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(73) Patenthaver: **Follicum AB, Scheelevägen 15, S-223 70 Lund, Sverige**

(72) Opfinder: **ALENFALL, Jan, Nybovägen 79, S-234 37 Lomma, Sverige**
DUNÉR, Pontus, Östen Undéns Gata 35, S-227 62 Lund, Sverige
HULTGARDH NILSSON, Anna, Toppeladugård 331, S-247 99 Genarp, Sverige

(74) Fuldmægtig i Danmark: **HØIBERG A/S, Adelgade 12, 1304 København K, Danmark**

(54) Benævnelse: **Modificerede osteopontin-polypeptider, som har et inaktiveret RGD-domæne**

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DESCRIPTION

Field of Invention

[0001] The present invention relates to agents for stimulating hair growth in mammals. In particular, there are provided compositions comprising a modified osteopontin polypeptide for stimulating hair growth in mammals (including humans).

Background

[0002] Hair growth is cyclical, occurring in three stages: anagen, the active growth phase; catagen, the degenerative phase; and telogen, the resting phase. After telogen, the old hair fibre is shed and a new hair is generated as part of the repeating cycle.

[0003] Alopecia, or hair loss, occurs in both men and women, and is attributed to numerous causes including aging, hormone levels, stress, and chemotherapy. In these circumstances, more and more hair follicles remain in the telogen stage, resulting in a gradual decrease of the hair fibre length and diameter, finally reaching a stage of partial or complete baldness.

[0004] Various types of hair loss are known, including alopecia areata, androgenetic alopecia, anagen effluvium, self-induced hair loss, telogen effluvium, and scarring alopecia. Alopecia areata, thought to be an auto-immune disorder, begins with hair loss in a rounded patch on the scalp. Alopecia areata includes mild patchy hair loss on the scalp, as well the loss of all scalp hair, known as alopecia totalis, and the loss of all scalp and body hair, known as alopecia universalis. Androgenetic alopecia, including male and female pattern baldness, is thought to be caused by a combination of genetic predisposition, aging, and androgen hormone levels. Androgenetic alopecia is associated with increased androgen stimulation, which adversely affects the hair follicles. Increased androgen stimulation can be produced by, among other mechanisms, elevated levels of 5-alpha-reductase, an enzyme that converts testosterone to dihydrotestosterone. Anagen effluvium is hair loss due to chemicals or radiation, such as chemotherapy or radiation treatment for cancer. Self-induced hair loss includes hair loss caused by conscious or unconscious self-inflicted damage to the hair. Two common types of self-induced hair loss are trichotillomania, or hair loss that results when someone continually pulls or plucks out his own hair, and traction alopecia, which is caused by hairstyles such as ponytails or braids that continually pull at the hair. Telogen effluvium is stress-related hair loss caused by events such as, for example, surgery, child birth, or pregnancy termination. Further causes of telogen effluvium include the use of oral contraceptives or other prescription drugs, thyroid abnormality, diabetes, lupus, and emotional stress. Scarring alopecia includes hair loss caused by infection and inflammation of the hair follicles, and hair loss caused by burns or other trauma.

[0005] Hair loss is a widespread problem that is considered by some to be cosmetically unappealing and often causes emotional distress to the individual concerned. As a consequence, there is great demand for alopecia treatments. Many compositions have been tested for their ability to stimulate hair growth, for example, by promoting or prolonging anagen. Examples of such compositions include potassium channel openers, such as minoxidil (Regaine RTM., Pharmacia Corp.) and diazoxide; 5-alpha-reductase inhibitors, such as finasteride (Propecia RTM., Merck & Co.); and the immunosuppressant cyclosporin A.

[0006] However, such known treatments for stimulating hair growth exhibit limited effectiveness and cause unwanted side effects. For example, among other undesirable side effects, potassium channel openers cause cardiovascular effects, finasteride is unsafe for women who are pregnant or may become pregnant, and cyclosporin A suppresses the immune system. Further, even when applied topically to areas in which hair growth is desired, known treatments for alopecia often cause hair growth in undesired areas of the body, such as facial hair on women. Such disadvantages of known compositions for treating alopecia lead many individuals experiencing hair loss to rely on wigs and toupees. Other individuals seek hair transplant surgery, which is expensive, is not fully effective, and sometimes is not possible, for example, for chemotherapy patients.

[0007] Accordingly, there is a need for new treatments to stimulate hair growth, suitable for use in both medical and cosmetic applications.

Summary of Invention

[0008] The first aspect of the invention provides a composition for stimulating hair growth in a mammal comprising:

(a) a modified osteopontin polypeptide in which an RGD domain is inactivated; and

(b) a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent

wherein the modified osteopontin polypeptide consists of an amino acid sequence of SEQ ID NO: 63.

[0009] Thus, the active component of the compositions of the invention is derived from a naturally-occurring osteopontin protein (*i.e.* the polypeptide comprises an amino acid sequence corresponding to that of a modified, for example mutated, version of a naturally-occurring osteopontin protein).

[0010] Osteopontin, also known as bone sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), secreted phosphoprotein 1 (SPP1), 2ar and Rickettsia resistance (Ric), is a gene product which is conserved in several mammalian species.

[0011] The gene has seven exons, spans 5 kilobases in length and in humans it is located on the long arm of chromosome 4 region 13 (4q13). The protein is composed of ~300 amino acids residues and is rich in acidic residues: 30-36% are either aspartic or glutamic acid. Osteopontin has ~30 attached carbohydrate residues, including 10 sialic acid residues, which are attached to the protein during post-translational modification in the Golgi apparatus.

[0012] Osteopontin was first discovered as a novel sialoprotein in bone anchoring osteoclasts onto the mineralized bone matrix (Franzen & Heinegard, 1985, Biochem. J. 232(3)715-24). The name *osteopontin* comes from the presence of the protein in bone (*osteo-*) and its ability to form a bridge (*-pons*) between bone cells and the mineral phase. Sequence analysis and subsequent structural studies showed osteopontin to be a 32 kDa glycoprotein composed of a highly acidic region of some ten aspartic acid residues thought to mediate the mineral binding properties of osteopontin. Furthermore, in the mid portion of the osteopontin molecule there is also a cell attachment domain mediated through an R-G-D sequence (Oldberg et al., 1986, Proc. Natl. Acad. Sci. USA 83(23):8819-23).

[0013] Osteopontin is constitutively expressed in osteoblasts and in several epithelial cell types, resulting in osteopontin being secreted into many body fluids. Bone is the only tissue type where osteopontin is deposited and from where it can be recovered in large amounts. The expression of osteopontin can also be induced In vascular smooth muscle cells, in different cancer cell types and among inflammatory cells (specifically macrophages and T lymphocytes). Several important cellular functions have been attributed to osteopontin such as adhesion, proliferation, migration, anti-apoptosis and chemo attraction. Some of these functions are believed to be mediated via the RGD cell-adhesion domain which interacts with different integrins, mainly with αvβ3 but also αvβ1, and αvβ5 (for review see Scatena et al., 2007, Arterio. Thromb. Vasc. Biol. 27:2302-2309).

[0014] In recent years, osteopontin has emerged as a potent cytokine capable of modulating several cell types involved in inflammation and immune responses. The broad range of functions being attributed to Osteopontin has been puzzling and cannot all be explained by the single cell-binding RGD sequence. The explanation came when an eleven amino acid peptide in osteopontin R^{145}-G-D-S-L-A-Y-G-L-R-S^{155} **(SEQ ID NO:122)** (corresponding to amino acids 144 to 154 of UniProt code P10923) was identified and later functionally mapped. In addition to the known R^{145} -G-D^{147} site mediating binding to the αvβ3 integrin, two additional essential regions in the osteopontin molecule were discovered, namely a highly specific thrombin cleavage site, *i.e.* R^{154} -S^{155}, and a cryptic integrin binding site, *i.e.* S^{148}-L-A-Y-G-L-R^{154} **(SEX ID NO:123),** which binds to an α9β1 integrin (see Scatena *et al., supra*). An additional binding site for α4β1 has also been identified (see Scatena et *al., supra*).

[0015] A characterising feature of the osteopontin-derived polypeptide in the compositions of the invention is that the RGD domain naturally present in osteopontin is inactivated such that it is non-functional (at least in part). For example, inactivation of the RGD domain may prevent the osteopontin-derived polypeptide from binding to one or more of the integrins which bind the naturally occurring osteopontin protein.

[0016] Thus, by "modified osteopontin polypeptide" we include polypeptides corresponding to a modified form of a naturally-occurring osteopontin protein in which the RGD domain is non-functional (at least in part), as well as fragments and variants thereof which retain a hair-stimulatory property of the 'full length' modified osteopontin. For example, the non-functional RGD domain of the modified osteopontin polypeptide may be unable to bind integrin αvβ3 (see Scatena *et al., supra*).

[0017] Advantageously, the naturally-occurring osteopontin protein is mammalian, *e.g.* human.

[0018] The modified osteopontin polypeptides present in the compositions of the invention are capable of stimulating hair growth in mammals.

[0019] In one embodiment, the polypeptide is capable of stimulating the growth of human hair.

[0020] In a further embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo*.

[0021] In a further embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo* with greater efficacy than wildtype human osteopontin. By "greater efficacy" in this context, we include a quicker onset of action and/or efficacy at a lower dose and/or greater maximum effect (*e.g.* greater number of new follicles or density of hair). In one embodiment, the polypeptide is capable of stimulating the growth of hair *in vivo* with a quicker onset of action and a greater maximum hair growth effect than wildtype human osteopontin at the same dose (*e.g.* see Example 8).

[0022] It will be appreciated by persons skilled in the art that the stimulation of hair growth may be mediated by an effect of existing hair follicles and/or by inducing the formation of new hair follicles.

[0023] Thus, in one embodiment, the modified osteopontin polypeptide is capable of stimulating existing hair follicles (for example, by prolonging the anagen phase and/or by shortening the telogen phase such that the resting follicles become active).

[0024] In a further embodiment, the polypeptide is capable of inducing the formation of new hair follicles, or stem cells for producing the same.

[0025] As discussed above, the modified osteopontin polypeptides lack the active tri-peptide sequence "arginine-glycine-aspartic acid" normally found in naturally-occurring osteopontin proteins. It will be appreciated that this RGD domain may be inactivated by a number of different strategies.

[0026] The polypeptide consists of the amino acid sequence of SEQ ID NO: 63.

[0027] VDTYDGDISVVYGLR **SEQ ID NO: 63** ["FOL-005"]

[0028] Persons skilled in the art will appreciate that the modified osteopontin polypeptide may be glycosylated at one or more amino acids. For example, the polypeptide may retain one or more of the glycosylation sites of the corresponding ('parent') osteopontin protein, to which may be attached a carbohydrate group.

[0029] Modified osteopontin polypeptides suitable for use in the compositions of the invention may be made by *in vitro* cell-based expression methods well known to persons skilled in the art (for example, see Sambrook & Russell, 2000, Molecular Cloning, A Laboratory Manual, Third Edition, Cold Spring Harbor, New York, which is incorporated herein by reference). The choice of expression vector and host cell to be used may depend on a number of factors. For example, if the modified osteopontin polypeptide is to be glycosylated, a mammalian expression system will be required.

[0030] Suitable expression vectors and host cells are commercially available from many sources.

[0031] Alternatively, the modified osteopontin polypeptides may be synthesised by known means, such as liquid phase and solid phase synthesis (for example, t-*Boc* solid-phase peptide synthesis and BOP-SPPS).

[0032] It will be appreciated by persons skilled in the art that the present invention also includes pharmaceutically and/or cosmetically acceptable acid or base addition salts of the above described modified osteopontin polypeptide. The acids which are used to prepare the pharmaceutically and/or cosmetically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmaceutically and/or cosmetically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulphate, bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate [i.e. 1,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others.

[0033] Pharmaceutically and/or cosmetically acceptable base addition salts may also be used to produce pharmaceutically and/or cosmetically acceptable salt forms of the modified osteopontin polypeptides. The chemical bases that may be used as reagents to prepare pharmaceutical and/or cosmetically acceptable base salts of the present compounds that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmaceutical and/or cosmetically acceptable cations such as alkali metal cations (e.g. potassium and sodium) and alkaline earth metal cations (e.g. calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic

amines, among others.

[0034] It will be further appreciated that the modified osteopontin polypeptide of the invention may be lyophilised for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophilisation method (*e.g.* spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of activity loss and that use levels may have to be adjusted upward to compensate. Preferably, the lyophilised (freeze dried) polypeptide loses no more than about 20%, or no more than about 25%, or no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilisation) when rehydrated.

[0035] The modified osteopontin polypeptide is provided in the form of a composition comprising the polypeptide and a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent, selected with regard to the intended route of administration and standard pharmaceutical or cosmetic practice (for example, see Remington: The Science and Practice of Pharmacy, 19th edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA , which is incorporated herein by reference).

[0036] By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. Suitable pharmaceutical carriers are well known in the art of pharmacy. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free; however, other acceptable carriers may be used. Thus, "pharmaceutically acceptable carrier" and "pharmaceutically acceptable excipient" includes any compound(s) used in forming a part of the formulation that is intended to act merely as a carrier, *i.e.,* not intended to have biological activity itself. The pharmaceutically acceptable carrier or excipient is generally safe, non-toxic, and neither biologically nor otherwise undesirable. A pharmaceutically acceptable carrier or excipient as used herein includes both one and more than one such carrier or excipient.

[0037] Likewise, the term "cosmetically acceptable" is used to denote formulations suitable for use as cosmetic products. Suitable cosmetic carriers are well known in the art, such as those commonly used in shampoos, lotions, creams and other such products.

[0038] The excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, sucrose, mannitol, and cyclodextrines, which are added to the composition, e.g.,, for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethylenglycol/polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, e.g., for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain lenght and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

[0039] The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the peptide in the pharmaceutical preparation. The diluent may be one or more of saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, com oil, peanut oil, cottonseed oil or sesame oil).

[0040] The diluent may also function as a buffer. The term "buffer" Is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Examples of buffers are Trizma, Bicine, Tricine, MOPS, MOPSO, MOBS, Tris, Hepes, HEPBS, MES, phosphate, carbonate, acetate, citrate, glycolate, lactate, borate, ACES, ADA, tartrate, AMP, AMPD, AMPSO, BES, CABS, cacodylate, CHES, DIPSO, EPPS, ethanolamine, glycine, HEPPSO, imidazole, imidazolelactic acid, PIPES, SSC, SSPE, POPSO, TAPS, TABS, TAPSO and TES.

[0041] Optionally, the composition may comprise an adjuvant. The term "adjuvant" is intended to mean any compound added to the formulation to increase the biological effect of the peptide. The adjuvant may be one or more of zinc, copper or silver salts with different anions, for example, but not limited to fluoride, chloride, bromide, iodide, tiocyanate, sulfde, hydroxide, phosphate, carbonate, lactate, glycolate, citrate, borate, tartrate, and acetates of different acyl composition.

[0042] The compositions of the invention may also be in the form of biodegradable microspheres. Aliphatic polyesters, such as

poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers of PLA and PGA (PLGA) or poly(carprolactone) (PCL), and polyanhydrides have been widely used as biodegradable polymers in the production of microspheres. Preparations of such microspheres can be found in US 5,851,451 and in EP0213303.

[0043] The compositions of the invention may also be in the form of polymer gels, where polymers such as starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate,

[0044] The modified osteopontin polypeptide may be formulated at various concentrations, depending on the efficacy/toxicity of the particular polypeptide being used. Preferably, the composition comprises the modified osteopontin polypeptide at a concentration of between 1 nM and 1 M, for example between 0.1 μM and 1 mM, 1 μM and 100 μM, between 5 μM and 50 μM, between 10 μM and 50 μM, between 20 μM and 40 μM and optionally about 30 μM. For *ex vivo* and *in vitro* applications, compositions may comprise a lower concentration of a modified osteopontin polypeptide, for example between 0.0025 μM and 1 μM.

[0045] It will be appreciated by persons skilled in the art that the compositions of the invention may be administered by a variety of routes, for example topical, transdermal, parenteral or oral administration.

[0046] Advantageously, the compositions of the invention are suitable for topical administration or intracutaneous administration.

[0047] Thus, the compositions of the invention may be administered topically to the skin (e.g. scalp). For example, the composition may be provided in the form of an ointment containing the active polypeptide suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the polypeptide can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0048] Optionally, the composition for topical administration may comprise a penetration enhancer (for example, as described in Osborne & Henke, 1997, Pharmaceutical Technology, November: 58-82 and Pathan & Setty, 2009, Tropical Journal of Pharmaceutical Research 8 (2): 173-179, the disclosures of which are incorporated herein by reference).

[0049] Alternatively, the compositions of the invention may be administered parenterally, for example intracutaneously. Such compositions are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

[0050] Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0051] It may be beneficial to use a sustained-release system, such as microsphere formulations, for delivering the modified osteopontin polypeptides.

[0052] Alternatively, compositions can be administered by a surgically implanted device that releases the active polypeptide directly to the required site (*i.e.* the epidermis).

[0053] The compositions of the invention may also be delivered by transdermal methodologies.

[0054] For example, electroporation therapy (EPT) and/or iontophoresis systems can be employed for the administration of proteins and polypeptides. In such methods, a device is used to deliver a pulsed electric field to cells, resulting in the increased permeability of the cell membranes to the drug and significant enhancement of intracellular drug delivery.

[0055] An alternative transdermal method, electroincorporation, utilises small particles of up to 30 microns in diameter on the

surface of the skin experience electrical pulses identical or similar to those used in electroporation. The particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

[0056] Additional transdermal methodologies have also been developed by PowderJect Pharmaceuticals (now owned by Novartis AG).

[0057] Suitable methods for administration of the polypeptides and compositions of the invention are well known in the art, for example, see Therapeutic Protein and Peptide Formulation and Delivery, Zahra Shahrokh et a/. (Eds), 1997, American Chemical Society, ISBN13: 9780841235281.

[0058] A second aspect of the invention provides a polypeptide consisting of the amino acid sequence of SEQ ID NO: 63 63.

[0059] In one embodiment, the polypeptide is isolated (*e.g.* outside the mammalian body).

[0060] The polypeptide of the invention may be for medical use in the treatment or prevention of a disease or condition associated with hair loss (as described in detail below).

[0061] Also described herein is the use of a polypeptide of the invention for stimulating hair growth in a mammal, wherein the use is cosmetic or commercial (as described in detail below).

[0062] Also described herein are compositions according to the first aspect of the invention for use in stimulating hair growth in a mammal.

[0063] Thus, the compositions may be for use in stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

[0064] The composition may be for use in the treatment or prevention of a disease or condition associated with hair loss, such as alopecia.

[0065] Alopecia is typically associated with the loss of anagen hairs. However, it will be appreciated that the compositions of the invention may also be used for treatment of conditions associated with the loss of telogen hairs.

[0066] In one embodiment, the alopecia is selected from the group consisting of:

1. (a) androgenic alopecia (also known as androgenetic alopecia, *alopecia androgenetica*, male pattern baldness or female pattern baldness);
2. (b) traction alopecia;
3. (c) anagen effluvium;
4. (d) telogen effluvium;
5. (e) alopecia areata;
6. (f) alopecia totalis;
7. (g) alopecia universalis;
8. (h) alopecia barbae;
9. (i) alopecia mucinosa;
10. (j) alopecia neoplastica;
11. (k) cicatricial alopecia; and
12. (l) scarring alopecia.

[0067] For example, the alopecia may be androgenic alopecia.

[0068] Alternatively, the alopecia may be anagen effluvium. This condition, resulting from the early entry of hairs into the telogen phase, may be due to a variety of causes, including eating disorders, fever, childbirth, chronic illness, major surgery, anemia, severe emotional disorders, crash diets, hypothyroidism, and drugs.

[0069] Thus, the hair loss may be induced by radiotherapy and/or chemotherapy agents. For example, hair loss is a common

and distressing side effect of treatment with chemotherapeutic drugs such as cisplatin, etoposide and paclitaxel.

[0070] Conveniently, the mammal is a human.

[0071] Also described herein is the use of a modified osteopontin polypeptide as defined above in relation to the first aspect of the invention in the preparation of a medicament for stimulating hair growth in a mammal.

[0072] Thus, the medicament may be for stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

[0073] Also described herein is a method for stimulating hair growth in a mammal comprising administering an effective amount of a modified osteopontin polypeptide as defined above in relation to the first aspect of the invention.

[0074] Thus, the method may be for stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

[0075] The polypeptide composition of the invention can be administered to the patient in an effective amount. A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a stimulatory effect on hair growth. This is a predetermined quantity of active material calculated to produce the desired therapeutic effect. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art.

[0076] The method may be for the treatment or prevention of a disease or condition associated with hair loss, such as alopecia (*e.g.* associated with the loss of anagen hairs).

[0077] It will be appreciated by persons skilled in the art that the composition of the first aspect of the invention are not limited to medical uses but may also be used as cosmetic agents (in the sense that they do not provide any physical health improvement, as such, but merely provide an aesthetic benefit to the mammal).

[0078] Also described herein is the use of a composition according to the first aspect of the invention for stimulating hair growth in a mammal, wherein the use is cosmetic.

[0079] Thus, the cosmetic composition may be for stimulating existing hair follicles and/or inducing the growth of new hair follicles (or stem cells for producing the same).

[0080] For example, the cosmetic composition may be used for the treatment or prevention of baldness, which may be associated with a receding hairline and/or thinning hair.

[0081] Such compositions are not limited to use on the scalp, but may also be applied elsewhere on the body (including to the face to encourage the growth of a beard, eyelashes, eyebrows, *etc.*)

[0082] Conveniently, the mammal is a human.

[0083] It will be appreciated by persons skilled in the art that the compositions of the invention may be used on their own or in combination with other therapeutic or cosmetic agents. For example, the compositions of the invention may be used in a combination therapy with existing treatments to prevent loss of existing hair and/or to stimulate growth of new hair, for example potassium channel openers, such as minoxidil (Regaine RTM., Pharmacia Corp.) and diazoxide; 5-alpha-reductase inhibitors, such as finasteride (Propecia RTM., Merck & Co.); and the Immunosuppressant cyclosporin A.

[0084] It will be further appreciated by skilled persons that the compositions of the invention may be used *in vivo, ex vivo* or *in vitro.*

[0085] Thus, in addition to being applied or administered directly to a mammal, the compositions may be used to stimulate hair growth *ex vivo,* for example in a skin explant prior to grafting of the skin on to the mammal.

[0086] Alternatively, the compositions may be used to grow hair follicles *in vitro, e.g.* in cell culture, which may then be transplanted to a patient.

[0087] Also described herein is the use of a polypeptide according to the first aspect of the invention for stimulating hair growth *in vitro* or *ex vivo*.

[0088] For example, the polypeptide may be used to stimulate the growth of hair follicles (or stem cell precursors of the same).

[0089] Also described herein is a method for making a composition according to the first aspect of the invention, the method comprising admixing a modified osteopontin polypeptide in which an RGD domain is inactivated with a pharmaceutically acceptable and/or cosmetically acceptable excipient, carrier or diluent.

[0090] Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1 shows a schematic representation of the sampling area of skin on the mice.

Figure 2 is a representative photograph of a cross section of skin from a mouse 48 hours after being treated with a modified osteopontin polypeptide. Follicles within the epidermis are indicated by the arrow. The linear density of the follicles was 18.3 follicles per mm (598 follicles in 32.65 mm of epidermis).

Figure 3 is a representative photograph of a cross section of skin from a mouse 48 hours after being treated with a negative control composition. Follicles within the epidermis are indicated by the arrow. The linear density of the follicles was 11.7 follicles per mm (379 follicles in 32.50 mm of epidermis).

Figure 4 shows representative photographs of (a) a control-treated animal on day 14 having a hair growth score of 0, (b) an animal treated with exemplary 'full length' polypeptide "Cx" (corresponding to SEQ ID NO: 1 but without the initial sixteen amino acid signal peptide, 60 nM) on day 14 having a hair growth score of 1.5 (c) an animal treated with exemplary short peptide "FOL-004" (SEQ ID NO: 5, 60 nM) on day 5 having a hair growth score of 2.5 and (d) an animal treated with exemplary short peptide "FOL-005° (SEQ ID NO: 5, 63 nM) on day 5 having a hair growth score of 2.0.

Figure 5 shows the effect of exemplary 'full length' polypeptide "Cx" and polypeptides SEQ ID NOs: 5 and 63 of the invention on hair growth.

Figure 6 shows the effect of exemplary polypeptide SEQ ID NO: 5 on hair growth, as compared to the corresponding polypeptide SEQ ID NO: 121 from wildtype mouse osteopontin.

EXAMPLE A - *In* vivo study of hair growth effect of mutated mouse osteopontin in mice

Materials and Methods

Creation and production of test polypeptide

[0091] The modified osteopontin polypeptide sequence SEQ ID. NO:1 was cloned into a pCEP4 expression vector by the use of *XhoI* and *BamHI* restriction enzyme sites. The pCEP4 expression vector contains an ampicillin resistance gene for expression in bacteria and a hygromycin resistance gene for expression in eukaryotic EBNA cells. The polypeptide containing vector were transformed into competent XL-10 bacteria cells, multiplied and isolated with Qiagen midi prep kit. Isolated vectors were then transacted into human EBNA cells by the use of Fugene transfection reagent according to manufacture protocol (Invitrogen).

Isolation of test polypeptide

[0092] Medium from EBNA cell culture containing the produced polypeptide were collected after three to four days of culture. Polypeptides produced by pCEP4 plasmid contain an inserted His-tag, which is used to facilitate the isolation with Ni-sepharose gel chromatography (Invitrogen). Collected medium was diluted with binding buffer (20mM sodium phosphate, 500mM sodium chloride, pH 7.8), Ni-sepharose suspension was added before incubation on shaker overnight at 4°C. The Ni-sepharose gel was pelleted by centrifugation at 1000g for seven minutes and poured into a BioRad disposable mini column. Unbound proteins were removed with binding buffer followed by washing buffer (20mM sodium phosphate, 500mM sodium chloride, pH 6.0). Polypeptides were eluated from the column with 500mM imidazole.

General study design

[0093] The study consisted of four experimental groups each containing five male wtC57BL/6N mice.

[0094] On day -1 the necks of all participating mice were shaved carefully in squares of approx. 1.5 x 1.0 cm and small remaining hairpieces were removed. On the following day (day 0), each animal received four intracutaneous injections of 25µl each at separate locations (each approx. 0.5 x 0.5 cm) within the shaved rectangle (see Figure 1).

[0095] Each animal received two injections of 25µl of a composition comprising an exemplary modified osteopontin polypeptide of SEQ ID NO:1 ("Test polypeptide" or "Cx", 60 nmol/l in PBS) and two negative control injections of 25µl PBS (according to the scheme outlined in Table 1).

Table 1: Study Design

Group		Intracutaneous Application		Number of animals
		Volume	Time schedule after application	
1	Test polypeptide + PBS	25µl	Necropsy after 24h (day 1)	5
2	Test polypeptide + PBS	25µl	Necropsy after 48h (day 2)	5
3	Test polypeptide + PBS	25µl	Necropsy after 96h (day 4)	5
4	Test polypeptide + PBS	25µl	Necropsy after 336h (day 14)	5

[0096] Animals from Treatment Groups 1 to 4 were sacrificed 24h (Group 1), 48h (Group 2), 96h (Group 3) and 336h (14 days, Group 4) after compound application, respectively.

[0097] In all cases, the marked skin areas in the neck were removed and processed as follows: One of the polypeptide-treated skin samples and one of the PBS-treated control skin samples were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. The two other skin samples (polypeptide-treated and PBS-treated) were snap-frozen in liquid nitrogen and stored appropriately at -80°C.

[0098] A Hematoxylin-staining of the paraffin-embedded sections was performed.

Animals

Rationale	Accepted test system for the purpose of the study
Strain	male wtC57BL/6N
Source	Charles River GmbH
	Sandhofer Weg 7
	D 97633 Sulzfeld
Total number of animals	20
Age at delivery	5-6 weeks
Body weight and range	approx. 30g
(at acclimatisation)	
Identification	Labeling by ear mark
Acclimatisation	February, 17th to March, 1st 2011

Husbandry

Conditions	Optimum hygienic conditions, air-conditioned with 10-15 air changes per hour, and continually monitored environment with target ranges for temperature 22 ± 3°C and for relative humidity 30-70%, 12 hours artificial fluorescent light / 12 hours dark.
Accommodation	max. 3 animals per cage
Diet	M-Zucht
	ssniff Spezialdiäten GmbH
	Ferdinand Gabriel Weg 16
	D-59494 Soest
Water	Community tap water (autoclaved)

Test polypeptide

Identification	Modified osteopontin polypeptide of SEQ ID NO:1
Storage conditions	stored in Hepes buffer at 4°C
Safety precautions	prevent ingestion, inhaling, wear gloves and skin mask, wash with soap and water after skin contact; no special precautions
Sample preparation	delivered stock solution was diluted 15x with PBS to obtain the 60nM working solution before injection

Vehicle

Identity	PBS pH 7.4

Treatment

Route of administration:	intracutaneously
Frequency of administration	single application on day 0
Dose volume	25 µl

Observations

[0099] The following parameters were recorded:

Viability / Mortality	daily
Clinical signs	daily

Protocol for Hematoxylin-staining of paraffin-embedded sections of Group 1

[0100]

1. 1. Xylen bath 5min
2. 2. Xylen bath 5min
3. 3. EtOH 100% bath 5min
4. 4. EtOH 95% bath 5min
5. 5. EtOH 70% bath 5min
6. 6. PBS bath 5min
7. 7. Hematoxylin bath about 20sec (Harrys Hematoxylin)[a),b)]
8. 8. Water bath 3 times 3 min
9. 9. EtOH 70% bath 5min
10. 10. EtOH 95% bath 5min
11. 11. EtOH 100% bath 5min

12. 12. Xylen bath 5min
13. 13. Xylen bath 5min
14. 14. Mount slide using Permount and coveslip.
    1. a) the time depends on the type of hematoxylin used, 20sec to 10min (which can be determined by the use of test slides)
    2. b) filtrated before use

Results

[0101] The effect of treatment with a modified osteopontin polypeptide (SEQ ID NO:1) on hair follicle density is summarised in Table 1 (a) and (b) below.

Table 1(a) - Polypeptide-treated animals

Polypeptide treated Slide 1	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	21	0.90
area a3	25	0.95
area b1	13	0.90
area b2	21	0.90
area b3	26	0.95
Sum	124	5.50
Polypeptide treated Slide 2	Number of hair follicles	Length of epidermis, mm
area a1	16	0.90
area a2	18	0.90
area a3	25	0.95
area b1	14	0.90
area b2	18	0.90
area b3	19	0.95
Sum	110	5.50
Polypeptide treated Slide 3	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	13	0.90
area a3	19	0.95
area b1	20	0.90
area b2	17	0.90
area b3	14	0.90
Sum	101	5.45
Polypeptide treated Slide 4	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	17	0.90
area a3	15	0.90
area b1	18	0.90
area b2	17	0.90

Polypeptide treated Slide 4	Number of hair follicles	Length of epidermis, mm
area b3	13	0.90
Sum	98	5.40

Polypeptide treated Slide5	Number of hair follicles	Length of epidermis, mm
area a1	13	0.90
area a2	10	0.90
area a3	21	0.90
area b1	12	0.90
area b2	10	0.90
area b3	16	0.90
Sum	82	5.40

Polypeptide treated Slide 6	Number of hair follicles	Length of epidermis, mm
area a1	18	0.90
area a2	10	0.90
area a3	10	0.90
area b1	18	0.90
area b2	17	0.90
area b3	10	0.90
Sum	83	5.40

Polypeptide treated Slide7	Number of hair follicles	Length of epidermis, mm
Slide 34	124	5.50
Slide 38	110	5.50
Slide 42	101	5.45
Slide 46	98	5.40
Slide 50	82	5.40
Slide 54	83	5.40
Total	598	32.65

Table 1(b) - Control-treated animals

Control treated Slide 1	Number of hair follicles	Length of epidermis, mm
area a1	9	0.90
area a2	17	0.90
area a3	7	0.90
area b1	11	0.90
area b2	14	0.95
area b3	10	0.90
Sum	68	5.45

Control treated Slide 2	Number of hair follicles	length of epidermis, mm
area a1	12	0.90
area a2	17	0.90
area a3	7	0.90
area b1	12	0.90

Control treated Slide 2	Number of hair follicles	length of epidermis, mm
area b2	13	0.90
area b3	10	0.90
Sum	71	5.40
Control treated Slide 3	Number of hair follicles	Length of epidermis, mm
area a1	8	0.90
area a2	7	0.90
area a3	11	0.90
area b1	19	0.95
area b2	8	0.90
area b3	14	0.90
Sum	67	5.45
Control treated Slide 4	Number of hair follicles	Length of epidermis, mm
area a1	21	0.90
area a2	5	0.90
area a3	2	0.90
area b1	16	0.90
area b2	11	0.90
area b3	3	0.90
Sum	58	5.40
Control treated Slide 5	Number of hair follicles	Length of epidermis, mm
area a1	13	0.90
area a2	2	0.90
area a3	9	0.90
area b1	12	0.90
area b2	7	0.90
area b3	15	0.90
Sum	58	5.40
Control treated Slide 6	Number of hair follicles	Length of epidermis, mm
area a1	7	0.90
area a2	11	0.90
area a3	10	0.90
area b1	6	0.90
area b2	8	0.90
area b3	15	0.90
Sum	57	5.40
Control treated Slide 7	Number of hair follicles	Length of epidermis, mm
Slide 18	68	5.45
Slide 22	71	5.40
Slide 26	67	5.45

Control treated Slide 7	Number of hair follicles	Length of epidermis, mm
Slide 34	58	5.40
Slide 38	58	5.40
Slide 42	57	5.40
Total	379	32.50

[0102] A summary of the results is shown below in Table 2

Table 2

	No. of follicles analysed	No. of follicles per mm
Treatment with SEQ ID NO: 1	356	18.3
Control group	356	11.7

[0103] Representative tissue sections showing follicles in treated and control animals are shown in Figures 2 and 3.

[0104] By comparison, wildtype mouse osteopontin was observed to have no detectable effect on hair growth (data not shown).

Conclusions

[0105] The data show that treatment with the exemplary modified osteopontin polypeptide of SEQ ID NO: 1 increases the number of hair follicles/mm epidermis by about 60%.

[0106] These findings confirm the usefulness of the osteopontin-like polypeptides of the invention in stimulating hair growth.

EXAMPLE B - *In vivo* study of hair growth effect of SEQ ID NOS: 5 and 63 in mice

Materials and Methods

Animals

[0107] Mice (C57BL/6) were used at an age of 6 to 8 weeks.

Overview of study design

[0108]

- Selection of animals in telogen phase of hair growth.
- Clipping of dorsal back of animals
- Subcutaneous injection of test peptide/vehicle
- Visual analysis: Percentage anagen induction; Mean hair growth score; Visual melanogenesis:
- Histological analysis: Follicle count in subcutis; Morphometry for skin thickness.

Treatment groups

• Animals were assigned randomly to treatment groups (see Table 3)

[0109]

Table 3

Treatment	*No. of animals*	**Dose**	*Volume*
"Cx"	5	60 nM	25 µl
"FOL-004" (= SEQ ID NO:5)	5	60 nM	25 µl
"FOL-005" (= SEQ ID NO:63)	5	60 nM	25 µl
Vehicle control	5	-	25 µl

• Animals were administered the specified treatment on Days 1, 5 and 9

• Treatment were administered by subcutaneous injection into dorsal clipped skin

Scoring criteria

• The effect of the treatments was observed and scored daily according to the criteria in Table 4

[0110]

Table 4

Observation of dorsal skin	*Hair growth score*
No hair growth, pink skin	0
Skin colour changes from pink to gray without visible hair growth	0.5
Skin colour changes from pink to gray or black without visible hair growth, indicating the onset of anagen	1
Sparse hair growth	1.5
Diffuse short hair growth	2
Moderate hair growth	2.5
Dense, normal coat hair	3

Results

[0111] The effect of the exemplary polypeptides of the invention on hair growth is shown in Table 5.

Table 5

	Day											
Animal	***1***	***2***	***5***	***6***	***7***	***8***	***9***	***12***	***13***	***14***	***15***	***16***
(A) Vehicle (control)												
1	0	0	0	0	0	0	0	0	0	0	NA	NA
2	0	0	0	0	0	0	0	0	0	0	NA	NA
3	0	0	0	0	0	0	0	0	0	0	NA	NA
4	0	0	0	0	0	0	0	0	0	0	NA	NA
5	0	0	0	0	0	0	0	0.5	1	1	NA	NA
Mean	***0.0***	***0.0***	***0.0***	***0.0***	***0.0***	***0.0***	***0.0***	***0.1***	***0.2***	***0.2***	-	-

(B) "Cx" (SEQ ID NO:1 minus the initial 16 amino acid signal peptide)												
1	0	0	0	1	1.5	2	2.5	3	3	3	NA	NA
2	0	0	0	0.5	0.5	0.5	0.5	1	1	1	NA	NA
3	0	0	0	0.5	0.5	0.5	1	1.5	1.5	1.5	NA	NA
4	0	0	0	0.5	0.5	0.5	1	1.5	1.5	1.5	NA	NA
5	0	0	0	0	0	0	0.5	1	1.5	2	NA	NA
Mean	***0.0***	***0.0***	***0.0***	***0.5***	***0.6***	***0.7***	***1.1***	***1.6***	***1.7***	***1.8***	-	-
(C) FOL-004 (SEQ ID NO: 5)												
1	0	0	0.5	1	1	1	1	1.5.	1.5	1.5	2.5	3
2	0	0	2.5	2.5	3	3	3	3	3	3	3	3
3	0	0	2.5	2.5	2.5	3	3	3	3	3	3	3
4	0	0	1.5	2.5	3	3	3	3	3	3	3	3
5	0	0	1.5	2	2.5	2.5	3	3	3	3	3	3
Mean	***0.0***	***0.0***	***1.7***	***2.1***	***2.4***	***2.5***	***2.6***	***2.7***	***2.7***	***2.7***	***2.9***	***3.0***
(D) FOL-005 (SEQ ID NO: 63)												
1	0	0	2	2.5	3	3	3	3	3	3	3	3
2	0	0	2	3	3	3	3	3	3	3	3	3
3	0	0	0.5	0.5	1	1	1.5	2.5	2.5	2.5	3	3
4	0	0	2	2.5	2.5	3	3	3	3	3	3	3
5	0	0	0	0	0	0.5	1	2	2.5	2.5	3	3
Mean	***0.0***	***0.0***	***1.3***	***1.7***	***1.9***	***2.1***	***2.3***	***2.7***	***2.8***	***2.8***	***3.0***	***3.0***

[0112] Figure 4 shows representative photographs of (a) a control-treated animal on day 14 having a hair growth score of 0, (b) an animal treated with exemplary 'full length' polypeptide "Cx" (SEQ ID NO: 1, 60 nM) on day 14 having a hair growth score of 1.5 (c) an animal treated with exemplary short peptide "FOL-004" (SEQ ID NO: 5, 60 nM) on day 5 having a hair growth score of 2.5 and (d) an animal treated with exemplary short peptide "FOL-005" (SEQ ID NO: 5, 63 nM) on day 5 having a hair growth score of 2.0.

[0113] The results are summarised in Figure 5.

[0114] Exemplary polypeptides FOL-004 (SEQ ID NO: 5) and FOL-005 (SEQ ID NO: 63) both induced rapid hair growth, which was evident from day 5 and maintained until the end of the assessment period (day 16)..

[0115] A 'full length' polypeptide of the invention, ("Cx") also induced pronounced hair growth, albeit with a slower onset than the FOL-004 (SEQ ID NO: 5) or FOL-005 (SEQ ID NO: 63).

[0116] Animals treated with the vehicle control showed little sign of hair growth over the period of the experiment.

[0117] None of the polypeptides tested lead to any adverse effects in the animals (no body weight loss or any other discernible adverse symptom).

Conclusions

[0118] All mutated osteopontin polypeptides tested showed pronounced hair growth effects in vivo, with the shorter exemplary polypeptides FOL-004 (SEQ ID NO: 5) and FOL-005 (SEQ ID NO: 63) exhibiting a particular fast onset of action.

EXAMPLE C - *In vivo* study of hair growth effect in mice of the wildtype osteopontin fragment equivalent to SEQ ID NO: 5

Materials and Methods

[0119] Animals, as described above in Example B, were treated as described in Table 6:

Table 6

Treatment	*No. of animals*	*Dose*	*Volume*
"FOL-001"* (= SEQ ID NO:121)	6	60 nM	25 µl
* "FOL-001" consists of the following amino acid sequence: VDVPNGRGDSLAYGLR **[SEQ ID NO: 121]**			

[0120] This sequence is a fragment of wildtype mouse osteopontin, and corresponds to the region of the protein from which "FOL-004" is derived (i.e. "FOL-004" is a mutated version of wildtype fragment "FOL-001").

- Animals were administered the specified treatment on Days 1, 5 and 9
- Treatment were administered by subcutaneous injection into dorsal clipped skin

Scoring criteria

[0121]

- The effect of the treatments was observed and scored daily as described in Example B.

Results

[0122] The effect of the polypeptide "FOL-001" on hair growth is shown in Table 7 and Figure 6.

Table 7

	Day											
Animal	***1***	**2**	***5***	**6**	***7***	***8***	***9***	***12***	***13***	***14***	***15***	***16***
FOL-001 (SEQ ID NO: 121, 60nM)												
1	0	0	0.5	0.5	0.5	0.5	1	2	2	2.5	2.5	NA
2	0	0	0.5	0.5	0.5	0.5	1	2	2	2.5	2.5	NA
3	0	0	0	0	0	0	0.5	1	1	1	1	NA
4	0	0	0	0	0	0	0	0	0	0	0	NA
5	0	0	0	0	0	0	0.5	1	1	1	1	NA
6	0	0	0	0	0	0	0.5	1	1	1	1	NA
Mean	**0**	**0**	**0.17**	**0.17**	**0.17**	***0.17***	***0.58***	***1.17***	***1.17***	***1.33***	***1.33***	**-**

[0123] Peptide FOL-001 (SEQ ID NO: 121) produced a delayed but detectable hair growth effect in mice, which became evident at about day 9 and reached a plateau at a score of about 1.3.

Conclusions

[0124] Compared to the corresponding mutated peptide sequence of the invention (FOL-004; SQE ID NO:5), the equivalent non-mutated wild type sequence exhibited a delayed onset of hair growth stimulation with a maximum effect of less than 50% of that observed with FOL-004.

[0125] Thus, the exemplary polypeptide shows much greater efficacy than the equivalent non-mutated wild type sequence.

EXAMPLE *D - In vivo* study of anagen induction in peeled skin

Materials and Methods

[0126] Animals and treatments are as described above in Example B.

[0127] All animals were sacrificed on day 16.

Observation of melanogenesis

[0128] Following completion of the term of hair growth assessment for Example B, the animals were euthanized and dorsal skin peeled and removed for observation of the internal surface.

Histological analysis

[0129] Peeled skin sections were prepared for histological analysis as described in Example A. Follicle count in the subcutis and skin thickness were then measured.

Results

[0130] Results are summarised in Tables 8 and 9 below.

Table 8

Observation of melanogenesis					
Group	**Skin colour (externa)**	**% anagen induction***	**Black colour in peeled skin (day 16)**	**% anagen induction****	**No.of animals showing hair growth**
Cx(60nM	Black (5/5 animals)	100	3/5	60	5/5
FOL-004 (60nM)	Black (5/5 animals)	100	1/5	20	5/5
FOL-005 (60nM)	Black (5/5 animals)	100	3/5	60	5/5
Vehicle	Pink (4/5 animals)	20	1/5	20	1/5

* with respect to external skin colour change from pink to black

** with respect to black colour in peeled skin

Table 9a (all animals)

Group	**No. of animals considered**	**Mean ± sem**	
		Follicle count in subcutis (no.)	**Skin thickness**
Cx (60nM	5	22.20 ± 10.69	308.8 ± 21.94

Group	No. of animals considered	Mean ± sem	
		Follicle count in subcutis (no.)	Skin thickness
FOL-004 (60nM)	5	6.20 ± 5.80	312.2 ± 15.60
FOL-005 (60nM)	5	34.80 ± 14.58	348.80 ± 40.05
Vehicle	4*	0 ± 0	246.5 ± 8.35
* Animal No. 5 was found to be a significant outlier (p<0/05, Grubb's test) and so was ignored in analysis			

Table 9b (animals in anagen only)

Group	No. of animals considered	Mean ±sem	
		Follicle count in subcutis (no.)	Skin thickness
Cx (60nM	3/5	36.67 ± 10.69	308.8 ± 21.94
FOL-004 (60nM)	1/5	29 ± 5.8	312.2 ± 15.60
FOL-005 (60nM)	3/5	58 ± 14.58	348.80 ± 40.05
Vehicle	0/4*	0 ± 0	246.5 ± 8.35

[0131] The hair growth cycle consists of three phases: a resting telogen phase (C57BL/6 skin is a pale pink colour), an active hair growth anagen phase (where the skin becomes dark gray or black), and finally a catagen phase (where hair growth stops, and the skin transitions back to the telogen phase, returning to a pale pink colour).

[0132] In the present study, the animals were preselected for pink skin (telogen phase) and the test polypeptide was administered by subcutaneous/topical route. A good hair growth promoter triggers the transition from the resting telogen phase to the active anagen phase, and thus a transition from light skin to dark skin. This dark pigmentation may result from the collection of melanin in the hair follicles, in preparation for new hair growth during the anagen phase. Melanin synthesis of follicular melanocytes is strictly coupled to the anagen phase, ceases during catagen and is absent in telogen phase. Hence a good hair growth promoter causes blackening of dorsal skin. Upon termination, the dorsal skin of the animals was then excised out by peeling and the peeled skin reversed and observed for the induction of melanogenesis indicated by visual blackening. Histological analysis reveals number of follicles and skin thickness. A good hair growth promoter increases no. of follicles in sub cutis and skin thickness.

[0133] In the present study, all three test polypeptides led to the appearance of dense hair growth after three subcutaneous injections (see Example B above). It was also observed that in some of the animals, subsequent to full growth of the hair, the skin colour changed from black to gray to pink, a characteristic of catagen phase. The peeled skin collected in the study showed the black patch, indicative of anagen phase (active phase) of hair growth cycle due to melanocyte sequestering. In these animals, the histological results showed a large number of hair follicles and increase in skin thickness. But in some animals, where the catagen phase had occurred, peeled skin was found to be of pink colour, even though the hair coat was intact. Thus, analysis of such peeled skin sections demonstrated lack of follicles in subcutis but still good increase in skin thickness.

Conclusions

[0134] The exemplary mutated osteopontin polypeptides of the invention showed pronounced induction of anagen, as evidenced by an increase in the number of hair follicles in the subcutis and/or enhanced skin thickness.

REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. Komposition til stimulering af hårvækst i et pattedyr, omfattende:

(a) et modificeret osteopontin-polypeptid, i hvilket et RGD-domæne er inaktiveret; og

(b) en farmaceutisk acceptabel og/eller kosmetisk acceptabel excipiens, bærer eller fortyndingsmiddel,

hvor det modificerede osteopontin-polypeptid består af en aminosyresekvens ifølge SEQ ID NO: 63.

2. Polypeptid, som består af aminosyresekvensen ifølge SEQ ID NO: 63.

DRAWINGS

FIGURE 1

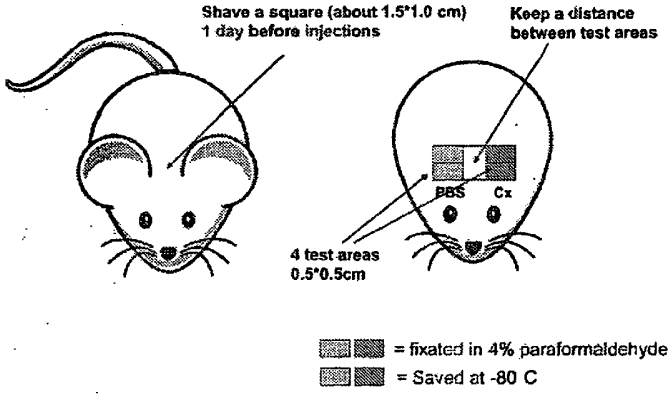


FIGURE 2

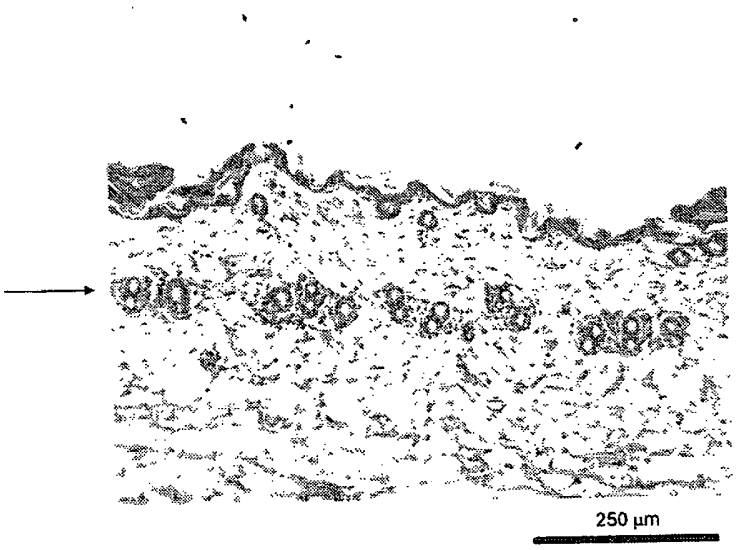


FIGURE 3

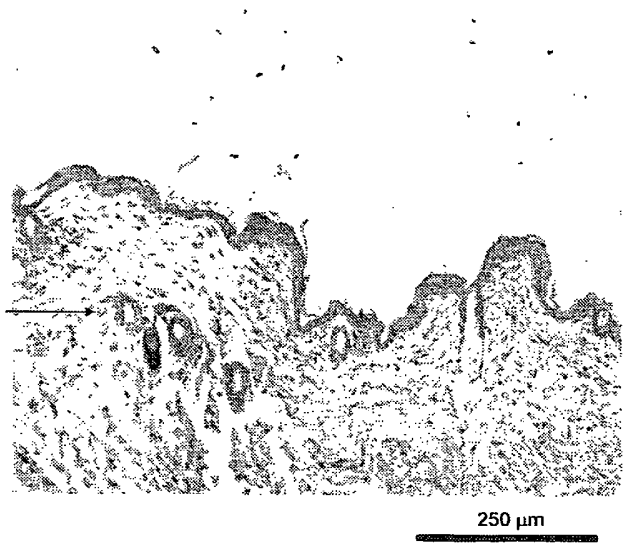


FIGURE 4(A)

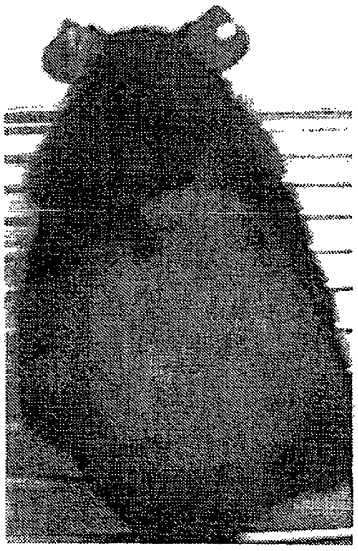


FIGURE 4(B)

FIGURE 4(C)

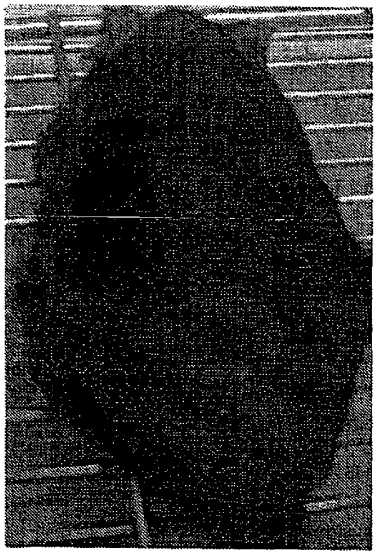


FIGURE 4(D)

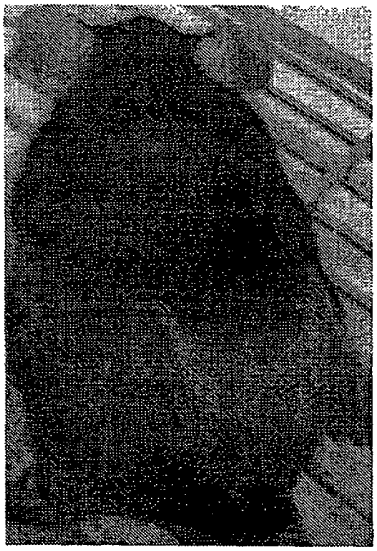
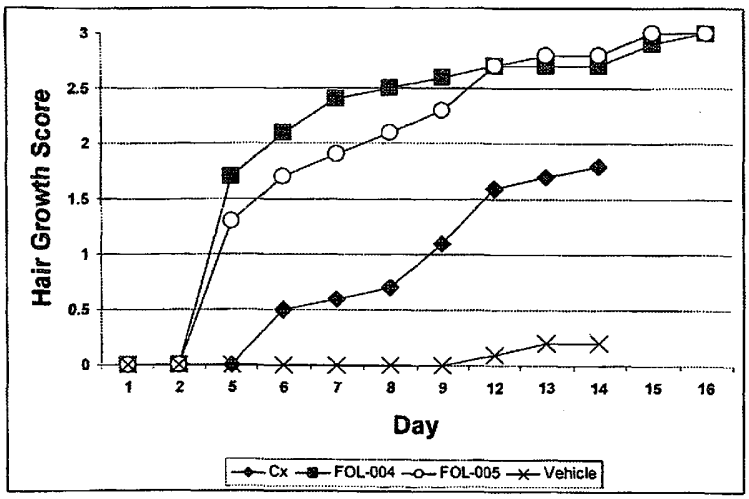


FIGURE 5


Hair Growth Score
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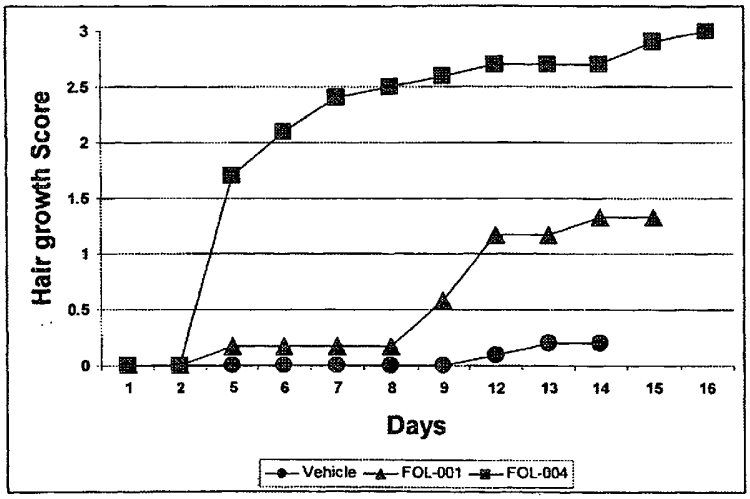
2.5
2
1.5
1
0.5
0
1
2
5
6
7
8
9
12
13
14
15
16
Day
Cx
FOL-004
FOL-005
Vehicle

FIGURE 6


Hair growth Score
3
2.5
2
1.5
1
0.5
0
1
2
5
6
7
8
9
12
13
14
15
16
Days
Vehicle
FOL-001
FOL-004