

PEPTIDES ENDOWED WITH ANGIOGENIC ACTIVITY

FIELD OF THE INVENTION

The present invention relates to peptides endowed with angiogenic activity, thereby inducing proliferation, migration, and capillary-like tube formation in human endothelial
5 cells (human ECs). The angiogenic peptides can be used for preventing and/or treating conditions associated to deficient angiogenesis or where induction of angiogenesis is beneficial.

STATE OF THE ART

Angiogenesis plays a central role in various physiological processes within the human
10 body. Recent evidence has shown that a number of pathological conditions are caused by deficient angiogenesis or benefit from stimulation of angiogenesis. In these conditions, the administration of angiogenic active molecules has shown to be a valid therapeutic approach.

A number of studies have demonstrated that angiogenic active molecules are useful in the treatment of ulcers, in particular, gastric and duodenal ulcers, coronary artery diseases,
15 ischemia, in particular cerebral and miocardial ischemia, cerebrovascular diseases and acute and chronic cutaneous wounds.

In these pathological conditions, the administration of molecules endowed with angiogenic activity improves tissue perfusion, delivers survival factors to sites of tissue repair, mobilize regenerative stem cell populations, and ultimately restores form and function
20 to the tissue.

For example, in phase I clinical trials with basic Fibroblast Growth Factor (bFGF), a potent angiogenic factor, in patients with gastric or duodenal ulcers that were refractory to conventional therapy was found to heal gastric ulcers that were caused by nonsteroidal anti-inflammatory drugs (Hull et al, Gut 1995, (37) 5, 610–612).

Angiogenic agents have also been shown to be useful in ischemic cardiovascular disease, inducing cardiac tissue repair and regeneration. Although more than 2,000 patients with heart disease have received some form of experimental angiogenic therapy, currently there are no FDA-approved angiogenic drugs to treat ischemic cardiovascular disease (Vale et al, Revista Espanola de Cardiologia, 2001, (54) 10: 1210–1224; Simons et al, Circulation,
25 2000, (102) 11: E73–E86).
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Angiogenic active molecules may also find application in inducing bone regeneration, dental pulp regeneration, embryo implant and fetus persistence in pregnancy.

Vasculogenesis is a pivotal procedure during dental implant osseointegration as well as bone repair process.

For example, vascular endothelial growth factor (VEGF), a signal protein that stimulates angiogenesis and vasculogenesis, has been used in tissue regeneration and pulp regeneration experimental models favoring endodontic regenerative procedures (Mamatha Yadlapati, et al., J Endod., Volume 43, Number 1, Pages 77-83, January 2017). Also, VEGF has been demonstrated to play a central role in bone repair (Meng kai Guang, et al., Journal of Oral Science, Vol. 59, No. 2, 215-223, 2017).

In addition, angiogenesis has been demonstrated to be important during pregnancy for both implantation and normal intrauterine development of the fetus.

For example, it has been demonstrated that angiogenesis induced by endometrial RAS plays a role around the time of embryo implantation, affecting subsequent pregnancy outcomes (Ruofan Qi, et al., Ther Adv Endocrinol Metab, 2020, Vol. 11: 1–12) and stimulation of angiogenesis and vasculogenesis by hCG contributes to provide the placenta with an adequate maternal blood supply and optimal embryo nutrition during the invasion of the uterine endometrium (Grیدهlet V, et al., Front. Immunol., March 2020, Vol. 11, Article 343).

The human immunodeficiency virus type 1 (HIV-1) matrix protein variant p17 (p17) is a 132 amino acids (aa)-long structural multi-target protein endowed with different biologically activities and display, differently from the wild-type protein (refp17), B cell growth-promoting activity.

It plays a crucial role in both early and late stages of the virus life cycle.

The biological activity of p17 occurs after binding to heparan sulfate proteoglycans (De Francesco et al, J Biol Chem, 2011, 286: 19541-19548; Bugatti et al, J Biol Chem, 2013, 288: 1150-1161), to CXCR1 and CXCR2 (Caccuri et al PNAS, 2012, 109: 14580-14585; Giagulli et al, Blood, 2012, 119: 2274-2283), and to many other still unknown receptors (He et al, BBA Gen Sub, 2019, 1863: 13-24; Liu et al, Lab Invest, 2019, 99: 180-190).

Recently, p17 was found to exert a potent angiogenic and lymphangiogenic activity upon stress condition mediated by interaction with the CXCR1 and CXCR2 receptors (Caccuri et al, PNAS, 2012, 109: 14580-14585; Caccuri et al, ATVB, 2014; 34: 846-856). All p17 activities on human endothelial cells were sustained by activation of a secretory autophagy-based pathway (Mazzuca et al, J Virol, 2017, 91, pii: e00801-17).

In He W et al., Biochim Biophys Acta Gen Subj. 2019; 1863:13-24, in order to identify the epitopes of p17 responsible for interaction with receptors and B cell growth, eight peptides corresponding to 20 amino acid sequences of p17 were tested for both their ability to bind to

the p17 receptors and inducing B cell growth. Peptide F1, corresponding to aminoacids 2 to 21 of p17 (SEQ ID No. 1), was found to be the only one endowed with cell growth promoting activity, while Peptide F2, corresponding to aminoacids 17-36 of p17 (SEQ ID No. 2), was found capable to bind p17 receptors such as heparin and CXCR1 and CXCR2 receptors, and therefore to contain the sequence of p17 mediating the binding of the protein to these receptors. No biological activity was studied or suggested for Peptide F2.

SUMMARY OF THE INVENTION

The present inventors have now surprisingly found a number of different peptides derived from p17 which are capable of exerting angiogenic activity on endothelial cells.

More in particular, the inventors have surprisingly found that the peptides having SEQ ID No. 2 and SEQ ID No. 3, corresponding to Peptides F2 and F3 described in He W. et al. cited above, have a biological activity relevant for therapeutic purposes. In details, as will be shown in the experimental section, these peptides are endowed with a potent angiogenic activity on human umbilical vein endothelial cells (HUVECs) and other endothelial cells of different origin, such as aorta (HAECs), lung (HMVEC-Ls) and lymph node (LN-LECs).

Further, the inventors have also surprisingly found that peptides having SEQ ID No. 2 and SEQ ID No. 3 promote endothelial cells migration in a wound healing assay and have angiogenic activity either *ex vivo*, by using an aortic ring assay and *in vivo*, by using the chick chorioallantoic membrane (CAM) assay.

The inventors have also found that SEQ ID No. 2 and SEQ ID No. 3 angiogenic activity is mediated by different pathways.

More in particular, peptide having SEQ ID No. 2 exerts this activity by binding and activating chemokine receptors CXCR1 and CXCR2 and under stressed condition only, while peptide having SEQ ID No. 3 exerts its activity without interaction with CXCR1 and CXCR2 under both stressed and normal cell culture conditions.

Furthermore, the angiogenic activity involves an autophagy-based pathway only in case of peptide of SEQ ID No. 2, while activity of peptide of SEQ ID No. 3 is independent from autophagy.

By further analysis of the activity of the above peptides, the present inventors also identified further peptides, having sequences of SEQ ID No. 9; SEQ ID No. 13; SEQ ID No. 14; having a partial sequence of the peptide of SEQ ID No. 3 and maintaining a potent angiogenic activity.

Finally, sequence alignment between peptide of SEQ ID No. 3 and human erythropoietin (EPO) or Simian Immunodeficiency Virus (SIV) of chimpanzee (SIVcpz) and gorillas (SIVgor) allowed to identify an EPO-derived peptide of SEQ ID No. 10, SIVcpz-derived peptides of SEQ ID No. 15, SEQ ID No. 16 and SEQ ID No. 17, and
5 SIVgor-derived peptide of SEQ ID No. 18, having sequence similarity to the peptide of SEQ ID No. 3, and also endowed with a potent angiogenic activity.

Accordingly, a first aspect of the present invention relates to a peptide, or derivative thereof, having length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof, for use
10 as a medicament.

Preferably, the above peptide is for use in the treatment of conditions associated to/caused by deficient angiogenesis or conditions benefiting from increased angiogenesis.

A second aspect of the present invention relates to a pharmaceutical composition
15 comprising (i) a peptide having length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a derivative thereof, and (ii) at least one pharmaceutically acceptable ingredient.

A third aspect of the present invention relates to a therapeutic method for treating or preventing angiogenesis-related conditions in a subject in need thereof, comprising the
20 systemic administration of a pharmaceutical composition comprising (i) a peptide having length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a derivative thereof, and (ii) at least one pharmaceutically acceptable ingredient.

A fourth aspect of the present invention relates to a polynucleotide having a sequence
25 that codes for a peptide having length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, or 18.

A fifth aspect of the present invention relates to a peptide having length equal to or lower than 30 amino acids, and comprising the SEQ ID No. 9, 10, 13, 14, 15, 16, 17, or 18.

30 **BRIEF DESCRIPTION OF THE FIGURES**

The embodiments of the present invention will now be described, by way of example, with reference to the accompanying drawings, wherein:

Figure 1 shows the amino acid sequence of p17 protein as well as of the eight p-17 derived peptides F1 to F8,

Figure 2 shows the graphical representation of the results of the angiogenic activity assay on HUVECs as described in example 2,

Figure 3 shows the graphical representation of the results of the angiogenic activity assay on HAECs as described in example 2,

5 Figure 4 shows the graphical representation of the results of the angiogenic activity assay on HMVEC-Ls as described in example 2,

Figure 5 shows the graphical representation of the results of the angiogenic activity assay on LN-LECs as described in example 2,

10 Figure 6 shows the graphical representation of the results of the wound-healing assay on HUVECs as described in example 3,

Figure 7 shows the graphical representation of the results of the aortic ring assay as described in example 4,

Figure 8 shows the graphical representation of the results of the chick chorioallantoic membrane (CAM) assay as described in example 5,

15 Figure 9 shows the graphical representation of the results of the angiogenic activity assay on HUVECs with CXCR1 and CXCR2 inhibition as described in example 6,

Figure 10 shows the graphical representation of the results of the angiogenic activity assay on HUVECs under normal (A) and stressed (B) conditions as described in example 7,

20 Figure 11 shows the graphical representation of the results of the angiogenic activity assay promoted by EPO at various concentrations on HUVECs under normal (A) and stressed (B) conditions as described in example 8,

25 Figure 12 shows the graphical representation of the results of the angiogenic activity assay promoted by F3S and EPO peptide on HUVECs under normal (A) and stressed (B) conditions as described in example 8,

Figure 13 shows the graphical representation of the results of the angiogenic activity assay on HUVECs with the peptides ASRELERF (SEQ ID No 13) and ASRELERFLLE (SEQ ID No 14), conducted as described in example 2,

30 Figure 14 shows the graphical representation of the results of the angiogenic activity assay on HUVECs with the peptides AANELDRF (SEQ ID No 16) and AANELDRFLLE (SEQ ID No 15), conducted as described in example 2,

Figure 15 shows the graphical representation of the results of the angiogenic activity assay on HUVECs conducted as described in example 2 with the peptide gor

(ASRELERFACNPELLE - SEQ ID No. 17) and the peptide cpz (ASRELERFACNPGLME - SEQ ID No. 18), in comparison with peptide F3S (ASRELERFAVNPGLLE - SEQ ID No. 9),

5 Figure 16 shows the graphical representation of the results of the wound healing assay performed in vivo on spontaneously diabetic (db/db) mice (BKS.Cg-m Dock7^m +/- Lepr^{db} /J mice) as described in example 9 with the EPO peptide (DSRVLERYLLE – SEQ ID No. 10).

DETAILED DESCRIPTION OF THE INVENTION

10 In a first aspect, the invention relates to a peptide, or derivative thereof, having an amino acid sequence with a length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof, for use as medicament.

15 As used herein, the expression “peptide(s) having a sequence...” or “peptide(s) having an amino acid sequence...” refers to peptide(s) with exactly the sequence described thereafter. Thus, this expression does not include peptide(s) with an amino acid sequence comprising the sequence described thereafter together with other sequences. For example, the expression “peptide(s) having an amino acid sequence with a length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof” refers to peptide(s) with a sequence
20 length of 30 amino acids or less and comprising one of the claimed sequences.

Preferably, the above peptide is for use in the prevention or treatment in an individual of conditions associated to/caused by deficient angiogenesis or conditions that benefit from increased angiogenesis.

25 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 2 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 2.

30 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 3 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 3.

35 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 9 have an amino acid sequence with a length preferably equal to or lower than 30

amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No 9.

5 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 10 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 10.

10 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 13 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 13.

15 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 14 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 14.

20 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 15 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 15.

25 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 16 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 16.

30 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 17 have an amino acid sequence with a length preferably equal to or lower than 30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 17.

35 Peptides according to the several aspects of the present invention comprising the SEQ ID No. 18 have an amino acid sequence with a length preferably equal to or lower than

30 amino acids, more preferably equal to or lower than 25 amino acids, and still more preferably equal to or lower than 20 amino acids, even more preferably they have a sequence consisting of SEQ ID No. 18.

Abbreviations of the amino acid sequences used herein are in accordance with the IUPAC-IUB nomenclature as reported in the following Table A.

Table A

Alanine	Ala	A	Arginine	Arg	R
Asparagine	Asn	N	Aspartic acid	Asp	D
Cysteine	Cys	C	Glutamic acid	Glu	E
Glutamine	Gln	Q	Glycine	Gly	G
Histidine	His	H	Isoleucine	Ile	I
Leucine	Leu	L	Lysine	Lys	K
Methionine	Met	M	Phenylalanine	Phe	F
Proline	Pro	P	Serine	Ser	S
Threonine	Thr	T	Tryptophan	Trp	W
Tyrosine	Tyr	Y	Valine	Val	V

The term “conservative variant” as employed herein in relation to the amino acid sequence of the peptides according to the invention, means a sequence that differs from said amino acid sequence of the peptides for a conservative substitution of amino acids that do not influence the final peptide activity. The substitutions able to maintain the peptide activity are selected on the basis of (a) the efficacy in maintaining the structure of the peptide backbone in the area of substitution, such as sheet or helical three-dimensional structures, (b) the efficacy in maintaining electrical charge or hydrophobicity of the molecule in the target area, or (c) the efficacy in maintaining the bulk of the side chain.

Amino acids are classified according to general side chain properties as described in the following Table B.

Table B

Hydrophobicity	NorLeucine, Met, Ala, Val, Leu, Ile;
neutral hydrophobicity	Cys, Ser, Thr;

Acidity	Asp, Glu;
Basicity	Asn, Gln, His, Lys, Arg;
residue that affects chain orientation	Gly, Pro;
Aromaticity	Trp, Tyr, Phe.

Examples of conservative substitution belong to the group consisting of basic amino acids (arginine, lysine and histidine), acidic amino acids (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).

The amino acid substitutions that do not generally alter the specific activity are known in the art of the present invention.

Most common occurred alterations are 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, Asp/Gly, and the opposite alterations. Other examples of conservative substitutions are shown in the following Table C.

TABLE C

Starting amino acid	Possible substitution	Preferred 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
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

The peptide according to the several aspects of the present invention may be in the form of a modified peptide, wherein the N- or/and C-terminal thereof is chemically modified or protected with organic compounds.

- 5 The term “derivative” or “derivative thereof” as employed herein in relation to a peptide according to the several aspects of the present invention means a peptide wherein the N- and/or C-terminal thereof is chemically modified or protected with an organic compound.

- 10 Examples of modification include phosphorylation, glycosylation, acylation (including acetylation, lauroylation, myristoylation, palmitoylation), alkylation, carboxylation, hydroxylation, glycation, biotinylatlon, ubiquitinylation, and amidation.

- 15 Preferably, the peptide according to the several aspects of the present invention may be modified at the N-terminal thereof, more preferably by acylation, including acetylation, lauroylation, myristoylation, and palmitoylation. N-terminal acetyl and palmitoyl peptide derivatives are a preferred aspect of the present invention.

- 20 Depending on its length, the peptide according to the several aspects of the present invention may be synthesized by a method well known in the art, for example, by an automated peptide synthesizer, or produced by a genetic engineering technology. For example, a fusion gene encoding a fusion protein including a fusion partner and the peptide is prepared by genetic engineering, and then transformed into a host cell to

express the fusion protein. Thereafter, the peptide is cleaved and isolated from the fusion protein using a protease or a compound so as to produce the desired peptide. To this end, a DNA sequence encoding amino acid residues which can be cleaved by a protease such as Factor Xa or enterokinase, or a compound such as CNBr or hydroxylamine may
5 be inserted between the polynucleotides encoding the fusion partner and the peptide.

The peptides according to the several aspects of the present invention may exist as stereoisomers or mixtures of stereoisomers; for example, the amino acids that make them up can have L-configuration, D-configuration or be racemic independently from each other. Therefore, it is possible to obtain isomeric mixtures as well as racemates or
10 diastereomeric mixtures or pure diastereomers or enantiomers, depending on the number of asymmetric carbons and what isomers or isomeric mixtures are present. The preferred structures of the peptides are pure isomers, i.e., enantiomers or diastereomers. The most preferred structures of the peptides include amino acids having the L-configuration. Unless otherwise indicated, it is understood that when it is indicated that one amino acid
15 can be Ala, it is understood that it is selected from L-Ala-, D-Ala- or racemic or non-racemic mixtures of both.

As used herein, the term, "angiogenesis" refers to the growth of new blood vessels, or "neovascularization," and involves the growth of new blood vessels of relatively small caliber composed of endothelial cells. Angiogenesis is an integral part of many important
20 biological processes including cancer cell proliferation, solid tumor formation, inflammation, wound healing, repair of injured ischemic tissue, myocardial revascularization and remodeling, ovarian follicle maturation, menstrual cycle, and fetal development. New blood vessel formation is required for the development of any new tissue, whether normal or pathological, and thus represents a potential control point in
25 regulating many disease states, as well as a therapeutic opportunity to encourage growth of normal tissue and "normal" angiogenesis.

According to a preferred embodiment of the first aspect of the invention, the above peptide is for use in the treatment of ischemic diseases, arterial diseases, cerebrovascular diseases, ulcers, acute and chronic cutaneous wounds, bone and dental pulp loss and in
30 the prevention of spontaneous abortion.

The peptides described herein induce angiogenic proliferation and migration of endothelial cells resulting in formation of new capillaries and collateral vessels to promote healing of wounds and ulcers, to restore function to damaged cardiovascular tissue in ischemic, arterial or cerebrovascular diseases, to induce bone or dental pulp regeneration
35 in case of bone or dental tissue loss and to support embryo implantation and persistence during pregnancy.

Ulcers and cutaneous wounds are injuries or lesions caused by physical means, such as mechanical, chemical, bacterial, or thermal means, which disrupt the normal continuity of structures of the internal mucosae or of the skin, respectively.

Said ulcers are preferably selected from gastric and duodenal ulcers.

- 5 Said ischemic diseases are preferably selected from ischemic heart disease, myocardial infarction, acute myocardial infarction, myocardosis, cardiomyopathy, angina pectoris, unstable angina.

10 Repair of injured tissue due to ischemia requires extensive remodeling and re-vascularization. An infarction is, by definition, an area of tissue ischemic necrosis caused by occlusion of local blood circulation. The resulting necrotic lesion leaves the affected tissue deprived of oxygen and nutrients. In the heart, obstruction of coronary circulation in particular, results in myocardial infarction. As the ischemic myocardium undergoes rapid oxygen starvation, the hypoxic microenvironment of the affected cardiac muscle induces the synthesis of angiogenic factors to attempt re-vascularization. In this situation, 15 the provision of angiogenic factors is useful in order to support the re-vascularization of the tissue.

Said arterial diseases are preferably selected from coronary arteriosclerosis, heart failure, arteriosclerosis obliterans (ASO), Berger's disease, vascular injury, arterial embolism, arterial thrombosis, arterial occlusion of the organ, and aneurysm.

- 20 Said cerebrovascular diseases are preferably selected from cerebrovascular occlusion, cerebral infarction, cerebral thrombosis, cerebral embolism, stroke, cerebral hemorrhage, moyamoya disease, cerebrovascular dementia, Alzheimer type dementia, sequela of cerebral hemorrhage, and sequela of cerebral infarction.

25 Preferably, said bone and dental pulp loss is a consequence of a trauma or disease. Regeneration of the lost tissue is particularly important to properly restore the lost tissue. The administration of the peptides of the invention is particularly useful in maxillofacial surgery, bone grafts, prostheses (hip, knee, ear, etc.) and in dental implants.

30 The administration of molecules that improve vascularization of the uterus and thus assist embryo implantation and persistence is essential to promote pregnancy and the prosecution of pregnancy and prevent spontaneous abortion in women with uterine vascularisation defects. Preferably, the peptide of the invention is for use in the prevention of recurrent spontaneous abortion.

According to a further preferred embodiment of the first aspect of the invention, the above peptide is also for use as an adjuvant therapy in assisted reproduction technology.

In accordance with this embodiment, the peptide is administered to a woman undergoing assisted reproduction technology in order to facilitate embryo implantation and/or development.

5 A second aspect the present invention relates to a pharmaceutical composition comprising (i) a peptide as above described according to the first aspect of the invention, and (ii) at least one pharmaceutically acceptable ingredient.

10 A third aspect of the present invention relates to a therapeutic method for treating conditions associated to/caused by deficient angiogenesis or benefiting from increased angiogenesis as defined above, in a subject in need thereof, comprising the systemic administration of the above described pharmaceutical composition.

The pharmaceutical composition of the present invention can comprise an amount of the peptide, or a derivative and/or salt thereof, ranging from 0.00000001% to 20% by weight, preferably from 0.000001 % to 15% by weight, more preferably from 0.0001% to 10% by weight, and even more preferably from 0.0001% to 5% by weight.

15 Preferably, the pharmaceutical composition of the present invention is prepared in suitable dosage forms comprising an effective amount of at least one of the above described peptides together with at least one pharmaceutically acceptable ingredient.

20 Examples of suitable dosage forms are tablets, capsules, coated tablets, granules, solutions and syrups for oral administration; solutions, pomade and ointment for topical administration; medicated patches for transdermal administration; suppositories for rectal administration and injectable sterile solutions. Other suitable dosage forms are those with sustained release and those based on liposomes for oral, injectable or transdermal administration.

25 As described herein, the pharmaceutical composition of the present invention comprises at least one of the above described peptides together with a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, diluents, or other vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired.

30 Some examples of materials which can serve as pharmaceutically acceptable excipient include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients such as cocoa butter and suppository waxes;
35 oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and

soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, coloring agents, releasing agents, coating agents, 5 sweetening, flavoring and perfuming agents, preservatives and antioxidants.

The terms "pharmaceutically acceptable" and "physiologically acceptable" are intended to define, without any particular limitation, any material suitable for preparing a pharmaceutical composition to be administered to a living being.

10 The dosage forms can also contain other traditional ingredients such as: preservatives, stabilizers, surfactants, buffers, salts for regulating osmotic pressure, emulsifiers, sweeteners, colorants, flavourings and the like.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an 15 implanted reservoir. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation or delivered by implantation (e.g., surgically), such as with an 20 implantable or indwelling device like a stent.

The dosage forms of the pharmaceutical composition of the present invention can be prepared by techniques that are familiar to a pharmaceutical chemist, and comprise mixing, granulation, compression, dissolution, sterilization and the like.

A fourth aspect of the present invention relates to a polynucleotide having a sequence 25 that codes for a peptide according to the first aspect of the invention.

Preferably, said polynucleotide codes for a peptide having an amino acid sequence consisting of any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof, more preferably SEQ ID No. 9, 10, 13, 14, 15, 16, 17, or 18.

A polynucleotide is a nucleic acid molecule that can be spontaneous or artificial DNA 30 or RNA molecules, either single-stranded or double-stranded. The nucleic acid molecule can be one or more nucleic acids of same type (for example, having a same nucleotide sequence) or nucleic acids of different types. The nucleic acid molecules comprise one or more DNA, cDNA, decoy DNA, RNA, siRNA, miRNA shRNA, stRNA, snoRNA, snRNA PNA, antisense oligomer, plasmid and other modified nucleic acids, but not limited to 35 those.

The above polynucleotide mentioned can be used in a method for the recombinant production of the peptides according to the invention.

A fifth aspect of the present invention relates to a peptide having length equal to or lower than 30 amino acids, and comprising the SEQ ID No. 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof.

Preferably, said peptide has a sequence that consists of SEQ ID No. 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

10

EXPERIMENTAL PART

Example 1 – Starting materials

Recombinant proteins and peptides

Purified endotoxin (lipopolysaccharide)-free recombinant p17 (from clone BH10 of clade B isolate - SEQ ID No. 11), GST, and p24 (SEQ ID No. 12) were produced as described by De Francesco et al, Proc. Natl. Acad. Sci. USA. 2002; 99:9972-9977. The absence of endotoxin contamination (<0.25 endotoxin units/ml) in protein preparations was assessed by the Limulus amebocyte assay (Associates of Cape Cod, Inc.).

The nine p17-derived peptides F1-F8 (SEQ ID No. 1-8) and F3S (SEQ ID No. 9) and the EPO peptide (SEQ ID No. 10) were synthesized according to the conventional methods, known in the art, such as solid phase peptide synthesis methods, enzymatic synthesis or any combination (Bondazky et al., Int. J. Pept. Protein Res. (1993), 42(1):10-3).

The first 7 peptides (F1-F7) each comprises 20 amino acids with 5 overlapping residues, and the C-terminal peptide (F8) contains 36 amino acids with 15 overlapping residues (**Figure 1**). Peptide F8 comprises 16 amino acids, 15 thereof overlapping peptide F3. EPO peptide comprises 11 amino acids.

Cell Cultures

Human umbilical vein endothelial cells (HUVECs) were isolated and characterized as described in Caruso et al, Proc. Natl. Acad. Sci. USA. 2009; 106:20446–20451, and cultured in endothelial growth medium (EGM) (Lonza, Milan, Italy) containing 10% (vol/vol) fetal bovine serum (FBS).

Human aortic endothelial cells (HAECs) and Human lung microvascular endothelial cells (HMVEC-Ls) were purchased from Clonetics (San Diego, CA, USA) and cultured in EGM-2MV (Lonza) containing 10% (vol/vol) FBS.

Human primary lymph node-derived endothelial cells (LN-LECs) have been developed
5 and characterized as described in Norder et al, FASEB J, 2012; 26:2835-2846.

LN-LECs were cultured in EGM (Lonza) containing 10% FBS and supplemented with VEGF-C (25 ng/ml) (Reliatech, Wolfenbuettel, Germany).

All experiments were carried out with cells at passages 2-6. All data were analyzed for statistical significance using one-way analysis of variance (ANOVA). Bonferroni's post-
10 test was used to compare data. Differences were considered significant at a P value of <0.05. Statistical tests were performed using Prism 5 software (GraphPad).

Example 2 - Angiogenic Activity Assay

Low passage HUVECs were grown under stressed (serum starved; 0.5% FBS) conditions for 16 h in endothelial basal medium (EBM) and then harvested and
15 resuspended in complete medium containing 10% FBS.

Cells were seeded (5×10^4 cells per well) in wells coated with growth factor-reduced Cultrex basement membrane extract (BME; Trevigen Gaithersburg, MD, USA) and left untreated (NT) or treated with 10 ng/ml of GST, p24, p17 or each p17-derived peptide F1 to F8.

Data are the means $\pm$ SD of three independent experiments with similar results. Statistical analysis was performed by one-way ANOVA and the Bonferroni post-test was used to compare data (**P<0.001).

Activity screening at a single concentration of 10 ng/ml identified two different peptides out of the eight, namely peptide F2 (SEQ ID No. 2) and F3 (SEQ ID No. 3), endowed with
25 angiogenic activity (**Figure 2**).

The angiogenic activity of the two peptides was found to be similar to that exerted by p17. As expected the negative controls glutathione S-transferase (GST) and the HIV-1 capsid protein p24 (p24), did not induce any pro-angiogenic activity on HUVECs.

Similar results were obtained by using human ECs derived from different origin, such
30 as aorta (HAECs), lung (HMVEC-Ls) and lymph node (LN-LECs) (**Figures 3, 4 and 5**, respectively).

The same experiment was conducted on HUVEC cells with 10 ng of the peptides ASRELERF (SEQ ID No 13) and ASRELERFLLE (SEQ ID No 14) or the peptides AANELDRF (SEQ ID No 16) and AANELDRFLLE (SEQ ID No 15). The results are shown

in **Figure 13** and **Figure 14**, respectively, and show that all these peptides were able to significantly promote angiogenesis on HUVECs.

The experiment was repeated with 10 ng of the peptide gor (ASRELERFACNPELLE - SEQ ID No. 17), the peptide cpz (ASRELERFACNPGLME - SEQ ID No. 18), and the peptide F3S (ASRELERFAVNPGLLE - SEQ ID No. 9). The results are shown in Figure 15 and confirmed that SIVcpz-derived and SIVgor-derived peptides were able to significantly promote angiogenesis on HUVECs.

Example 3 - Wound-healing Assay

The involvement of peptide F2 and F3 in promoting EC migration was evaluated by performing a wound healing assay.

HUVECs were plated on 24-well plates (10^5 cells per well) in complete medium containing 10% FBS. Confluent monolayers were nutrient starved for 16 h and then scratched using a 200 μ l pipette tip. After being washed, cells were left untreated or treated with 10 ng/ml of GST, p24, p17, or each p17-derived peptide F1 to F8.

As shown in **Figure 6**, not treated (NT) HUVECs reached approximately 31,73% (standard deviation [SD], $\pm 1\%$) healing after 12 h of culture, whereas at the same time, HUVECs treated with either peptide F2 or F3 showed almost 100% of healing. Activity of peptide F2 and F3 was comparable to that exerted by p17. As expected, GST and p24 used as negative controls did not exert any effect on HUVEC migration.

Example 4 - Aortic ring assay

The vasculogenic activity of peptide F2 and F3 was further characterized by *ex vivo* experiments by using the rat aortic ring assay as described in Nicosia RF et al, Lab Invest, 1990; 63:115–122.

Briefly, the dorsal aorta was excised from 6-wk-old Sprague-Dawley rats. Rings, 1-mm thick, were embedded in collagen gel and then incubated with serum-free EBM containing PBS, peptide F2 (10 ng/ml), peptide F3 (10 ng/ml), or FGF-2 (50 ng/ml) (Thermo Fisher Scientific, Rodano, Italy). The plates were incubated for 10 days and angiogenesis was quantified by counting the number of microvessels originating from aortic rings.

As shown in **Figure 7**, the number of microvessels in not treated (PBS) cultures, used as negative control, was significantly lower (11 ± 1) than that of rings treated with peptide F2 (55 ± 5) or peptide F3 (46 ± 8). As expected, stimulation of aortic rings with 50 ng/ml of FGF-2 strongly increased microvessel outgrowth (84 ± 6).

Example 5 - Chick chorioallantoic membrane (CAM) assay

The vasculogenic activity of peptide F2 and F3 was further characterized by *in vivo* experiments by using the chick chorioallantoic membrane (CAM) assay.

Fertilized White Leghorn chicken eggs (30 per group) were incubated at 37°C at constant humidity. On day 3, a square window was opened in the shell, and 2-3 ml of albumin was removed to allow detachment of the developing CAM. The window was sealed with a glass, and the eggs were returned to the incubator. On day 8, eggs were treated with 1 mm³ sterilized gelatin sponges (Gelfoam; Upjohn, Kalamazoo, MI, USA) placed on top of the growing CAM, as described in Ribatti D et al, Nat Protoc, 2006; 1:85–91, and loaded with 1 µl of PBS (negative control), 1 µl of PBS containing 100 ng of peptide F2 or F3. CAMs were examined daily until day 12 and photographed in ovo with a stereomicroscope equipped with a camera and image analyzer system (Olympus, Hamburg, Germany). At day 12, the angiogenic response was evaluated by the image analyzer system and counted as the number of vessels converging toward the sponge.

As shown in **Figure 8**, a significant angiogenic response in the form of numerous allantoic neovessels developing radially toward the implant in a “spoke-wheel” was promoted by peptide F2 and F3 (mean number of vessels: 27 ± 4 and 30 ± 3, respectively) as compared to PBS (mean number of vessels of 12 ± 2).

Example 6 - Angiogenic Activity Assay with CXCR1 and CXCR2 Inhibition

Low passage HUVECs were grown under stressed (serum starved; 0.5% FBS) conditions for 16 h in endothelial basal medium (EBM) and then harvested and resuspended in complete medium containing 10% FBS.

Cells were seeded (5 x 10⁴ cells per well) in wells coated with growth factor-reduced Cultrex basement membrane extract (BME; Trevigen Gaithersburg, MD, USA) and left untreated (NT) or treated with 10 ng/ml of each p17-derived peptide F1 or F2, after preincubation for 1 hour at 37°C with 2.5 µg/ml of a control isotype-matched mAb (Ctrl mAb), a neutralizing mAb to CXCR1 and/or a neutralizing mAb to CXCR2.

As shown in **Figure 9**, the neutralizing mAb to CXCR1 and CXCR2 were inhibitory toward peptide F2-induced angiogenesis. On the contrary, CXCR1 and CXCR2 neutralizing mAbs did not impair the angiogenic activity of peptide F3.

These results suggested that peptide F3 does not use CXCR1 and CXCR2 to exert its angiogenic activity.

Example 7 - Angiogenic Activity Assay under Normal and Stressed Condition

Low passage HUVECs were grown under normal (10% FBS) or stressed (serum starved; 0.5% FBS) conditions for 16 h in endothelial basal medium (EBM) and then

harvested and resuspended in complete medium containing 10% FBS. Cells were seeded (5×10^4 cells per well) in wells coated with growth factor-reduced Cultrex basement membrane extract (BME; Trevigen Gaithersburg, MD, USA) and left untreated or treated with 10 ng/ml of p17, F2 or F3.

- 5 As shown in **Figure 10A**, under normal culture conditions, peptide F3 was found to promote angiogenesis as compared to not treated cells or to cells treated with peptide F2 or p17. As expected, HUVECs cultured under serum-deprived conditions were highly susceptible to stimulation with p17 as compared to control cells. Under stressed conditions, both peptides F2 and F3 were found to exert a potent angiogenic activity as compared to control, not treated cells (**Figure 10B**).

- 10 These results, together with those of example 6, suggested that peptide F2 acts as p17 by binding to CXCR1 and CXCR2 and promoting angiogenesis under stress condition only, while peptide F3 promotes angiogenesis under both stressed and normal cell culture condition, without interacting with CXCR1 and CXCR2, but possibly with a still unknown receptor.
- 15

Example 8 - Angiogenic Activity Assay – Mimicry with EPO

- Human erythropoietin (EPO) is endowed with angiogenic activity on human ECs even if the epitope involved in such activity is unknown up-to-date. The procedure of example 7 was repeated by leaving the cells untreated or treated with 5, 20 or 40 ng/ml of EPO, showing that EPO significantly promoted angiogenesis at a concentration of 20 ng/ml under both normal (A) or starved (B) culture conditions (**Figure 11**).
- 20

Sequence alignment between peptide F3 and EPO allowed to identify the best matching in the EPO N-terminal region included between amino acid 8 to 10.

- An EPO-derived 11 amino acids-long peptide (EPO peptide - SEQ ID No. 10) and a F3-derived 16 amino acids-long peptide (F3S - SEQ ID No. 9) were synthesized and tested at 10 ng/ml for their angiogenic activity under the same procedure of Example 7.
- 25

As shown in **Figure 12**, EPO peptide exerted a potent angiogenic activity, comparable to that of F3-derived 16 amino acids-long peptide, under both normal (A) and stressed (B) culture conditions.

- 30 **Example 9 – In vivo wound-healing assay**

Patients with diabetes are prone to impaired wound healing, with foot ulceration being particularly prevalent. This delay in wound healing also extends to animal models of diabetes. The impact of the EPO peptide (DSRVLERYLLE, corresponding to SEQ ID No.

10) on wound healing was therefore investigated by in vivo experiments on spontaneously diabetic (db/db) mice (i.e., BKS.Cg-m Dock7^m $+/+$ Lepr^{db} /J mice).

In this study, the healing of full-thickness excisional skin wounds on the flanks of diabetic mice in receipt of the EPO peptide was compared to that of wounds in receipt of negative control (Water for Injection) treatment. The overall wound closure (i.e. the reduction in open wound area) was determined over time by taking scaled wound images of each wound at each assessment point.

EPO Peptide (DFL24435) was solubilized at 200 μ g/mL (10 μ g per 50 μ L) in sterile water for injection, aliquoted into 100 μ L volumes, stored at -20°C, defrosted on the day of use and diluted as necessary.

The animals were divided in a control group, that was treated with water for injection, and a EPO peptide group, that was treated with the peptide. Treatment was carried out once a day on post-wounding days 0 to 13 and the animals were sacrificed on day 14.

In details, the EPO peptide group was treated with the peptide solution at a concentration of 1.0 μ g/50 μ L on post-wounding days 0 to 7 and at a concentration of 10 μ g /50 μ L on post-wounding days 8 to 13.

All wounds in both experimental groups were dressed with a piece of the transparent film dressing TegadermTM Film (TFD). EPO peptide formulations or water for injection (Vehicle group) were then applied directly to the wound surface by injection through the film dressing using a 30G hypodermic needle (dose volume 50 μ L).

The treatment regimens are reported in Table D below.

TABLE D

Treatment (TFD = Tegaderm TM Film Dressing)	Group name	Treatment Application (Study Day)	"n"
Water for Injection (WFI) + TFD	Vehicle (control)	Daily from day 0 until the day prior to harvesting	10
EPO peptide (in WFI) + TFD	DFL24435	Daily from day 0 until the day prior to harvesting	10

As can be seen in Figure 16, wounds in receipt of the EPO peptide (DFL24435 in the graph) demonstrated an increased closure rate compared to wounds in receipt of vehicle with significantly increased closure on days 8 to 14 ($p \leq 0.035$). This results demonstrate that the EPO peptide is able to significantly increase wound closure.

SEQUENCE LISTING

SEQ ID No. 1
GARASVLSSGGELDRWEKIRL
5
SEQ ID No. 2
EKIRLRPGGKKKYKLKHIVW
SEQ ID No. 3
10 KHIVWASRELERFAVNPGLL
SEQ ID No. 4
NPGLLETSEGCRQILGQLQP
15 SEQ ID No. 5
GQLQPSLQTGSEELRSLYNT
SEQ ID No. 6
SLYNTVATLYCVHQRIEIKD
20
SEQ ID No. 7
IEIKDTKEALDKIEEEQNKS
SEQ ID No. 8
25 TKEALDKIEEEQNKSKKKAQQAAADTGHSSQVSQNY
SEQ ID No. 9
ASRELERFAVNPGLLE

SEQ ID No. 10

DSRVLERYLLE

5 SEQ ID No. 11

MGARASVLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGC
RQILGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQ
QAAADTGHSSQVSQNY

10 SEQ ID No. 12

HQALSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQM
LKDTINEEAAEWDRMHPVQAGPIPPGQIREPRGSDIAGTTSTLQEQUITWMTSNPPIPVG
EIYKRWIILGLNKIVRMYSILDIRQGPKPEFRDYVDRFFRVLRAEQATQEVKNWMT
ETLLVQANPDCRTILKALGSGATLEEMMTAC

15

SEQ ID No. 13

ASRELERFLLE

SEQ ID No. 14

20 ASRELERF

SEQ ID No. 15

AANELDRFLLE

25 SEQ ID No. 16

AANELDRF

SEQ ID No. 17

ASRELERFACNPELLE

SEQ ID No. 18

ASRELERFACNPGLME

CLAIMS

1. A peptide, or derivative thereof, having an amino acid sequence with a length equal to or lower than 30 amino acids, and comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof, for use as a medicament.
- 5 2. A peptide for use as claimed in claim 1, for use in the prevention or treatment of conditions associated to/caused by deficient angiogenesis or conditions benefiting from increased angiogenesis.
3. A peptide for use as claimed in claim 1 or 2, having an amino acid sequence consisting of any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative
10 variant thereof.
4. The peptide for use as claimed in claim 1 to 3, for use in the treatment of ischemic diseases, arterial diseases, cerebrovascular diseases, ulcers, acute and chronic cutaneous wounds, bone and dental pulp loss and in the prevention of spontaneous abortion.
- 15 5. The peptide for use as claimed in claim 4, wherein said ulcers are preferably selected from gastric and duodenal ulcers.
6. The peptide for use as claimed in claim 4, wherein said ischemic diseases are preferably selected from ischemic heart disease, myocardial infarction, acute myocardial infarction, myocarditis, cardiomyopathy, angina pectoris and unstable angina.
- 20 7. The peptide for use as claimed in claim 4, wherein said arterial diseases are preferably selected from coronary arteriosclerosis, heart failure, arteriosclerosis obliterans (ASO), Berger's disease, vascular injury, arterial embolism, arterial thrombosis, arterial occlusion of the organ, and aneurysm.
8. The peptide for use as claimed in claim 4, wherein said cerebrovascular diseases
25 are preferably selected from cerebrovascular occlusion, cerebral infarction, cerebral thrombosis, cerebral embolism, stroke, cerebral hemorrhage, moyamoya disease, cerebrovascular dementia, Alzheimer type dementia, sequela of cerebral hemorrhage, and sequela of cerebral infarction.
- 9 The peptide for use as claimed in claim 4, wherein said spontaneous abortion is
30 recurrent spontaneous abortion.
10. The peptide for use as claimed in claims 1 to 3, for use as adjuvant therapy in assisted reproduction technology.
11. A pharmaceutical composition comprising (i) a peptide, or derivative thereof, having an amino acid sequence with a length equal to or lower than 30 amino acids and

comprising any one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof, and (ii) at least one pharmaceutically acceptable excipient.

12. A polynucleotide that codes for a peptide, or derivative thereof, having an amino acid sequence with a length equal to or lower than 30 amino acids and comprising any
5 one of SEQ ID Nos. 2, 3, 9, 10, 13, 14, 15, 16, 17, 18, or a conservative variant thereof.

13. A peptide having length equal to or lower than 30 amino acids, and comprising any one of the SEQ ID No. 9, 10, 13, 14, 15, 16, 17, or 18.

14. A peptide having an amino acid sequence consisting of any one of SEQ ID No. 9, 10, 13, 14, 15, 16, 17, or 18.

10