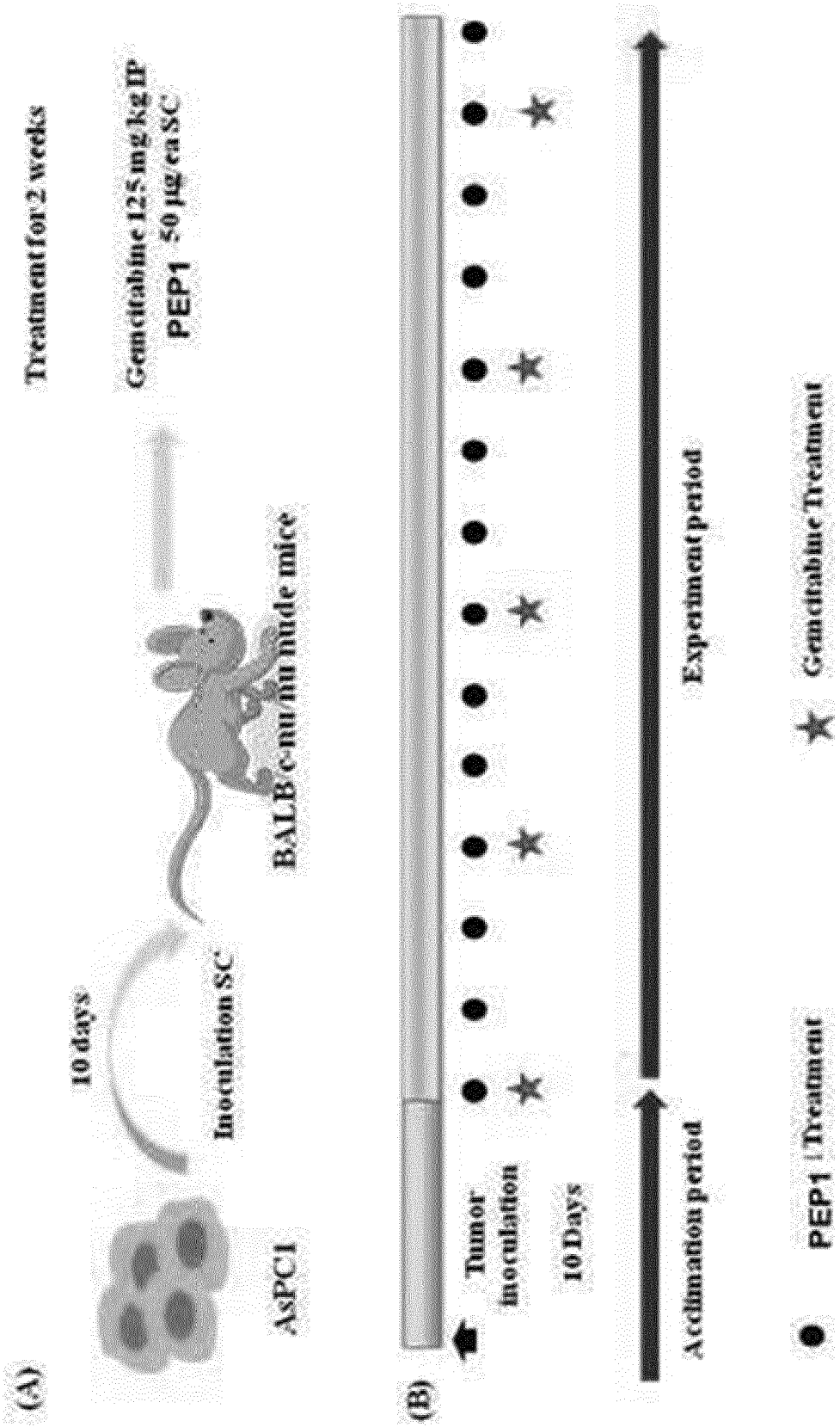
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au moins un peptide efficace pour inhiber la fibrose et sélectionné dans le groupe consistant en SEQ ID NO : 1 et une variante de celle-ci dans lesquelles un, deux ou trois acides aminés sont modifiés, dans laquelle dans le variant, les acides aminés sont modifiés par substitution d'acides aminés conservative.

- 5
2. Composition anti-fibrose pour son utilisation selon la revendication 1, dans laquelle la composition comprend en outre des excipients et additifs pharmaceutiquement acceptables, et est une composition pharmaceutique.
- 10
3. Composition anti-fibrose pour son utilisation selon la revendication 1, dans laquelle la composition comprend en outre des excipients, des lubrifiants et des additifs acceptables, et est une composition cosmétique.
- 15
4. Composition anti-fibrose pour son utilisation selon l'une quelconque des revendications 1 à 3 dans la prévention ou le traitement d'une maladie fibrotique, comprenant l'administration de la composition anti-fibrose à un sujet nécessitant un traitement.
- 20
5. Composition anti-fibrose pour son utilisation selon la revendication 4, dans laquelle la fibrose est induite par au moins un sélectionné dans le groupe consistant en un cancer, l'administration d'un agent anticancéreux et l'exposition à un rayonnement.
- 25
6. Composition anti-fibrose pour son utilisation selon la revendication 5, dans laquelle la composition inhibe la fibrose tissulaire d'une cellule cancéreuse sélectionnée dans le groupe consistant en un cancer pancréatique, un cancer colorectal, un cancer de l'estomac, un cancer de la prostate, un cancer du poumon non à petites cellules, un cancer du sein, un mélanome, et un cancer de l'ovaire.
- 30
7. Composition anti-fibrose pour son utilisation selon la revendication 5, dans laquelle la composition est destinée à être utilisée en combinaison avec un agent chimiothérapeutique ou une radiothérapie pour inhiber une fibrose tissulaire d'une cellule cancéreuse.
- 35
8. Composition anti-fibrose pour son utilisation selon la revendication 7, dans laquelle l'agent chimiothérapeutique est au moins un sélectionné parmi les analogues désoxynucléosidiques et les fluoropyrimidines.
- 40
9. Composition anti-fibrose pour son utilisation selon la revendication 8, dans laquelle l'analogue désoxynucléosidique est la gemcitabine, et la fluoropyrimidine est le 5-fluorouracil ou la capécitabine.
- 45
10. Composition anti-fibrose pour son utilisation selon la revendication 4, dans laquelle la composition est impliquée dans un processus de signalisation du TGF- β pour inhiber la fibrose d'un tissu dermique.
- 50
- 55
11. Peptide pour son utilisation selon l'une quelconque des revendications 1 à 10, dans lequel le peptide est efficace pour inhiber la fibrose et est sélectionné dans le groupe consistant en SEQ ID NO : 1 et une variante de celui-ci dans lesquelles un, deux ou trois acides aminés sont modifiés, dans lequel, dans le variant, les acides aminés sont modifiés par une substitution d'acides aminés conservative.

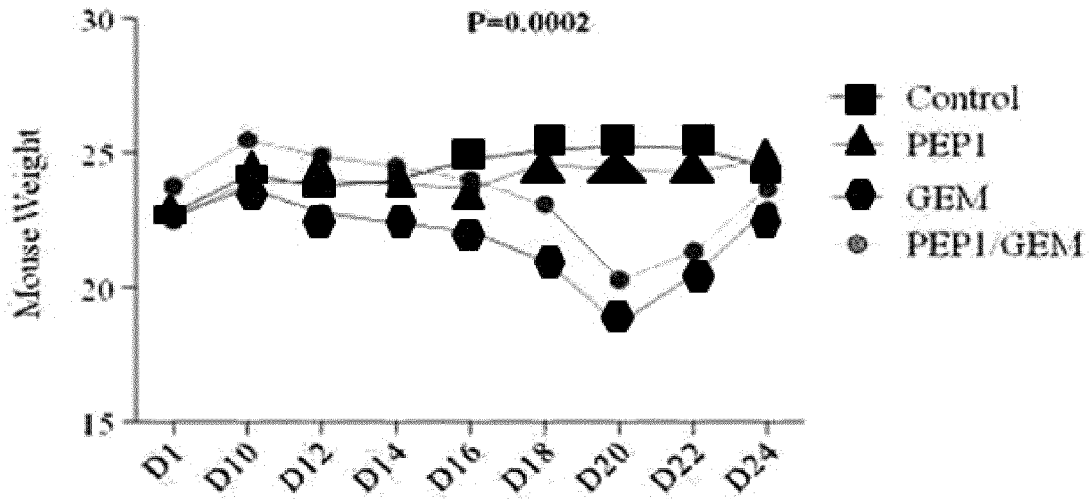
[Fig. 1]

Treatment protocol in PDAC xenograft tumor model



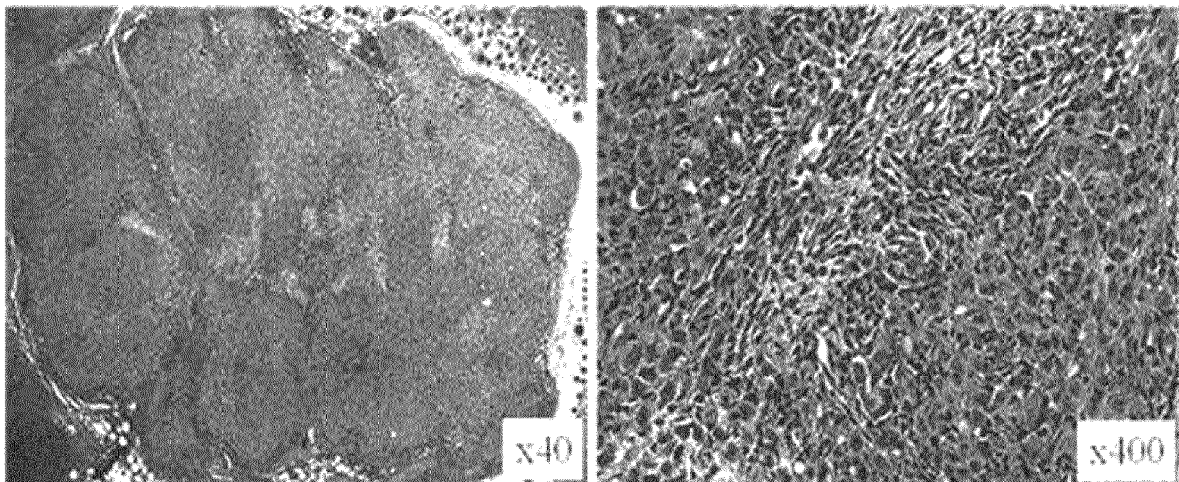
[Fig. 2]

***In vivo* anti-tumor effects of PEP1 combined with Gemcitabine:
Weight Changes (AsPC1)**



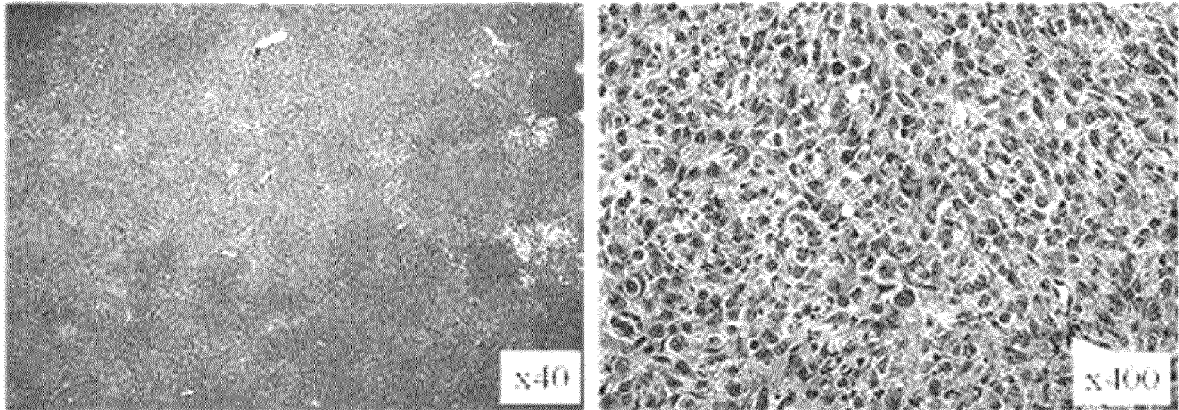
[Fig. 3]

ASPC1, Control (without Gemcitabine & PEP1)



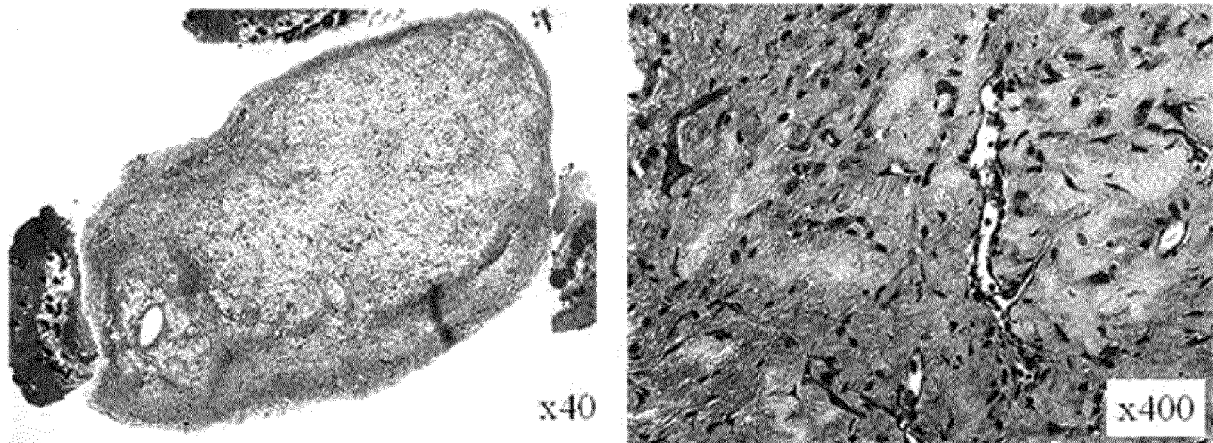
【Fig. 4】

ASPCI + PEPI



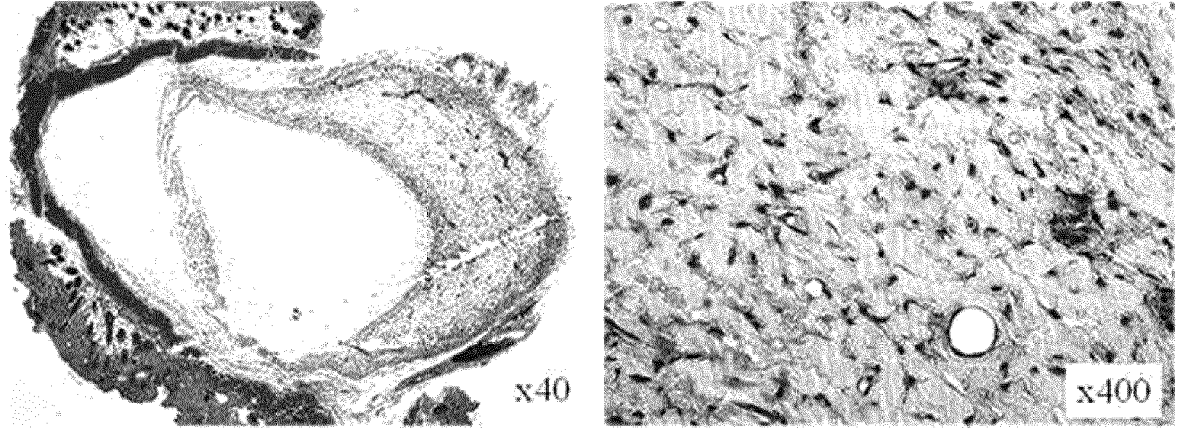
【Fig. 5】

ASPCI + Gemcitabine

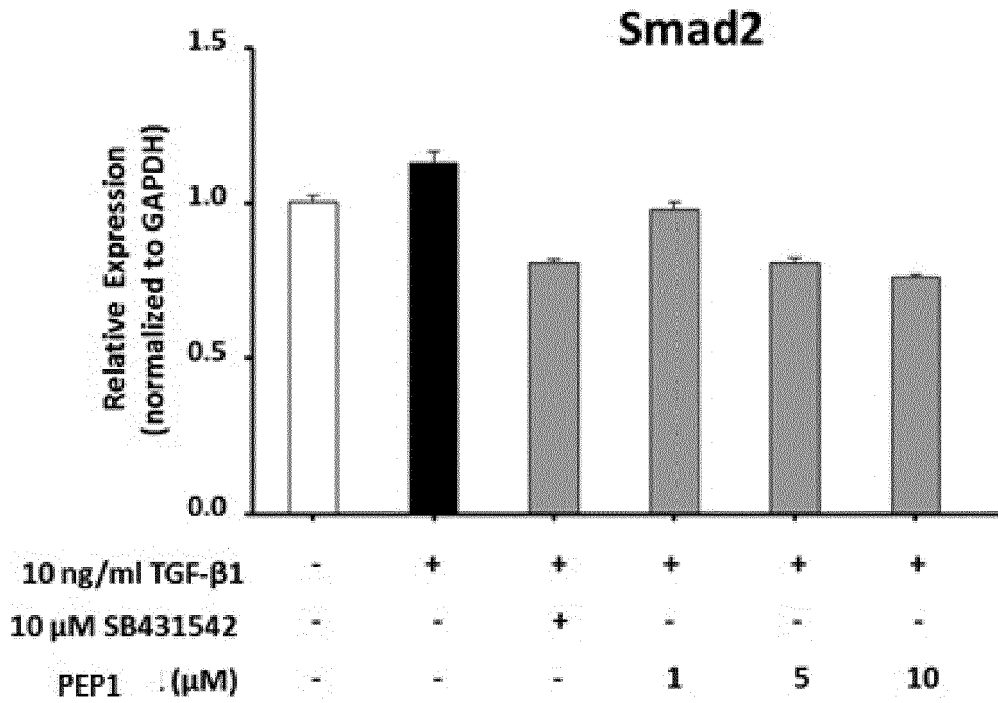


【Fig. 6】

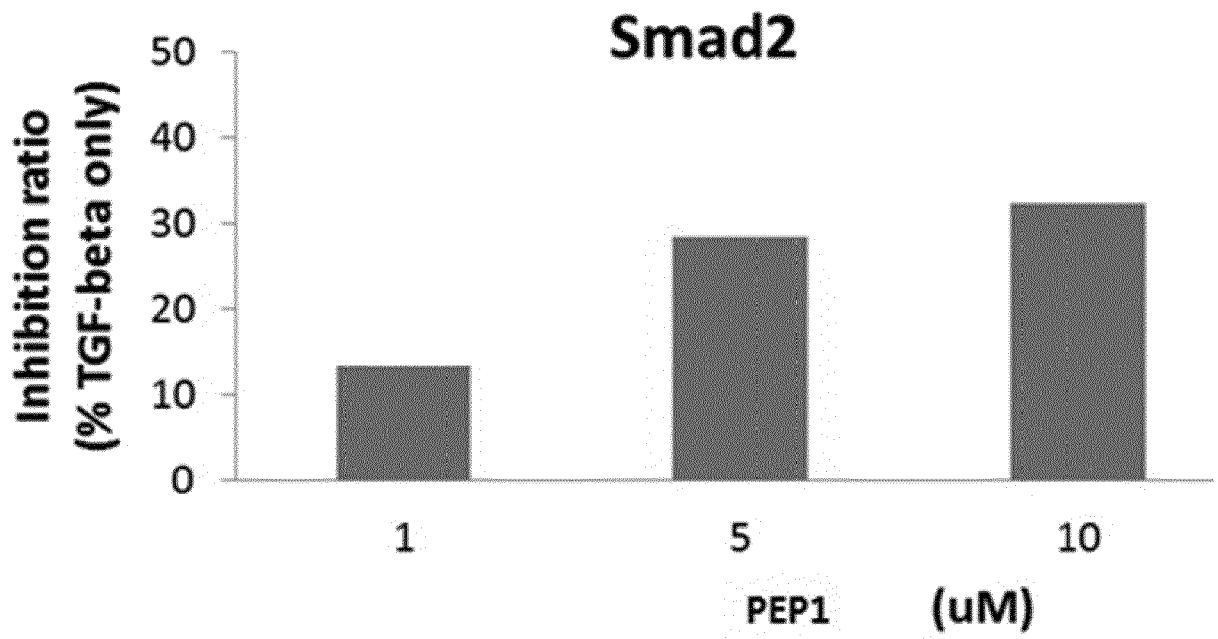
ASPC1 + Gemcitabine + PEP1



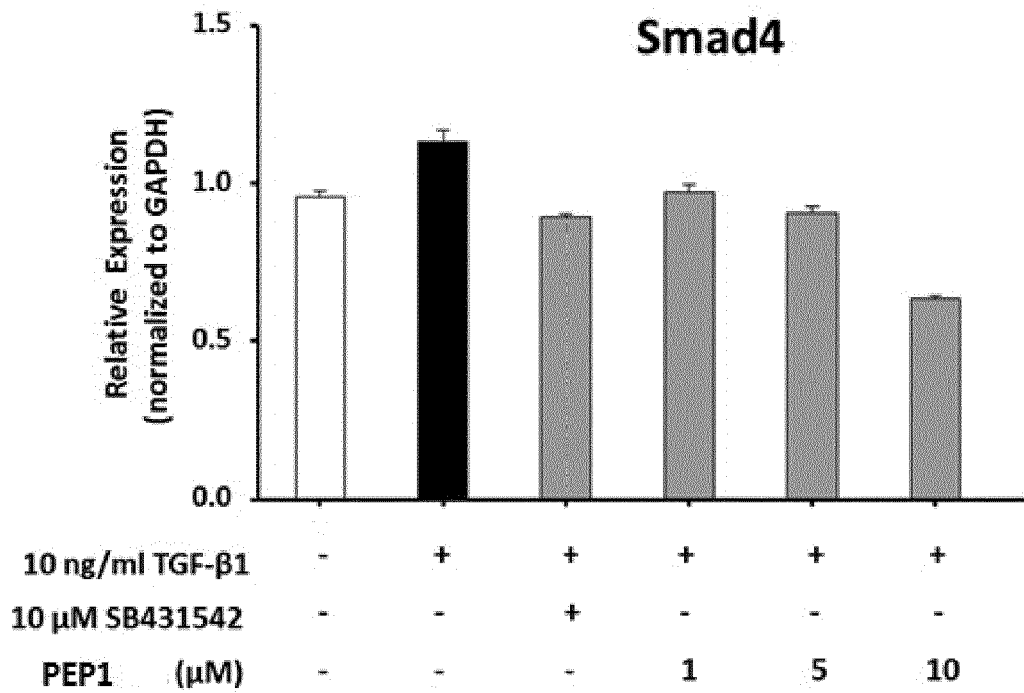
【Fig. 7】



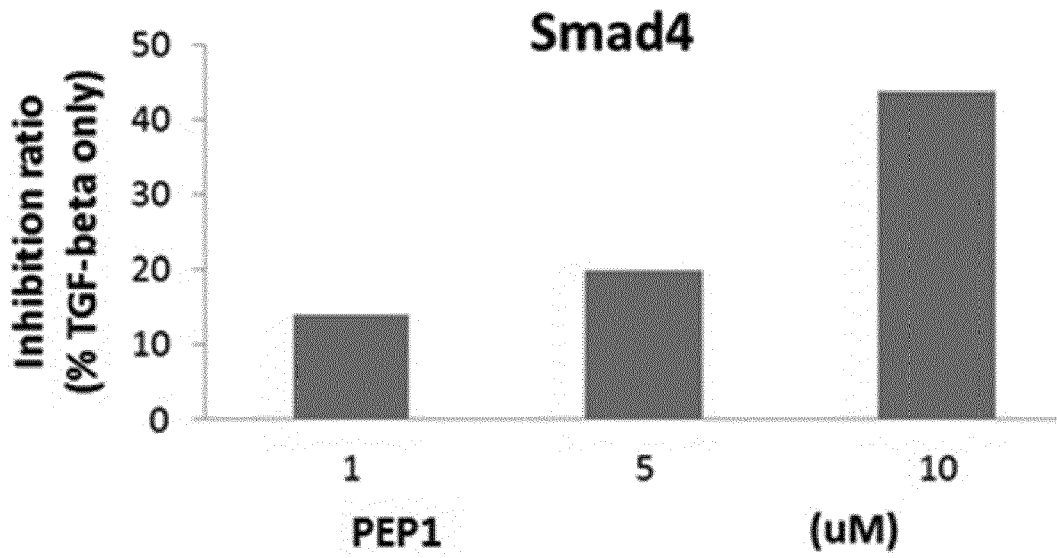
【Fig. 8】



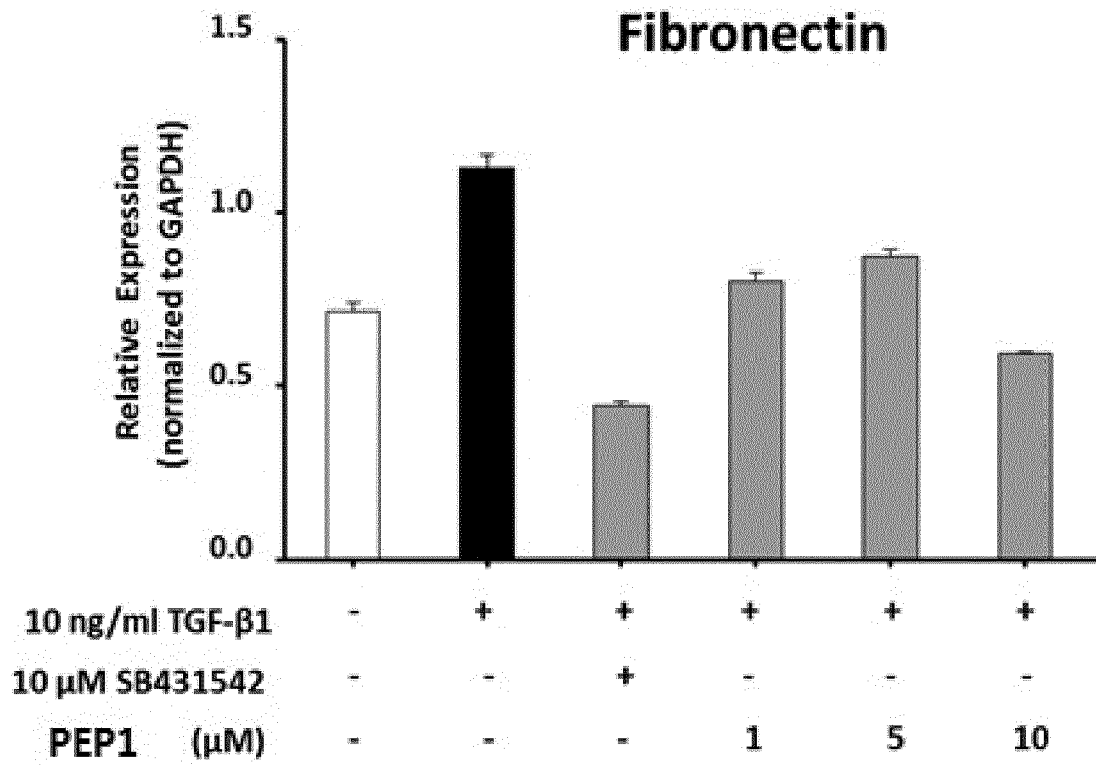
【Fig. 9】



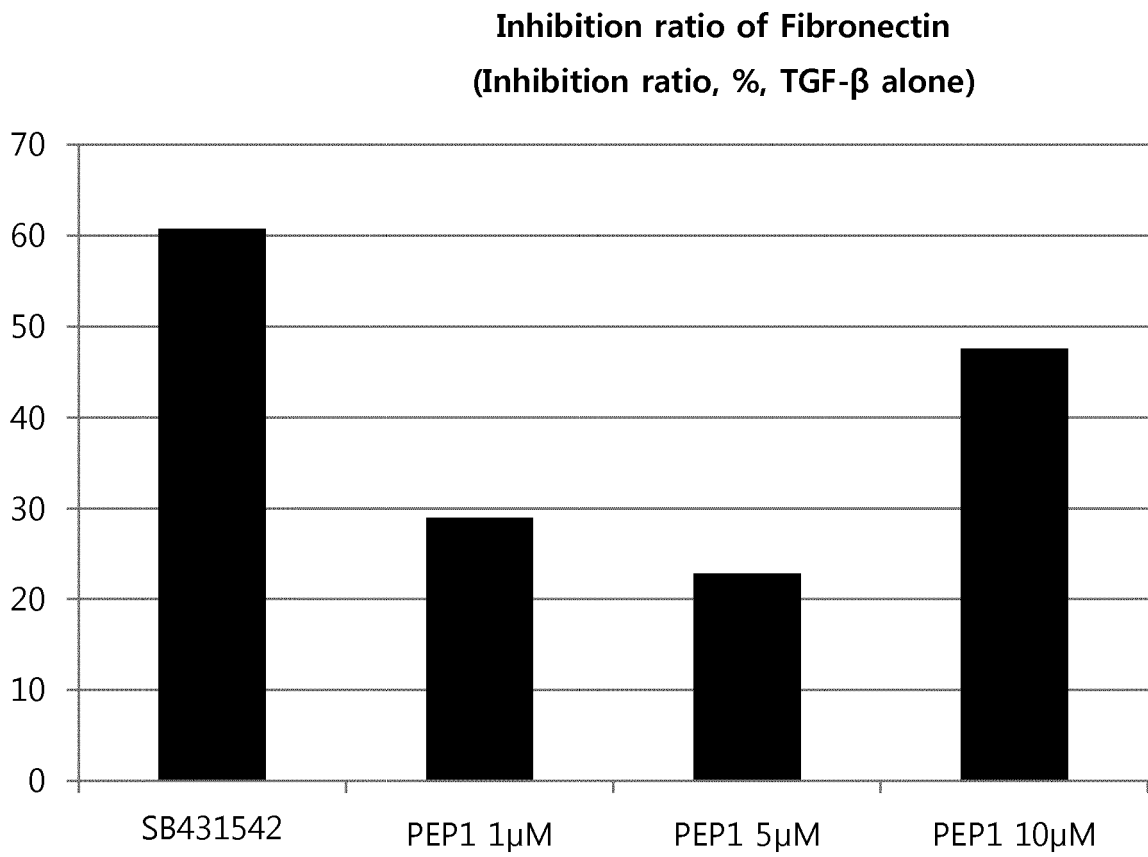
【Fig. 10】



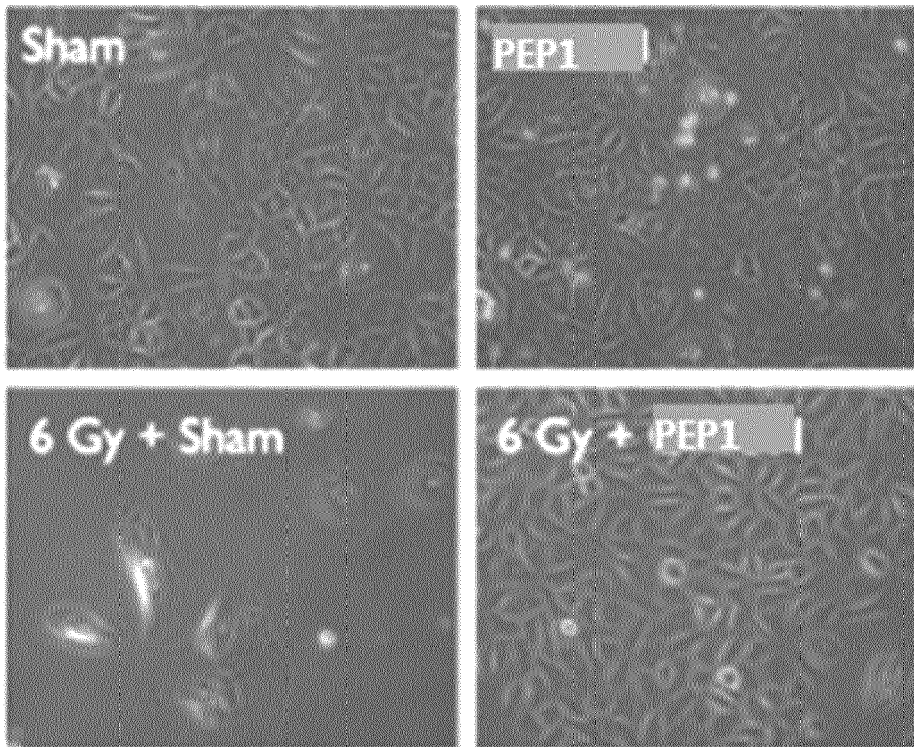
【Fig. 11】



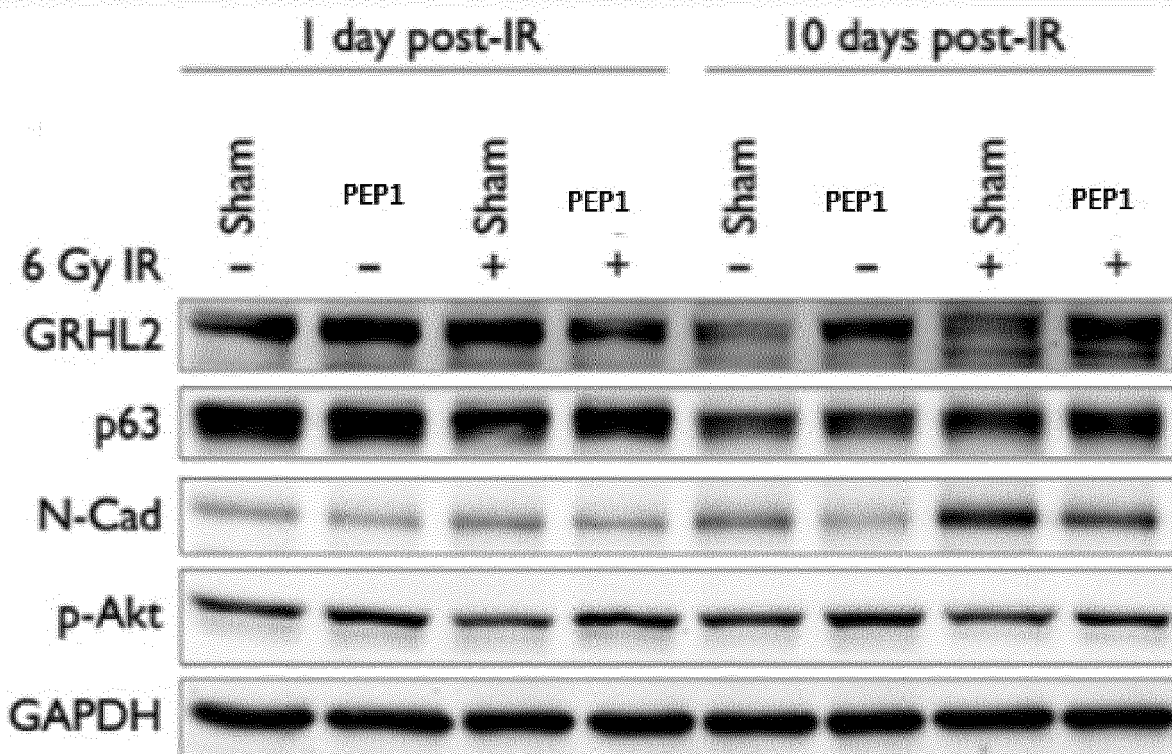
【Fig. 12】



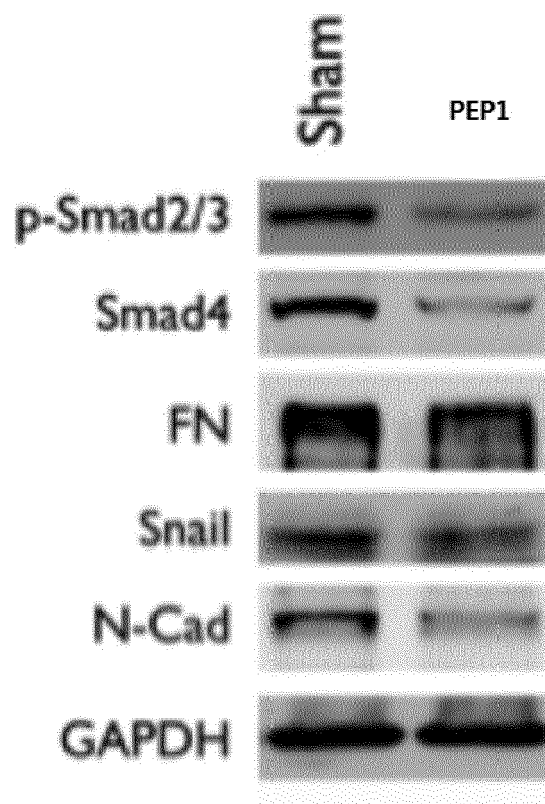
【Fig. 13】



【Fig. 14】



【Fig. 15】



REFERENCES CITED IN THE DESCRIPTION

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