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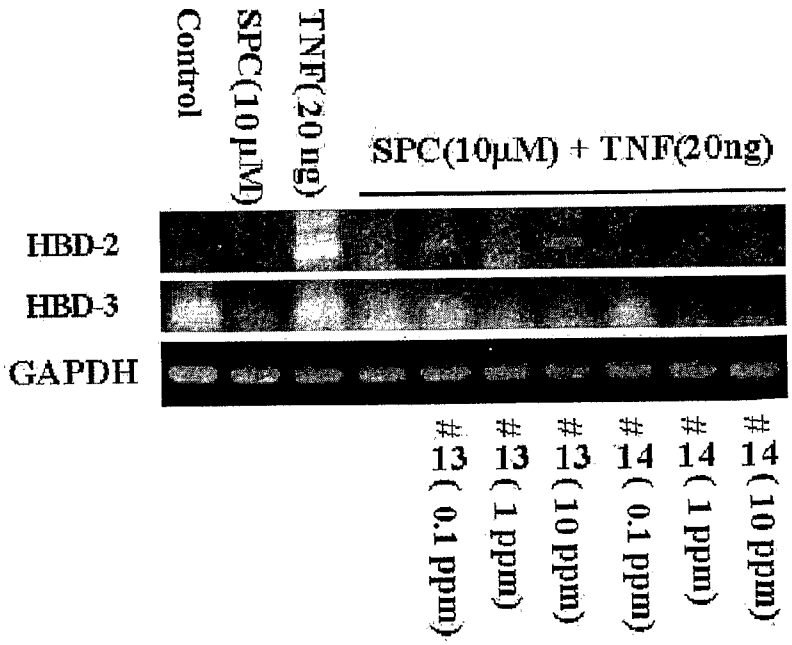
[Continued on next page]

(54) Title: USE OF SPHINGOSYLPHOSPHORYLCHOLINE ANTAGONIST FOR RESTORING THE EXPRESSION OF ANTIMICROBIAL PEPTIDES



(57) Abstract: SPC suppresses the expression of human beta defensins (HBD-1, HBD-2, HBD-3 and HBD-4), cathelicidin (LL-37), and dermcidin in atopic dermatitis patients, and accordingly, SPC antagonists are useful for preventing and treating disorders related to the decreased expression of AMPs.

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Description

USE OF SPHINGOSYLPHOSPHORYLCHOLINE ANTAGONIST FOR RESTORING THE EXPRESSION OF ANTIMICROBIAL PEPTIDES

Technical Field

[1] The present invention relates to a method for restoring the expression levels of antimicrobial peptides (AMPs), e.g., in the skin of an atopic dermatitis patient, using sphingosylphosphorylcholine (SPC) antagonists, a use of SPC antagonists for the manufacture of a medicament for restoring the AMPs expression, and a method for screening an SPC antagonist by analyzing the ability of candidate compounds for restoring the expression level of AMPs.

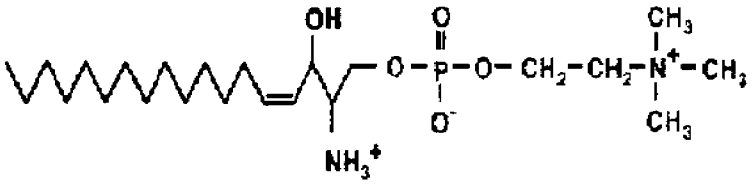
[2]

Background Art

[3] Sphingosylphosphorylcholine (SPC) is a member of the lysosphingolipid family and has the form of N-deacylated sphingomyeline of the following formula.

[4]

[5] ChemistryFigure 1



[6]

[7] SPC is a mitogen generated from sphingomyelin (SM) by SM N-deacylase (Higuchi K et al., *Biochem. J.* (2000), 350, 747-756), and is known to play a role in the Ca^{2+} release from endoplasmic reticulum and is also involved in the cell growth, proliferation(Desai et al., *Biochem. Biophys. Res. Commun.* (1991), 181, 361-366) and apoptosis(Jeon ES et al., *Biochim Biophys Acta.* (2005), 1734(1); 25-33). In particular, SPC has been demonstrated to affect the cell reconstitution or cell migration in various cells. For instance, it mediates wound healing in fibroblasts, and regulates inflammation, epidermal differentiation, morphogenesis and melanogenesis in keratinocytes(Berger et al., *Proc. Natl. Acad. Sci. U. S. A.* (1995), 92, 5885-5889). Several reports have recently shown that 1) the expression of SPC is significantly enhanced in the stratum corneum obtained from patients with atopic dermatitis (AD) as compared with that of healthy control subjects (Reiko Okamoto et al., *Journal of Lipid Research* (2003), 44, 93-102), 2) the activity of SM deacylase is abnormally high in the

epidermis of patients with AD (Higuchi K et al., *Biochem. J.* (2000), 350 747-756), and 3) some of the disorders of the skin barrier function are caused by a lowered level of the synthesis of ceramide by sphingomyelinase (Junko Hara et al., *J. Invest. Dermatol.* (2000), 115, 406-413). However, there has never been reported to date the mechanism that the increased SPC expression causes skin disorders such as AD.

[8] Antimicrobial peptides (AMPs) are small molecular weight proteins with antimicrobial activity for preventing infection by several pathogens such as bacteria, viruses and fungi (Ganz T et al., *Curr. Opin. Immunol.* (1994), 6, 584-589). AMPs have been demonstrated to induce angiogenesis, wound healing and chemotaxis (Izadpanah A et al., *J. Am. Acad. Dermatol.* (2005), 52, 381-390), and have a structural feature of being positively charged and possessing hydrophilic and hydrophobic residues.

[9] Recently, it has been established that an abnormal growth of *S. aureus* in the inflamed skin of a patient with AD is caused by the decrease in the expression of human β-defensin (HBD-2) and cathelicidin (LL-37) known as AMPs, and especially, the expression levels of HBD-2 and LL-37 are significantlylower in atopic lesionsthan in psoriatic lesions (Ong P. Y. et al., *N. Engl. J. Med.* (2002), 347, 1151-1160).

[10] Human defensins can be classified into alpha and beta defensins according to their structural features. The alpha defensins are arginine-rich monomeric polypeptides each composed of about 30-35 amino acids, and 6 isoforms thereof (HNP-1 to -4, HD-5, and HD-6) have been identified in human. In case of beta-defensins each composed of about 41-50 amino acids, 11 isoforms have been biochemically identified in human serum (*Biochemistry and Molecular Biology News* (2006), 1-7), and 28 human beta-defensin genes and 43 mouse beta-defensin genes have been identified to date by the human genome project (B.C. Schutte et al., *Proceedings of the National Academy of Sciences USA* (2002), 99, 2129-2133).

[11] Among these human beta defensins (HBDs), whose expression has been reported to be depressed in the skin of AD patients, HBD-1 is mainly expressed in the epithelial cells of kidney, female genital organs, respiratory organs, pancreas, the gum, tongue and tympanum, while HBD-2 is expressed in the skin, lung, respiratory tract, gum and tympanum, but is totally absent in urinary organs such as kidney and bladder, and salivary glands (J. Harder et al., *Nature* (1997), 387, 861). Further, HBD-3 is expressed in skin, while HBD-4 is expressed in male genital organs and stomach.

[12] HBD-1 and HBD-3 are constitutively expressed whereas the expression of most HBDs including HBD-2 and HBD-4 are inducible only in response to specific condition such as infection (J.M. Schroder et al., *Int. J. Biochem. Cell Biol.* (1999), 31, 645-651). Among these inducible HBDs, the expression of HBD-2 has been suggested to be upregulated by NF-κB through MEK1/2-ERK1/2 signal pathway (Tsutsumi-Ishii

Y et al., *J. Leukoc. Biol.* (2002), 71, 154-162), and enhanced by TNF-α, IL-1α, IL-1β or LPS (KW Cha et al., *Proc. Natl. Acad. Sci.* (2000), 97, 3016-3021) because the *HBD-2* gene promoter region includes NF-κB binding sites. Further, the expression of HBD-4 has been reported to be inducible by microbes such as *S. pneumoniae* via PMA-PKC signal pathway (J.R. Garcia et al., *FASEB J.* (2001), 15, 1819-1821), and the expression level of HBD-3 is elevated in response to TNF-α, IL-1β and IFN-γ.

[13] The present inventors have therefore endeavored to establish the pathological mechanism of atopic dermatitis, and have found that SPC dramatically reduces the expression of AMPs, especially HBD-1 to -4 which belong to the human defensin beta families and the expression thereof has been reported to decrease in the skin of AD patients, cathelicidin (LL-37), and dermcidin.

[14]

Disclosure of Invention

Technical Problem

[15] Accordingly, it is an object of the present invention to provide a method for restoring the expression of antimicrobial peptides (AMPs) to an effective level in a mammal having reduced AMPs expression.

[16] It is another object of the present invention to provide a use of sphingosylphosphorylcholine (SPC) antagonists for the manufacture of a medicament for restoring the expression of AMPs in a mammal.

[17] It is a further object of the present invention to provide an efficient method for screening a candidate for restoring the expression of AMPs.

[18]

Technical Solution

[19] In accordance with one aspect of the present invention, there is provided a method for restoring the expression of AMPs in a mammal in need of the restoration of the AMPs expression, comprising the step of administering SPC antagonists in a therapeutic dose to the mammal.

[20] In accordance with another aspect of the present invention, there is provided a use of an SPC antagonist for the manufacture of a medicament for restoring the expression of AMPs to normal levels in a mammal in need of such restoration.

[21] In accordance with a further aspect of the present invention, there is provided a method for screening an antagonist of SPC comprising the steps of: 1) treating a subject with SPC or a derivative thereof to downregulate the expression of AMPs; and 2) treating the subject with a SPC antagonist candidate and analyzing the restored levels of the AMPs expression in the subject

[22]

Brief Description of the Drawings

[23] The above and other objects and features of the present invention will become apparent from the following description of the invention, when taken in conjunction with the accompanying drawings, which respectively show:

[24] FIG. 1: RT-PCR analysis results showing that the mRNA expression levels of HBD-1 to -4, hCAP-18/LL-37, and DCD in HaCaT cells, which are constitutive or induced by TNF-α, are suppressed in an SPC dose-dependent manner;

[25] FIG. 2: RT-PCR analysis results showing that the mRNA expression levels of HBD-2, HBD-3 and DCD in HaCaT cells, which are constitutive or induced by PMA, are suppressed in an SPC dose-dependent manner;

[26] FIG. 3: RT-PCR analysis results showing that the mRNA expression levels of HBD-2 and HBD-3 in HaCaT cells, which are induced by TNF-α, and suppressed by SPC, are restored by treating 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine; and

[27] FIG. 4: Immunohistochemistry assay results showing that the protein expression of HBD-1 and HBD-2 in ICR mice, induced by PMA and suppressed by SPC, is restored to normal levels by treating 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine.

[28]

Mode for the Invention

[29] The SPC antagonists used in the inventive method for restoring the expression of AMPs to normal levels may be any one of the known SPC antagonists which can inhibit the activity of SPC or antagonize the mechanism that SPC downregulates the AMPs expression.

[30] The representative examples of the SPC antagonists include a competitive inhibitor which binds to active sites of SPC, a noncompetitive inhibitor which binds to a site other than active sites of SPC to inactivate the active sites, an inhibitor for the intracellular localization of SPC, a material degrading SPC or inducing the SPC degradation, and a blocking agent for the SPC-induced pathway of downregulating the expression of AMPs.

[31] In the present invention, the SPC antagonists may be systemically or locally administered to a subject in need of restoration of the AMPs expression, such as a mammal including human with a disorder related to reduction of the AMPs expression, e.g., atopic dermatitis (AD). A suitable single dose of the SPC antagonists in the inventive method may be determined in light of various relevant factors including the condition to be treated, the route of administration, the age and weight of the patient, and the severity of the patient's symptoms.

[32] Further, the present invention provides a method for screening a viable antagonist of SPC comprising the steps of 1) treating a subject with SPC or a derivative thereof to downregulate the expression of AMPs; and 2) treating the subject with an SPC antagonist candidates and analyzing the restored level of the AMPs expression in the subject. In the inventive method, the subject may be a test cell or mammal. The test cell may be any one of the known cells, preferably keratinocytes, expressing AMPs or capable of expressing AMPs responsively to inducing materials, and the mammal may be a rodent such as a mouse, rat or marmot, perferably a hairless mouse.

[33] According to one embodiment of the present invention, in step 1, SPC or a derivative thereof may be administered to a subject in a concentration ranging from about 1 nM to about 40 mM (from about 0.002%(w/w) to about 2%(w/w)). When the concentration is less than 1 nM, the desired suppression of the AMPs expression cannot be achieved, and when more than 40 mM, the test cell may undergo morphological change, or adverse effects may occur in the mammal. Further, when AMPs exhibiting inducible expression, e.g., HBD-2 and HBD-4 are employed in the analysis of the inventive method, step 1 may further comprise treating the subject with an inducing material for the AMPs expression before or concurrently with the SPC treatment. The inducing material for the AMPs expression may be selected from the group consisting of Ca^{2+}, TNF-α, IL-1β, IFN-γ and phorbol 12-myristate 13-acetate (PMA), preferably TNF-α and PMA.

[34] In step 2, the subject undergone downregulation of the AMPs expression in step 1 may be treated with an SPC antagonist candidate and the restored levels of the AMPs expression are analyzed. The expression levels of AMPs may be analyzed using one of the known methods employed for measuring gene or protein expression. For example, the gene expression of AMPs may be analyzed by RT-PCR, or blot analysis such as northern blot and using their genes or fragments thereof as probes, and the protein expression of AMPs, by way of conducting enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), sandwich assay, western blot, immunoblot or immunohistochemical staining, using antibodies against AMPs.

[35] SPC significantly reduces the expression of human beta defensins (HBD-1, HBD-2, HBD-3 and HBD-4), cathelicidin (LL-37), and dermcidin, which are reported to be depressed in patients with atopic dermatitis. Accordingly, SPC antagonists are useful for preventing and treating disorders related to the decreased expression of AMPs, such as atopic dermatitis, and a viable SPC antagonist can be efficiently screened by analyzing the restory levels of the AMPs gene or protein expression.

[36]

[37] The following Examples are intended to further illustrate the present invention without limiting its scope.

[38] Further, percentages given below for solid in solid mixture, liquid in liquid, and solid in liquid are on a wt/wt, vol/vol and wt/vol basis, respectively, and all the reactions were carried out at room temperature, unless specifically indicated otherwise.

[39]

[40] **Reference Example: Analysis for the expression level of AMPs**

[41]

[42] In the following Examples, the mode of mRNA expression of AMPs in test and control cells were analyzed by performing RT-PCR more than 3 times ({94℃, 1 min, 51~60℃, 1 min, and 72℃, 2 min} 50 cycles; primer conc.: 5 pmol/reaction, template content: 50 ng/reaction) using primers specific for the genes of human defensin families (HBD-1 to -4), cathelicidin (hCAP-18/LL-37), dermcidin (DCD), and GAPDH as a control. The sequences of the employed primers are listed in Table 1.

[43]

[44] Table 1

AMPs	Primer Sequence	
	Forward	Reverse
HBD-1	SEQ ID NO: 1	SEQ ID NO: 2
HBD-2	SEQ ID NO: 3	SEQ ID NO: 4
HBD-3	SEQ ID NO: 5	SEQ ID NO: 6
HBD-4	SEQ ID NO: 7	SEQ ID NO: 8
hCAP-18/LL-37	SEQ ID NO: 9	SEQ ID NO: 10
DCD	SEQ ID NO: 11	SEQ ID NO: 12
GAPDH (control)	SEQ ID NO: 13	SEQ ID NO: 14

[45]

[46] The mRNA expression levels of AMPs were examined by scanning the resulting RT-PCR photographs to estimate the respective band densities, and testing the estimated density values using the student's t-test. The difference between the test and control cells was judged to be significant only when the estimated p-values of the test and control groups were under 0.05.

[47]

[48] **Example 1: Analysis for the reduction of the AMPs expression by SPC**

[49]

[50] (1) Analysis in test cells

[51]

[52] Human keratinocyte cell line, HaCaT (Dr. N.E. Fusenig, Duetsches Krebs-

forschungszentrum, Heidelberg, Germany) was seeded in a 6 well-plate at an amount of $1x10^5$ cells/well, and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin-streptomycin, Gibco ,USA) for 24 hours under the conditions of 37℃ and 5% CO_2.

[53] Some of the cultured cells were treated with 20 ng of TNF-α or 40 or 80 nM phorbol 12-myristate 13-acetate (PMA) to induce the expression of AMPs exhibiting inducible expression, concurrently with 0, 1, 10 or 20 μM SPC. Some of the cultured cells were kept untreated (control cells). In accordance with the method of Reference Example, the test and control cells were subjected to RT-PCR, to analyze their mRNA expression levels of AMPs as function of the SPC treating concentration. The results are shown in FIGs. 1 and 2.

[54] As shown in FIGs. 1 and 2, the mRNA expression levels of HBD-1 to -4, hCAP-18/LL-37, and DCD were significantly suppressed in HaCaT cells in an SPC dose-dependent manner. Especially, the mRNA expression levels of HBD-2, HBD-3 and DCD were clearly suppressed by the SPC treatment.

[55]

[56] (Step 2) Analysis in test animals

[57]

[58] 8 week old male weighing about 25~30 g ICR mice (Charles River Laboratories, MA, USA) were subjected to a treatment to their skin of the ear, back and abdomen regions by 20 μl of PMA in acetone (2.5 μg/μl) for inducing the expression of AMPs, and concurrently, some of these mice were applied with 20 μl of 0.1% or 1% SPC, respectively, followed by the same SPC treatment after 6 hours therefrom. Some mice not treated with PMA were applied with only acetone (control group).

[59] After 24 hours, tissue samples were collected from the applied skins by 6 mm punching, and each sample was embedded in an OCT (optimal cutting temperature) compound, and frozen at -20℃. Each frozen tissue sample was sliced to a thickness of 4 μm, fixed on a slide glass, and incubated with anti-HBD-1 or anti-HBD-2 antibody (Santa cruse, USA) at room temperature for more than 1 hour. The obtained samples were each subjected to an immunohistochemical analysis using Envision kit (DAKO, USA). The results are shown in FIG. 4 ((a) to (d)).

[60] As the results in FIG. 4 ((a) to (d)) shows, the protein expressions of HBD-1 and HBD-2 in the skin of ICR mice were significantly reduced in an SPC dose-dependent manner.

[61]

[62] **Example 2: Screening for SPC antagonists by analyzing restory level of the AMPs expression**

[63]

[64] (1) Analysis in test cells

[65]

[66] HaCaT cells were seeded in a 6 well plate at a concentration of $1x10^5$ cells/well, and cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco) for 24 hours under the conditions of 37℃ and 5% CO_2.

[67] The cultured cells were treated with 20 ng of TNF-α for inducing the expression of HBD-2, together with 10 μM SPC for suppressing the AMPs expression. Then, they were each treated with 0, 0.1, 1 or 10 ppm of 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine. Some of the cultured cells were treated with nothing (control cells), or only with 20 ng of TNF-α or 10 μM SPC. The test and control cells were subjected to RT-PCR according to the method of Reference Example, to determine the mRNA expression levels of HBD-2 and HBD-3. The results are shown in FIG. 3.

[68] As shown in FIG. 3, the mRNA expression of HBD-2 and HBD-3 were significantly restored in the test cells treated with 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine.

[69] Therefore, these results suggest that SPC antagonists can be easily detected by the method of the present invention.

[70]

[71] (2) Analysis in test cells

[72]

[73] 20 μl of PMA (2.5 μg/μl) was applied on the skin of the ear, back and abdomen regions of each ICR mice 8 week old male weighing about 25~30 g to induce the expression of HBD-1 and HBD-2, together with 20 μl of 1% SPC for the suppression of the AMPs expression. Some of these mice were treated with 20 μl of 1% of 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine, followed by the same SPC treatment after 6 hours therefrom.

[74] After 24 hours, tissue samples were collected from the applied skins by 6 mm punching, embedded in an OCT (optimal cutting temperature) compound, and frozen at -20℃. Each frozen tissue sample was sectioned to a thickness of 4 μm, fixed on a slide glass, and incubated with anti-HBD-1 or anti-HBD-2 antibody (Santa cruse, USA) at room temperature for more than 1 hour. Each sample was subjected to an immunohistochemical analysis using Envision kit (DAKO, USA) and the results are shown in FIG. 4((d) to (f)).

[75] As the results in FIG. 4 ((d) to (f)) show, the suppressed levels of the expression of HBD-1 and HBD-2 by SPC were restored to normal levels in the test groups treated with 2-amino-2-octyloxymethyl-propane-1,3-diol or N-(2-(naphthalen-2-yloxy)ethyl)prop-2-en-1-amine.

[76] Therefore, these results suggest that SPC antagonists can be easily detected by the method of the present invention.

[77]

[78] While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the invention by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.

[79]

Sequence Listing

[80] <110> AMOREPACIFIC CORPORATION

[81]

[82] <120> METHOD FOR RECOVERING EXPRESSION OF ANTIMICROBIAL PEPTIDES BY

[83] USING SPHINGOSYLPHOSPHORYLCHOLINE ANTAGONIST

[84]

[85] <130> PCA60939/TPY

[86]

[87] <160> 14

[88]

[89] <170> KopatentIn 1.71

[90]

[91] <210> 1

[92] <211> 27

[93] <212> DNA

[94] <213> Artificial Sequence

[95]

[96] <220>

[97] <223> forward primer specific for HBD-1

[98]

[99]

[100] <400> 1

[101] ttgtctgaga tggcctcagg tggtaac 27

[102]

[103]

[104] <210> 2

[105] <211> 26

[106] <212> DNA

[107] <213> Artificial Sequence

[108]

[109] <220>

[110] <223> reverse primer specific for HBD-1

[111]

[112]

[113] <400> 2

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[115]

[116]

[117] <210> 3

[118] <211> 20

[119] <212> DNA

[120] <213> Artificial Sequence

[121]

[122] <220>

[123] <223> forward primer specific for HBD-2

[124]

[125]

[126] <400> 3

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[128]

[129]

[130] <210> 4

[131] <211> 20

[132] <212> DNA

[133] <213> Artificial Sequence

[134]

[135] <220>

[136] <223> reverse primer specific for HBD-2

[137]

[138]

[139] <400> 4

[140] gagaccacag gtgccaattt 20

[141]

[142]

[143] <210> 5

[144] <211> 21

[145] <212> DNA

[146] <213> Artificial Sequence

[147]

[148] <220>

[149] <223> forward primer specific for HBD-3

[150]

[151]

[152] <400> 5

[153] agcctagcag ctatgaccat c 21

[154]

[155]

[156] <210> 6

[157] <211> 21

[158] <212> DNA

[159] <213> Artificial Sequence

[160]

[161] <220>

[162] <223> reverse primer specific for HBD-3

[163]

[164]

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[167]

[168]

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[173]

[174] <220>

[175] <223> forward primer specific for HBD-4

[176]

[177]

[178] <400> 7

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[180]

[181]

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[183] <211> 20

[184] <212> DNA

[185] <213> Artificial Sequence

[186]

[187] <220>

[188] <223> reverse primer specific for HBD-4

[189]

[190]

[191] <400> 8

[192] catgcatagg tgttgggaca 20

[193]

[194]

[195] <210> 9

[196] <211> 20

[197] <212> DNA

[198] <213> Artificial Sequence

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[211] <213> Artificial Sequence

[212]

[213] <220>

[214] <223> reverse primer specific for hCAP-18/LL-37

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[224] <213> Artificial Sequence

[225]

[226] <220>

[227] <223> forward primer specific for DCD

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[232]

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[235] <211> 21

[236] <212> DNA

[237] <213> Artificial Sequence

[238]

[239] <220>

[240] <223> reverse primer specific for DCD

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[245]

[246]

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[248] <211> 20

[249] <212> DNA

[250] <213> Artificial Sequence

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[253] <223> forward primer specific for GAPDH

[254]

[255]

[256] <400> 13

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[258]

[259]

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[262] <212> DNA

[263] <213> Artificial Sequence

[264]

[265] <220>

[266] <223> reverse primer specific for DCD

[267]

[268]

[269] <400> 14

[270] atgagcccca gccttctcca t 21

[271]

[272]

[273]

[274]

[275]

Claims

[1] A use of a sphingosylphosphorylcholine antagonist for the manufacture of a medicament for restoring the expression of antimicrobial peptides to normal levels in a mammal in need of such restoration.

[2] The use of claim 1, wherein the sphingosylphosphorylcholine antagonist restore the expression of antimicrobial peptides by inhibiting the activity of sphingosylphosphorylcholine or antagonizing the pathway through which sphingosylphosphorylcholine downregulates the expression of antimicrobial peptides.

[3] The use of claim 2, wherein the sphingosylphosphorylcholine antagonist is selected from the group consisting of: a competitive inhibitor which binds to active sites of sphingosylphosphorylcholine, a noncompetitive inhibitor which binds to a site other than active sites of sphingosylphosphorylcholine to inactivate the active sites, an inhibitor for the intracellular localization of sphingosylphosphorylcholine, a material that induces the sphingosylphosphorylcholine degradation, a blocking agent for the sphingosylphosphorylcholine-induced pathway of downregulating the expression of antimicrobial peptides, and a mixture thereof.

[4] The use of claim 1, wherein the mammal suffers from atopic dermatitis.

[5] The use of claim 1, wherein the sphingosylphosphorylcholine antagonist is systemically or locally administered to the mammal.

[6] A method for screening an antagonist of sphingosylphosphorylcholine comprising the steps of:

1) treating a subject with sphingosylphosphorylcholine or a derivative thereof to downregulate the expression of antimicrobial peptides; and

2) treating the subject with a sphingosylphosphorylcholine antagonist candidate and analyzing the restored levels of the antimicrobial peptides expression in the subject.

[7] The method of claim 6, wherein the subject is a test cell or a mammal excluding human.

[8] The method of claim 7, wherein the test cell expresses antimicrobial peptides or is capable of expressing antimicrobial peptides responsively to an inducible material.

[9] The method of claim 8, whererin the test cell is keratinocyte.

[10] The method of claim 7, wherein the mammal is a mouse, rat or marmot.

[11] The method of claim 6, wherein sphingosylphosphorylcholine or a derivative thereof is administered to the subject at a concentration ranging from 1 nM to 40 mM in step 1).

[12] The method of claim 6, which further comprises treating the subject with an inducing material for the antimicrobial peptides expression before or concurrently with the sphingosylphosphorylcholine treatment in step 1.

[13] The method of claim 12, wherein the inducing material is selected from the group consisting of Ca^{2+}, TNF-α, IL-1β, IFN-γ, phorbol 12-myristate 13-acetate (PMA) and a mixture thereof.

[14] The method of claim 6, wherein the restored levels of the antimicrobial peptides expression are analyzed in step 2 by measuring the gene or protein expression levels of antimicrobial peptides.

[15] The method of claim 14, wherein the gene or protein expression levels of antimicrobial peptides are measured by a method selected from the group consisting of reverse transcription-polymerase chain reaction (RT-PCR), northern blot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), sandwich assay, western blot, immunoblot, immunohistochemical staining, and a combination thereof.

FIG. 1

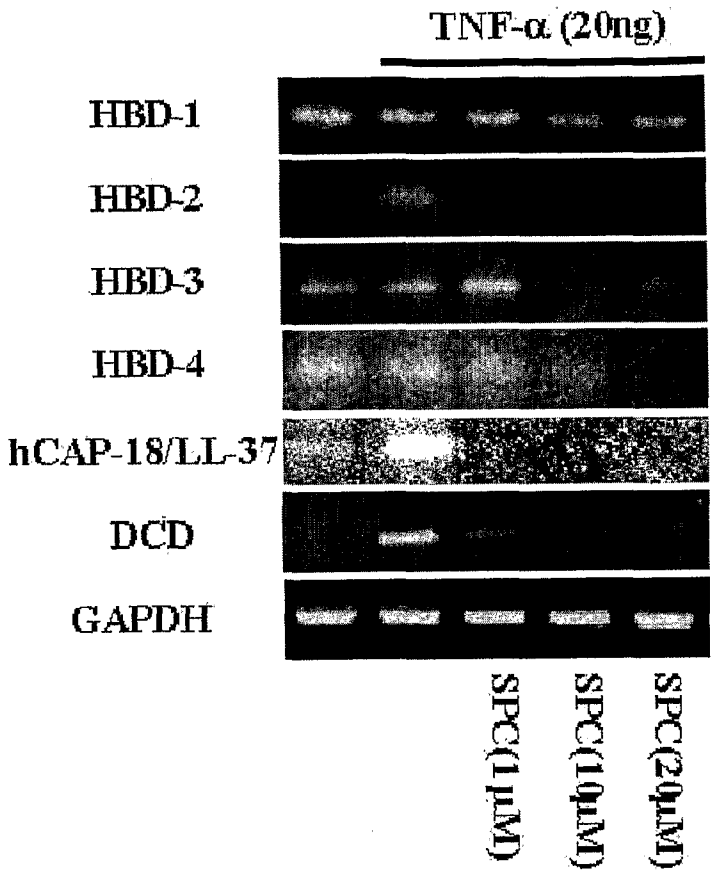

TNF-α (20ng)
HBD-1
HBD-2
HBD-3
HBD-4
hCAP-18/LL-37
DCD
GAPDH
SPC(1μM)
SPC(10μM)
SPC(20μM)

FIG. 2

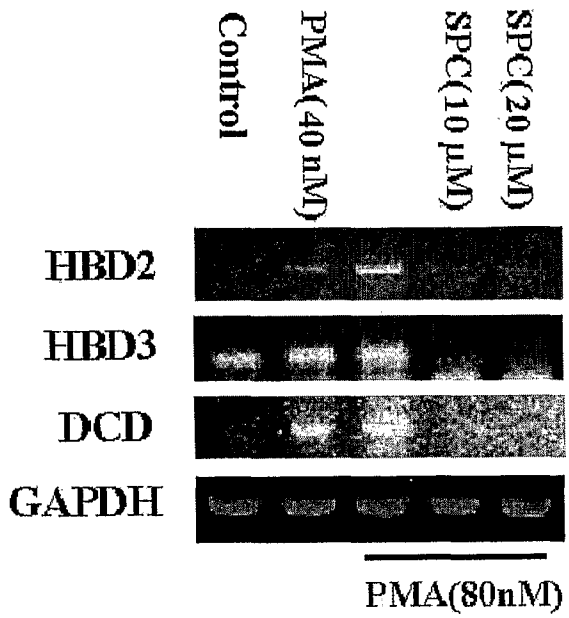

Control
PMA(40 nM)
SPC(10 μM)
SPC(20 μM)
HBD2
HBD3
DCD
GAPDH
PMA(80nM)

FIG. 3

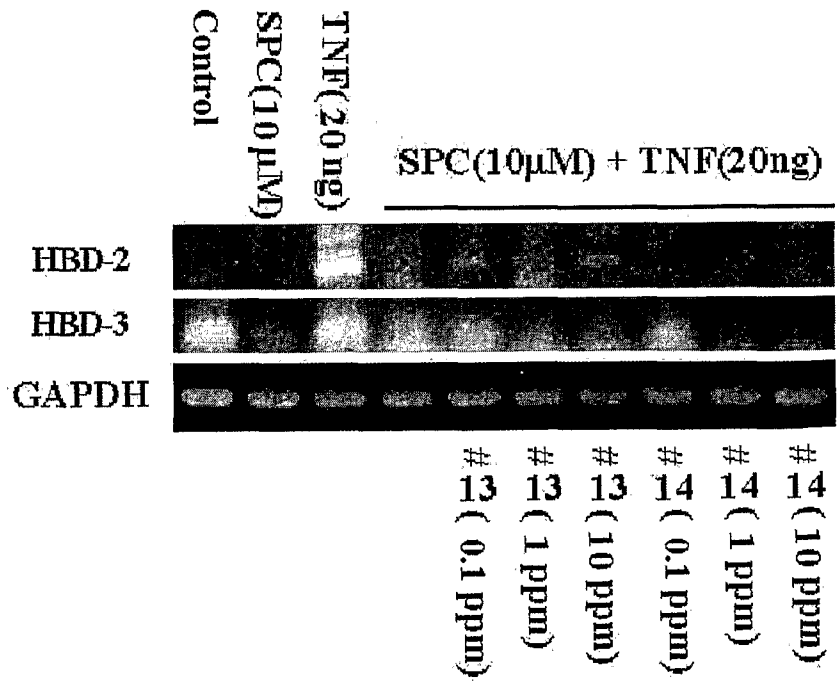
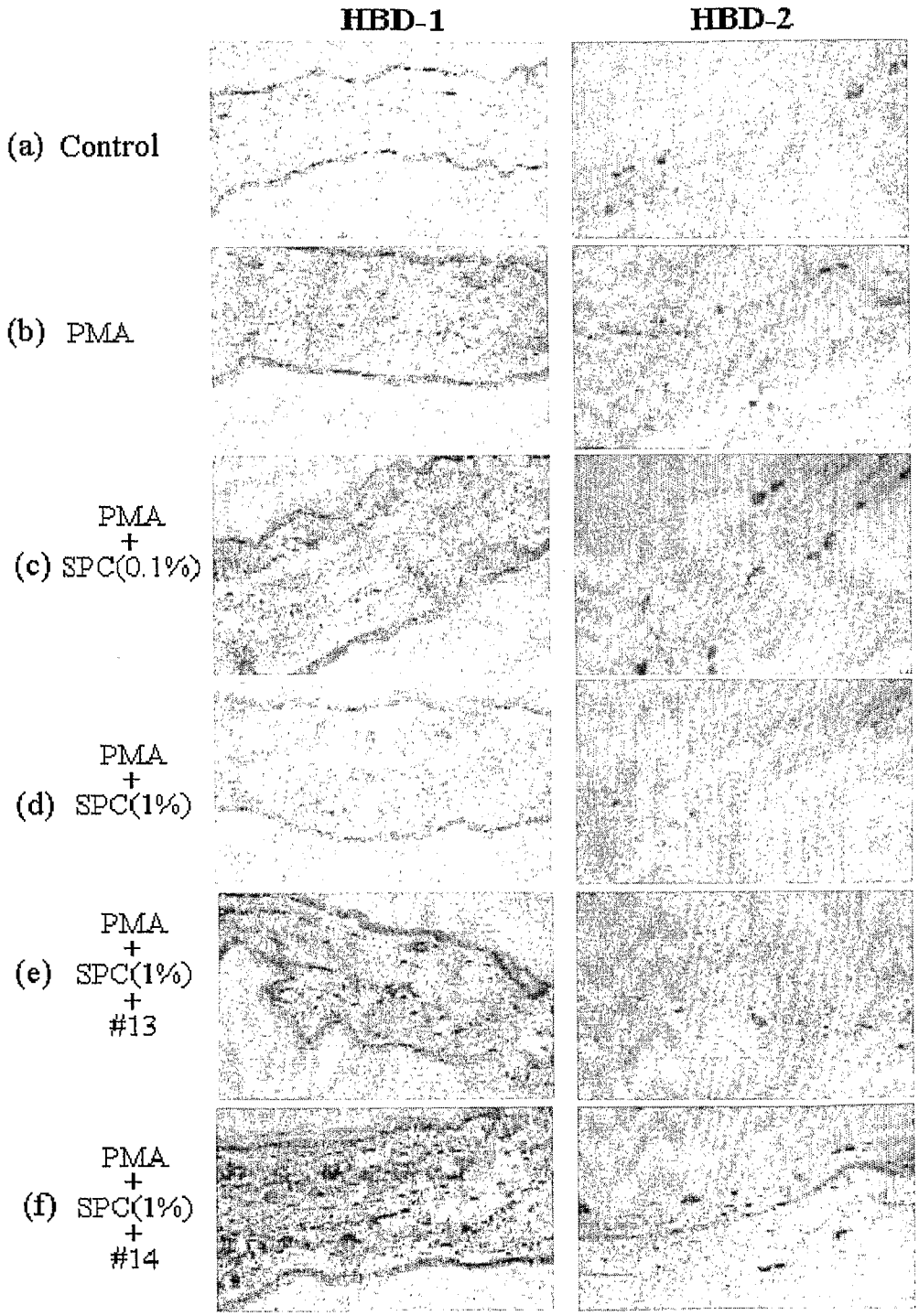

Control
SPC(10 μM)
TNF(20 ng)
SPC(10μM) + TNF(20ng)
HBD-2
HBD-3
GAPDH
#13 (0.1 ppm)
#13 (1 ppm)
#13 (10 ppm)
#14 (0.1 ppm)
#14 (1 ppm)
#14 (10 ppm)

FIG. 4


HBD-1
HBD-2
(a) Control
(b) PMA
PMA + (c) SPC(0.1%)
PMA + (d) SPC(1%)
PMA + (e) SPC(1%) + #13
PMA + (f) SPC(1%) + #14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/005313

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [x] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [x] contained in the international application as filed
   - [x] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/005313

A. CLASSIFICATION OF SUBJECT MATTER

C07K 1/14(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC8 C07K 1/14, G01N 33/53, C07H 21/00, C12N 15/00

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

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

eKIPASS, NCBI PubMed database, Delphion Researh Intellectural Property Network database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Karin Thevissen et al. FEBS Letters. Vol.580; 1903-1907(2006.03.03), See the page 1903 ; right column ; lines 5 - 13.	1 - 15
A	US 6,881,546 B2 (Sabbadini et al.) Apr. 19, 2005, See the column 1 ; lines 22-33, column 5 ; lines 65-66 and column 9 ; lines 12-29.	1 - 15
A	Paulo F.F. Almeida et al. Biochemistry. Vol.44; 9538-9544(2005),See the whole document. et al. Biochemistry. Vol.44; 9538-9544(2005),	1 - 15
A	Karl Lohner et al. Biochemistry. Vol.36; 1525-1531(1997), See the whole document.	1 - 15
A	Junko Arikawa et al. The Journal of Investigative Dermatology, Vol. 119(2);433-439(2002), See the whole document.	1 - 15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 MARCH 2007 (15.03.2007)	**16 MARCH 2007 (16.03.2007)**

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Telephone No. 82-42-481-5549

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2006/005313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US06881546	19.04.2005	US20030026799A1	06.02.2003
		US2004247603A1	09.12.2004
		US2005226862AA	13.10.2005
		US7169390BB	30.01.2007