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(54) **Title:** COMPOUNDS FOR TREATING OR PREVENTING AMINE OXIDASE RELATED DISEASES OR DISORDERS

(57) **Abstract:** This invention relates to small interfering RNAs (siRNAs) that down regulates the expression of vascular adhesion protein 1 (VAP-1) for prevention or treatment of a disease or disorder that benefits from the inhibition or down regulation of VAP-1. Pharmaceutical compositions comprising said siRNAs in combination with pharmaceutically acceptable carriers are also included. Furthermore, the invention concerns expression vectors comprising nucleic acids encoding the siRNA duplexes or the antisense strands of said duplexes in a manner which allows expression of said siRNA duplexes or antisense strands within a mammalian cell. Pharmaceutical compositions comprising said expression vectors in combination with pharmaceutically acceptable carriers are also included.

COMPOUNDS FOR TREATING OR PREVENTING AMINE OXIDASE RELATED DISEASES OR DISORDERS

FIELD OF THE INVENTION

This invention relates to the use of a small interfering RNA (siRNA) duplexes for down regulation of the expression of vascular adhesion protein 1 (VAP-1) and for treating or preventing diseases or disorders benefiting from such down regulation. Further, the invention concerns pharmaceutical compositions of said siRNA:s. The invention concerns also expression vectors encoding said siRNA duplexes or the antisense strands thereof as well as the use of such vectors and their pharmaceutical compositions.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

VAP-1 is a human endothelial cell adhesion molecule that has several unique properties that distinguish it from the other inflammation-related adhesion molecules. It has a unique and restricted expression pattern and mediates lymphocyte binding to vascular endothelium (Salmi, M., and Jalkanen, S., Science 257:1407-1409 (1992)). Inflammation induces the upregulation of VAP-1 to the surface of vascular endothelial cells mediating leukocyte entry to skin, gut and inflamed synovium (Salmi, M., and Jalkanen, S., Science 257:1407-1409 (1992); Salmi, M, et al., J. Exp. Med 178:2255-2260 (1993); Arvilommi, A., et al., Eur. J. Immunol 26:825-833 (1996); Salmi, M., et al., J. Clin. Invest. 99:2165-2172 (1997): (Salmi. M., and Jalkanen, S., J. Exp. Med. 183:569-579 (1996); J. Exp. Med 186:589-600 (1997)). One of the most interesting features of VAP-1 is a catalytic extracellular domain which contains a monoamine oxidase activity (Smith, D. J., et

al., J. Exp. Med 188:17-27 (1998)).

The cloning and sequencing of the human VAP-1 cDNA revealed that it encodes a transmembrane protein with homology to a class of enzymes called the copper-containing amine oxidases (E.C. 1.4.3.6). Enzyme assays have shown that VAP-1 possesses a monoamine oxidase (MAO) activity which is present in the extracellular domain of the protein (Smith, D. J., et al., J. Exp. Med. 188:17-27 (1998)). Thus, VAP-1 is an ecto-enzyme. Analysis of the VAP-1 MAO activity showed that VAP-1 belongs to the class of membrane-bound MAO's termed semicarbazide-sensitive amine oxidases (SSAO). These are distinguished from the widely distributed mitochondrial MAO-A and B flavoproteins by amino acid sequence, cofactor, substrate specificity and sensitivity to certain inhibitors. However, certain substrates and inhibitors are common to both SSAO and MAO activities. The mammalian SSAO's can metabolize various monoamines produced endogenously or absorbed as dietary or xenobiotic substances. They act principally on primary aliphatic or aromatic monoamines such as methylamine or benzylamine (Lyles G. A., Int. J. Biochem. Cell Biol, 28:259-274 (1996)). Thus, VAP-1 located on the vascular endothelial cell surface can act on circulating primary monoamines with the following reaction pathway.

$RNH_2 + O_2 + H_2O$ -------> $RCHO + H_2O_2 + NH_3$

The physiological substrates of VAP-1 SSAO in man have not been clearly identified. However, methylamine is a good substrate for VAP-1 SSAO. Methylamine is a product of various human biochemical pathways for the degradation of creatinine, sarcosine and adrenaline, and is found in various mammalian tissues and in blood. It can also be derived from the diet by gut bacterial degradation of dietary precursors. The concentration of methylamine in the blood can be increased in certain physiological and pathological situations such as diabetes. Another potential physiological substrate is aminoacetone.

VAP-1 SSAO activity has been proposed to be directly involved in the pathway of

leukocyte adhesion to endothelial cells by a novel mechanism involving direct interaction with an amine substrate presented on a VAP-1 ligand expressed on the surface of a leukocyte (Salmi et al. Immunity, (2001)). This publication describes the direct involvement of VAP-1 SSAO activity in the process of adhesion of leukocytes to endothelium. Moreover, deletion of VAP-1 from mouse confirms the importance of VAP-1 in vivo. VAP-1 deficient animals have decreased leukocyte infiltration at the sites of inflammation (e.g. in peritonitis) when compared to their wild-type littermate controls (Stolen et al. Immunity 22:105, 2005). Thus inhibitors of VAP-1 SSAO activity could be expected to reduce leukocyte adhesion in areas of inflammation and thereby reduce leukocyte trafficking into the inflamed region and therefore the inflammatory process itself.

In human clinical tissue samples expression of VAP-1 is induced at sites of inflammation. This increased level of VAP-1 can lead to increased production of H_2O_2 generated from the action of the VAP-1 SSAO extracellular domain on monoamines present in the blood. This generation of H_2O_2 in the localized environment of the endothelial cell could initiate other cellular events. H_2O_2 is a known signaling molecule that can upregulate other adhesion molecules and this increased adhesion molecule expression may lead to enhanced leukocyte trafficking into areas in which VAP-1 is expressed. It also may be that other products of the VAP-1 SSAO reaction could have biological effects also contributing to the inflammatory process. Thus the products of the VAP-1 SSAO activity may be involved in an escalation of the inflammatory process which could be blocked by specific SSAO inhibitors.

VAP-1 SSAO may be involved in a number of other pathological conditions associated with an increased level of circulating amine substrates of VAP-1 SSAO. The oxidative deamination of these substrates would lead to an increase in the level of toxic aldehydes and oxygen radicals in the local environment of the endothelial cell which could damage the cells leading to vascular damage. Increased levels of methylamine and aminoacetone have been reported in patients with Type I and Type II diabetes and it has been proposed that the vasculopathies such as

retinopathy, neuropathy and nephropathy seen in late stage diabetes could be treated with specific inhibitors of SSAO activity.

Various strategies for inhibiting VAP-1 activity have been disclosed. For example, WO 93/25582 discloses a monoclonal antibody specifically binding to VAP-1.

Alternatively, VAP-1 can be counteracted by using small molecules as inhibitors. The patent publications WO 02/020290 and WO 03/006003 disclose certain hydrazino compounds useful as specific VAP-1 SSAO inhibitors that modulate VAP-1 activity. These compounds are described as useful for the treatment of acute and chronic inflammatory conditions or diseases as well as diseases related to carbohydrate metabolism, aberrations in adipocyte differentiation or function and smooth muscle cell function, and various vascular diseases.

Grifantini, M., et al., Farmaco, Ed.Sci.23(3):197-203 (1968), report the synthesis of several alkyl- and acyl-derivatives of N-amino-1-ephedrine and N-amino-d-pseudoephedrine having antidepressant and monoamine oxidase inhibitory properties. Jeffrey O'Sullivan et al., Biochimica et Biophysica Acta 1647 (2003) 367-371 report the inhibition of semicarbazide-sensitive amine oxidases by certain monoaminosubstituted hexoses, namely glucosamine, galactosamine and mannosamine.

SUMMARY OF THE INVENTION

The aim of the present invention is to provide a new approach for counteracting the influence of VAP-1 in the individual. The expression of VAP-1 is down regulated by the influence of a small interfering RNA (siRNA) directed to a selected target site of the mRNA of VAP-1. Therefore, diseases or disorders benefiting from inhibiting VAP-1 by antibodies or small molecule inhibitors can be treated or prevented by the use of this new concept.

The novelty of siRNA based inhibition of VAP-1 function is the fact that it is the only way to inhibit both adhesive and enzymatic functions of VAP-1. It has been shown that surface epitopes of VAP-1, the function of which can be inhibited with anti-VAP-1 antibodies, are important in binding of leukocytes (Koskinen et al Blood 103:3388, 2004). However, also enzymatic SSAO activity of VAP-1 is involved in leukocyte adhesion (Koskinen). Moreover, the enzymatic activity results in the production of the biologically active end-products of the SSAO reaction (see above). It is known that anti-VAP-1 antibodies do not inhibit the SSAO activity of the molecule, and the small molecular SSAO inhibitors do not down-regulate surface expression of VAP-1 or block the epitopes seen by the anti-VAP-1 antibodies (Koskinen). Thus, these treatments only inhibit or down-regulate one aspect of VAP-1 function. In contrast, siRNA treatment, which leads to down-regulation of VAP-1 expression, simultaneously reduces the availability of the adhesive surface epitopes of VAP-1 and decreases the enzymatic SSAO activity.

Thus, according to one aspect, this invention concerns the use of a small interfering RNA (siRNA) that down regulates the expression of vascular adhesion protein 1 (VAP-1),
said siRNA being a duplex comprising an antisense sequence of about 21 nucleotides, said antisense being complementary to a region of the VAP-1 mRNA, and a sense sequence that is complementary to a sequence of about 19 nucleotides of said antisense, wherein the antisense sequence and the sense sequence both comprise a 3'-terminal overhang of a few, typically 2 nucleotides, and wherein the 5'-terminal of the antisense is a phosphate group (P),
in the manufacture of a pharmaceutical composition for use in prevention or treatment of a disease or disorder that benefits from the inhibition or down regulation of VAP-1.

According to another aspect, the invention concerns a pharmaceutical composition comprising the novel siRNA duplex as defined above a pharmaceutically acceptable carrier.

According to a third aspect, this invention concerns an expression vector comprising nucleic acid encoding the siRNA duplex as defined above or the antisense strand of said duplex, in a manner which allows expression of said siRNA duplex or antisense strand within a mammalian cell.

According to a fourth aspect, this invention concerns a pharmaceutical composition comprising an expression vector comprising nucleic acid encoding the siRNA duplex as defined above or the antisense strand of said duplex, in a manner which allows expression of said siRNA duplex or antisense strand within a mammalian cell, and a pharmaceutically acceptable carrier.

According to a fifth aspect, the invention concerns the use of an expression vector as defined above in the manufacture of a pharmaceutical composition for use in prevention or treatment of a disease or disorder that benefits from the inhibition or down regulation of VAP-1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the target mRNA (illustrated as cDNA) sequence of mouse VAP-1 (SEQ ID NO 1). Four alternative target sites estimated to be of particular interest are indicated in bold (target site 1: nt 981-963; target site 2 nt: 1771-1753; target site 3: nt 1818-1800; target site 4: nt 2558-2540).

Figure 2 shows four alternative siRNA-duplexes, each comprising an antisense sequence complementary to a target site of the target mRNA, and a sense sequence. The antisense strand of siRNA no 1 is complementary to target site 1 in Fig. 1, the antisense of siRNA no 2 is complementary to target site 2, antisense of siRNA no 3 is complementary to target site 3, and antisense of siRNA no 4 is complementary to target site 4 shown in Fig. 1.

Figure 3 shows the effect of siRNA no 1 (shown in Fig. 2) on down regulating VAP-1 expression in CHO cells compared to the effect of control siRNA against GFP, green fluorescent protein (a target protein that is not expressed in human cells

and therefore serves as a negative control to exclude non-specific effects of any RNAi molecule).

Figure 4 shows the target mRNA (illustrated as cDNA) sequence of human VAP-1 (SEQ ID NO 10). Four alternative target sites estimated to be of particular interest are indicated in bold italics (target site 1: nt 1227-1245; target site 2 nt: 1557-1575; target site 3: nt 2161-2179 ; target site 4: nt 2446-2464).

Figure 5 shows four alternative siRNA-duplexes, each comprising an antisense sequence complementary to a target site of the target mRNA, and a sense sequence. The antisense strand of siRNA no I is complementary to target site 4 in Fig. 4, the antisense of siRNA no II is complementary to target site 1, antisense of siRNA no III is complementary to target site 2, and antisense of siRNA no IV is complementary to target site 3 shown in Fig. 4.

Figure 6 shows the effect of siRNA no III (shown in Fig. 5) on down regulating VAP-1 expression in CHO cells compared to the effect of control siRNA against GFP.

DETAILED DESCRIPTION OF THE INVENTION

Uses and principle of action of siRNA:

The application of siRNA:s has become important in the development of new therapies in the last years. O Heidenreich presents an overview of pharmaceutical applications in the article "Forging therapeutics from small interfering RNAs in European Pharmaceutical Review Issue 1, 2005. The principle has particularly been suggested for the treatment of tumors and carcinomas, sarcomas, hypercholesterolemia, neuroblastoma and herpetic stromal keratitis.

The principle of siRNA is extensively presented in literature. As examples can be mentioned the US patent publications 2003/0143732, 2003/0148507,

2003/0175950, 2003/0190635, 2004/0019001, 2005/0008617 and 2005/0043266. An siRNA duplex molecule comprises an antisense region and a sense strand wherein said antisense strand comprises sequence complementary to a target region in an mRNA sequence encoding a certain protein, and the sense strand comprises sequence complementary to the said antisense strand. Thus, the siRNA duplex molecule is assembled from two nucleic acid fragments wherein one fragment comprises the antisense strand and the second fragment comprises the sense strand of said siRNA molecule. The sense strand and antisense strand can be covalently connected via a linker molecule, which can be a polynucleotide linker or a non-nucleotide linker. The length of the antisense and sense strands are typically about 19 to 21 nucleotides each. Typically, the antisense strand and the sense strand both comprise a 3'-terminal overhang of a few, typically 2 nucleotides. The 5'-terminal of the antisense is typically a phosphate group (P).

The siRNA duplexes having terminal phosphate groups (P) are easier to administrate into the cell than a single stranded antisense. In the cell, an active siRNA antisense strand is formed and it recognizes a target region of the target mRNA. This in turn leads to cleaving of the target RNA by the RISC endonuclease complex (RISC = RNA-induced silencing complex) and also in the synthesis of additional RNA by RNA dependent RNA polymerase (RdRP), which can activate DICER and result in additional siRNA duplex molecules, thereby amplifying the response.

Thus, compared to usual antisense therapy, the siRNA therapy has the following advantages: 1) administration into the cell is easier because of the duplex form, 2) smaller doses are required because additional duplex molecules are synthesized in the cell and 3) the target RNA is destructed by cleavage.

Preferred embodiments:

Preferred siRNA structures:

The siRNA duplex should preferable have an antisense sequence of about 21 nucleotides, typically 19-21 nucleotides. The sense sequence that is complementary should preferably be of the same length so that it is complementary to the antisense, except for the nucleotides of the sense sequence that creates the overhang, which are not necessary complementary to the antisense. The overhangs at the 3'-terminal of the antisense and sense strands contain typically 2 nucleotides.

The term "complementary" means that the nucleotide sequence can form hydrogen bonds with the target RNA sequence by Watson-Crick or other base-pair interactions. The term shall be understood to cover also sequences which are not 100 % complementary. It is believed that also lower complementarity might work. However, 100 % complementarity is preferred.

Certain preferred siRNA:s are shown in Figure 2, which are directed to the targets marked in bold in the VAP-1 mRNA (cDNA) shown in Figure 1.

Particularly preferred are the siRNA:s shown in Figure 5, which are directed to the targets marked in bold in the humanVAP-1 mRNA (cDNA) shown in Figure 4.

However, also other useful target regions at the target RNA can be used. A useful target region can easily be identified by using any of the numerous academic or commercially affiliated algorithms that have been developed to assist scientists to locate utilizable siRNA sequences. As examples of such software systems can be mentioned siDirect (http://design.RNAi.jp/) (Nucleic Acids Res. 2004 Jul 1;32: W124-9); TROD (T7 RNAi Oligo Designer (http://www.cellbio.unige.ch/RNAi.html; Nucleic Acids Res. 2004 Jul 1;32: W121-3); DEQOR (http://cluster-1.mpi-cbg.de/Deqor/deqor.html; Nucleic Acids Res. 2004 Jul 1;32: W113-20) or programs available at http://www.genscript.com; http://www.genscript.com/rnai.html#design or http://www.genscript.com/sirna_ca.html#design; Bioinformatics 2004 Jul 22;20(11)1818-20. An essential criterion of the tools is to achieve siRNA:s with

maximum target-specificity for mammalian RNA interference where off-target gene silencing is avoided. The usefulness of any sequence identified by such algorithms should thereafter be verified by experiments, for example by introducing it into VAP-1 positive cells, estimating the decrease in VAP-1 mRNA, the decreased VAP-1 protein expression, or the decrease in SSAO enzyme activity by using routine techniques such as quantitative reverse-transcriptase PCR, immunohistochemistry, immunocytological stainings, immunoblotting or SSAO enzyme assays. For more information, see K Huppi et al., Defining and Assaying RNAi in Mammalian Cells, Molecular Cell, Vol. 17, 1-10 January 7, 2005.

Modifications:

The siRNA molecule shall, when used as a pharmaceutical, be introduced in a target cell. The delivery can be accomplished, as will be dealt with in more detail in the following section, in two principally different ways: 1) exogenous delivery of the siRNA duplex or 2) endogenous transcription of a DNA sequence encoding this siRNA duplex or the antisense strand thereof, where the DNA sequence is located in a vector.

Normal, unmodified RNA has low stability under physiological conditions because of its degradation by ribonuclease enzymes present in the living cell. If the siRNA duplex shall be administered exogenously, it is highly desirable to modify the molecule according to known methods so as to enhance its stability against chemical and enzymatic degradation.

Modifications of nucleotides, not only siRNA:s but also antisense oligonucleotides, ribozymes, etc. to be administered exogenously in vivo are extensively described in the art. Principally, any part of the nucleotide, i.e the ribose sugar, the base and/or internucleotidic phosphodiester strands can be modified. For example, removal of the 2'-OH group from the ribose unit to give 2'-deoxyribosenucleotides results in improved stability. Prior discloses also other modifications at this group: the replacement of the ribose 2'-OH group with alkyl, alkenyl, allyl, alkoxyalkyl, halo, amino, azido or sulfhydryl groups. Also other modifications at the ribose unit can be

performed: locked nucleid acids (LNA) containing methylene linkages between the 2'- and 4'- positions of the ribose can be employed to create higher intrinsic stability.

Furthermore, the internucleotidic phosphodiester linkage can, for example, be modified so that one ore more oxygen is replaced by sulfur, amino, alkyl or alkoxy groups. Also the base in the nucleotides can be modified.

Preferably, the siRNA comprises modifications of one or more 2'-hydroxyl groups at ribose sugars, and/or modifications in one or more internucleotidic phosphodiester linkages, and/or one or more locked nucleic acid (LNA) modification between the 2'- and 4'-position of the ribose sugars.

Particularly preferable modifications are, for example, replacement of one or more of the 2'-OH groups by 2'-deoxy, 2'-O-methyl, 2'-halo, eg. fluoro or 2'-methoxyethyl. Especially preferred are siRNA:s where some of the internucleotide phoshodiester linkages also are modified, e.g. replaced by phosphorothioate linkages.

It should be stressed that the modifications mentioned above are only non-limiting examples. The siRNA:s according to this invention can bear any modification.

The unmodified as well as the modified siRNA molecules can be prepared according to the methods disclosed in the cited patent publications and other prior art publications.

Administration and formulations of the siRNA:

The siRNA duplex according to this invention can be administered to the individual by various methods. According to one method, the siRNA may be administered exogenously as such, or in the form of a pharmaceutical composition admixed with a suitable carrier which may be, for example, a liposome, cholesterol, lithocholic acid, lauric acid, a cationic lipid, polyethylenimine (PEI) or its conjugates with polyethylene glycol (PEG) derivatives. However, also other carriers can be used.

The siRNA can be administered systemically or locally. As suitable routes of administration can be mentioned intravenous, intramuscular, subcutaneous injection, inhalation, oral, topical, ocular, sublingual, nasal, rectal, intraperitoneal delivery and transdermal delivery systems. The composition containing the siRNA can, instead of using direct injection, also be administered by use of, for example, a catheter, infusion pump or stent.

Another method to achieve high concentrations of the siRNA in cells is to incorporate the siRNA-encoding sequence into an expression vector and to administer such a vector to the individual. In this application, the expression vector could be construed so that either the siRNA duplex or only the antisense strand thereof is expressed, e.g. in the form of short hairpin RNAs. The expression vector can be a DNA sequence, such as a DNA plasmid capable of eukaryotic expression, or a viral vector. Such a viral vector is preferably based on an adenovirus, an alphavirus, an adeno-associated virus or a retrovirus. Preferably, the vector is delivered to the patient in similar manner as the siRNA described above. The delivery of the expression vector can be systemic, such as intravenous, intramuscular or intraperitoneal administration, or local delivery to target tissue or to cells explanted from the patient, followed by reintroduction into the patient.

Since intravenous administration of siRNA preferentially targets liver vasculature (Lewis DL and Wolff JA, Methods Enzymol. 2005;392:336-50; Soutschek J et al., Nature. 2004 Nov 11;432(7014):173-8; and Song E et al., Nat Med. 2003 Mar;9(3):347-51), diseases of liver are especially suitable targets for intervention.

Required dose:

The required dosage of the compounds will vary with the particular disease or condition being treated, the severity of the condition, the duration of the treatment, the administration route and the specific compound being employed.

Thus, a typical daily dose is in the dosage range of about 1 mg/kg to about 20 mg/kg, preferably about 5 mg/kg body weight. When siRNA is used, the suitable

administration frequence is believed to be 1 to 2 doses daily. When the RNAi is delivered by an expression vector, a single dose (or a single doses repeated at certain intervals, eg. once in week) is believed to be enough.

Diseases or conditions with responsiveness to inhibition or down regulation of VAP-1:

In the following, the tem "treatment" or "treating" shall be understood to include complete curing of a disease or condition, as well as amelioration or alleviation of said disease or condition.

The term "prevention" shall be understood to include complete prevention, prophylaxis, as well as lowering the individual's risk of falling ill with said disease or condition.

As examples of groups of diseases or conditions the treatment or prevention of which would benefit from inhibition or down regulation of VAP-1 can be mentioned inflammatory diseases or conditions; diseases related to carbohydrate metabolism; diseases related to aberrations in adipocyte differentiation or function or smooth muscle cell function and vascular diseases. However, the diseases or conditions are not restricted to these groups.

According to one embodiment, the inflammatory disease or condition can be a connective tissue inflammatory disease or condition, such as, but not limited to ankylosing spondylitis, Reiter's syndrome, psoriatic arthritis, osteoarthritis or degenerative joint disease, rheumatoid arthritis, Sjögren's syndrome, Bechet's syndrome, relapsing polychondritis, systemic lupus erythematosus, discoid lupus erythematosus, systemic sclerosis, eosinophilic fasciitis, polymyositis and dermatomyositis, polymyalgia rheumatica, vasculitis, temporal arteritis, polyarterisis nodosa, Wegner's granulamatosis, mixed connective tissue disease, or juvenile rheumatoid arthritis.

According to another embodiment, said inflammatory disease or condition is a gastrointestinal inflammatory disease or condition, such as, but not limited to

Crohn's disease, ulcerative colitis, irritable bowel syndrome (spastic colon), fibrotic conditions of the liver, inflammation of the oral mucosa (stomatitis), or recurrent aphtous stomatitis. In particular, said inflammatory diseases or conditions include inflammatory liver diseases like autoimmune chronic hepatitis, drug- and toxin-induced liver diseases, cirrosis, primary biliary cirrosis and primary sclerosing cholangitis.

According to a third embodiment, said inflammatory disease or condition is a central nervous system inflammatory disease or condition, such as, but not limited to multiple sclerosis, Alzheimer's disease, or ischemia-reperfusion injury associated with ischemic stroke.

According to a fourth embodiment, said inflammatory disease or condition is a pulmonary inflammatory disease or condition, such as, but not limited to asthma, chronic obstructive pulmonary disease, or adult respiratory distress syndrome.

According to a fifth embodiment, said inflammatory disease or condition is a skin inflammatory disease or condition such as, but not limited to contact dermatitis, atopic dermatitis, psoriasis, pityriasis rosea, lichen planus, or pityriasis rubra pilaris.

According to a seventh embodiment said inflammatory condition is related to tissue trauma or resulting from organ transplantations or other surgical operations.

According to an eighth embodiment, said disease related to carbohydrate metabolism is a disease such as but not limited to diabetes, atherosclerosis, vascular retinopathies, retinopathy, nephropathy, nephrotic syndrome, polyneuropathy, mononeuropathies, autonomic neuropathy, foot ulcers or joint problems.

According to a tenth embodiment said disease relating to aberrations in adipocyte differentiation or function or smooth muscle cell function is a disease such as but not limited to atherosclerosis or obesity.

According to an eleventh embodiment, the vascular disease is a disease such as but not limited to atheromatous ateriosclerosis, nonatheromateous ateriosclerosis, ischemic heart disease, peripheral aterial occlusion, thromboangiitis obliterans (Buerger's disease), or Raynaud's disease and phenomenon.

The invention will be illuminated by the following non-restrictive Experimental Section.

EXPERIMENTAL SECTION

Knock-down of mouseVAP-1 expression by siRNA:s in vitro

CHO cells stably transfected with mouse VAP-1 cDNA in pCDNA3.1 expression vector were cultured to 70-90% confluency in 24 well plates in alpha-MEM medium containing 10% fetal calf serum. The siRNA oligonucleotides (the siRNA duplexes no. 1-4 as shown in Figure 2) were transfected into cells using Lipofectamine 2000 reagent according to the manufacturer's instructions. Briefly, 1 µl Lipofectamine reagent was mixed with 50 µl Optimem medium in one tube and VAP-1 or GFP siRNA (both at 10 pmol and 50 pmol) with 50 µl Optimem medium in another tube, and the tubes were allowed to stand at room temperature for 5 min. Then the contents of the two tubes were mixed and allowed to stand for 25 min at room temperature. Meanwhile, the CHO mouse VAP-1 transfectants were rinsed twice with Optimem medium and thereafter 0.4 ml Optimem was added per well. Thereafter the mixed transfection solution was added into the wells (giving 20 nM or 100 nM final concentration of the siRNA). The cells were cultured for 4 h in a humified cell incubator at 37° C and then the medium was replaced with the normal MEM alpha medium. The plates were then transferred back to the cell incubator for 2 days.

After the incubation the cells were detached using trypsin-edta solution and stained for immunofluorescence. In brief, the cells were incubated with a negative control mAb 3G6 or with an anti-mouse VAP-1 mAb 7-106 at 10 µg/ml for 15 min. After washings, FITC-conjugated goat anti-rat second stage reagent was added for 15

min. After washings the cells were fixed in paraformaldehyde-containing buffer and analyzed using FACSCalibur flow cytometer.

All four siRNA duplexes no. 1-4, used at 20 nM, reduced VAP-1 expression on the transfectants, whereas the control siRNA against GFP was without effect. The results with siRNA no. 1 are shown in Figure 3.

Similar results were obtained in three independent assays with the pool and separate siRNA molecules. The siRNAs were also tested at 100 nM concentration, and the results were essentially the same.

Knock-down of human VAP-1 expression by siRNA:s in vitro

CHO cells stably transfected with human VAP-1 cDNA in pCDNA3.1 expression vector were cultured to 70-90% confluency in 24 well plates in alpha-MEM medium containing 10% fetal calf serum. The siRNA oligonucleotides (the siRNA duplexes no. I-IV as shown in Figure 5) were transfected into cells using Lipofectamine 2000 reagent according to the manufacturer's instructions. Briefly, 1 µl Lipofectamine reagent was mixed with 50 µl Optimem medium in one tube and VAP-1 or GFP siRNA (both at 10 pmol and 50 pmol) with 50 µl Optimem medium in another tube, and the tubes were allowed to stand at room temperature for 5 min. Then the contents of the two tubes were mixed and allowed to stand for 25 min at room temperature. Meanwhile, the CHO human VAP-1 transfectants were rinsed twice with Optimem medium and thereafter 0.4 ml Optimem was added per well. Thereafter the mixed transfection solution was added into the wells (giving 20 nM or 100 nM final concentration of the siRNA). The cells were cultured for 4 h in a humified cell incubator at 37° C and then the medium was replaced with the normal MEM alpha medium. The plates were then transferred back to the cell incubator for 2 days.

After the incubation the cells were detached using trypsin-edta solution and stained for immunofluorescence. In brief, the cells were incubated with a negative control mAb 3G6 or with an anti-human VAP-1 mAb TK-8-14 at 10 µg/ml for 15 min. After washings, FITC-conjugated goat anti-mouse Ig second -stage reagent was

added for 15 min. After washings the cells were fixed in paraformaldehyde-containing buffer and analyzed using FACSCalibur flow cytometer.

All four siRNA duplexes no. I-IV, used at 100 nM, reduced humanVAP-1 expression on the transfectants when compared to cells treated with the control siRNA against GFP. The results from one representative experiment with siRNA no. III are shown in Figure 6, which shows a 63% reduction in the mean fluorescence intensity of VAP-1 expression after the treatment. Mean fluorescence intensity is a measure of the number of molecules per cell and thus indicates that after VAP-1 siRNA treatment the cells have lost 63% of their VAP-1 molecules. Mean reduction in the mean fluorescence intensities from two independent assays were 53% with siRNA III. siRNAs I and II both gave a mean reduction of 43% in VAP-1 expression, whereas no IV only marginally reduced VAP-1 expression by 10%.

The siRNAs were also tested at 20 nM concentration, and again no III was the most potent showing a 33% reduction in the expression. Similar results were obtained in two independent assays.

Based on these promising results for the siRNA duplexes in vivo, the inventors are currently testing their efficacy in vivo.

It has been shown that mouse VAP-1 protein is efficiently down-regulated by siRNA in vitro. Therefore, RNAi technology appears to be a promising new way to knock-down this adhesion molecule also in vivo.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the expert skilled in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

CLAIMS

1. Use of a small interfering RNA (siRNA) that down regulates the expression of vascular adhesion protein 1 (VAP-1),

said siRNA being a duplex comprising an antisense sequence of about 21 nucleotides, said antisense being complementary to a region of the VAP-1 mRNA, and a sense sequence that is complementary to a sequence of about 19 nucleotides of said antisense, wherein the antisense sequence and the sense sequence both comprise a 3'-terminal overhang of a few, typically 2 nucleotides, and wherein the 5'-terminal of the antisense is a phosphate group (P),

in the manufacture of a pharmaceutical composition for use in prevention or treatment of a disease or disorder that benefits from the inhibition or down regulation of VAP-1.

2. The use according to claim 1, wherein the disease or disorder is selected from the group consisting of inflammatory diseases or conditions; diseases related to carbohydrate metabolism; diseases related to aberrations in adipocyte differentiation or function or smooth muscle cell function and vascular diseases, in particular inflammatory diseases or conditions including inflammatory liver diseases like autoimmune chronic hepatitis, drug- and toxin-induced liver diseases, cirrosis, primary biliary cirrosis and primary sclerosing cholangitis.

3. The use according to claim 1 or 2, wherein the siRNA can be selected from a group consisting of siRNA no.I (SEQ ID NO 11 and 12), siRNA no. II (SEQ ID NO 13 and 14), siRNA no.III (SEQ ID NO 15 and 16) and siRNA no. IV (SEQ ID NO 17 and 18) as disclosed in Figure 5.

4. The use according to claim 1, 2 or 3, wherein the siRNA comprises modifications of one or more 2'-hydroxyl groups at ribose sugars, and/or modifications in one or

more internucleotidic phosphodiester linkages, and/or one or more locked nucleic acid (LNA) modification between the 2'- and 4'-position of the ribose sugars.

5. A pharmaceutical composition comprising the siRNA according to claims 1-4 and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition according to claim 5 wherein the carrier is a liposome, cholesterol, lithocholic acid, lauric acid, a cationic lipid, polyethylenimine (PEI) or its conjugates with polyethylene glycol (PEG) derivatives.

7. An expression vector comprising nucleic acid encoding the siRNA duplex or the antisense strand of said duplex according to claims 1-3 in a manner which allows expression of said siRNA duplex or antisense strand within a mammalian cell.

8. The expression vector according to claim 7 wherein the nucleic acid encoding said siRNA duplex or antisense strand is inserted either in a DNA sequence or in a viral vector.

9. The expression vector according to claim 8 wherein the nucleic acid encoding said siRNA or antisense strand is inserted in a viral vector and said viral vector is based on an adenovirus, an alfavirus, an adeno-associated virus or a retrovirus.

10. A pharmaceutical composition comprising an expression vector according to any of the claims 7-9 and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition according to claim 10 wherein said carrier is a liposome, cholesterol, lithocholic acid, lauric acid, a cationic lipid, polyethylenimine (PEI) or its conjugates with polyethylene glycol (PEG) derivatives.

12. The use of an expression vector according to any of the claims 7-9 in the manufacture of a pharmaceutical composition for use in prevention or treatment of a disease or disorder that benefits from the inhibition or down regulation of VAP-1.

13. The use according to claim 12, wherein the disease or disorder is selected from the group consisting of inflammatory diseases or conditions; diseases related to carbohydrate metabolism; diseases related to aberrations in adipocyte differentiation or function or smooth muscle cell function and vascular diseases.

VAP-1 mRNA (shown as cDNA)

AUTHORS Bono,P., Salmi,M., Smith,D.J. and Jalkanen,S.
TITLE Cloning and characterization of mouse vascular adhesion protein-1 reveals a novel molecule with enzymatic activity
JOURNAL J. Immunol. 160 (11), 5563-5571 (1998)
PUBMED 9605161
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from AF054831.1.
FEATURES Location/Qualifiers
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/organism="Mus musculus"
/mol_type="mRNA"
/strain="BALB/c"
/db_xref="taxon:10090"
/chromosome="11"
/map="4 B2-B5"

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  61 tccatacaca gacgagatcc ctccccttc ccccaacccc agctctcccc caatcccaac
 121 cctgacccag agagcactct gatttggaaa ctggaggaca aagccatgta aggtctgtgg
 181 atatcactgc agccagctca gacccgcctc ccaaccctag ctccggcttc cctgctcctt
 241 cccaccctgg caaaagcaga ctacaaagaa gctctgctat tgcctagcgc ctgggagacc
 301 ccagccaaaa ggagtccatt tctggtagaa gcctgtccat caagaagcaa tgacccagaa
 361 gaccacccta gtgctcctgg ctctggctgt catcaccatc tttgctttgg tttgtgtctt
 421 gctagctggc aggagcggag atgggggtgg actgagccaa cctcttcatt gcccctctgt
 481 tcttcctagt gtccagcccc ggacacaccc tagccagagc cagccgtttg cagacctgag
 541 cccagaggag ctgacagctg tgatgagctt cctgaccaag cacctggggc cagggctggt
 601 ggatgcagcc caggctcgac cctcggacaa ctgtgtcttc tcagtagagt tgcagctgcc
 661 tgccaaggct gcagccctgg cccacttgga cagaggggg cccccacctg tgagagaggc
 721 attggccatc atcttctttg gtggacaacc caagcccaac gtgagtgagc tggtggtggg
 781 gcccctgcct cacccctcct acatgcggga tgtgactgtg gagcgccacg gcggccccct
 841 gccctattac cggcgtcctg tgttggacag agagtatcag gatatcgagg agatgatctt
 901 ccacagagag ctgccccaag cttctggact cctccatcac tgttgcttct acaaacacca
 961 gggacagaac ctgctaacaa tgactacagc cccccgtggt ttgcaatcag gggaccgggc
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1141 attctatcaa ggccgttact atgagagtct cactcagctg gaggaccagt ttgaggctgg
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1261 ctcagtgcca ccgggcccag ctcccctct gcagttccat ccccagggac cccggttcag
1321 tgtccagggg agccaagtgt cttcttcctt gtgggctttt tcttttggcc ttggagcttt
1381 cagtggccca aggatctttg atatccgctt tcaaggggag agggtggcct atgaaatcag
1441 tgtccaggag gccatagccc tctacggtgg aaattcccca gcatcaatgt cgacctgcta
1501 cgtggacggt agctttggca ttggcaaata ctctaccccc ctgatccgag gggtagactg
1561 tccttacctc gccacctatg tggactggca cttccttctg gaatctcagg cccccaagac
1621 actacgtgat gcattttgtg tgtttgaaca gaaccagggc ctcccactcc ggcggcacca
1681 ctcagatttc tactcccatt attttggggg tgttgtgggg acggtgcttg tggtcagatc
1741 cgtgtctacc ttgctcaatt acgactacat atgggacatg gtcttccacc ccaatggggc
1801 catagaagtc aaattccacg ccacaggcta tatcagctca gctttcttct tcggtgctgg
1861 tgagaagttt gggaaccgag ttggggcgca cacgctgggc acggtacaca cccacagcgc
1921 tcacttcaaa gtggatctgg atgtggcagg gctgaagaac tgggcctggg cagaggatat
1981 ggcttttgtc cccacgattg taccttggca accggagtac cagatgcaga ggctgcaggt
2041 gactcggaag ctgctggaga cagaggagga ggctgccttc ccactggggg gcgccacccc
```

FIG. 1

cont.

cont.

2101 acgctacctg tacctggcca gtaaccacag caacaagtgg ggtcataggc ggggctaccg
2161 catccagata ctcagctttg ctggaaagcc cttgccccag gaaagtccca tagagaaagc
2221 cttcacctgg gggaggtatc acttggctgt gacccagagg aaggaggagg agcctagcag
2281 ctctagcatc ttcaaccaga acgacccgtg gacccccact gtgaacttca ccgacttcat
2341 cagcaatgag accattgctg gagaggactt ggtagcctgg gtgacggctg gctttttgca
2401 catccctcat gcagaagata tccccaacac ggtgactgcg gggaactcag tgggcttctt
2461 cctccggccg tataacttct ttgacgagga cccctccttt cattctgctg actccatcta
2521 tttccgggag ggccaggatg **ccacggcctg tgaggtta**ac cccttggctt gcctgtccca
2581 gactgccacc tgtgcccccg aaattcctgc cttctcccat gggggctttg cttacagaga
2641 caattgaact gtttctaagt atccctccct cgctcctgct cagaccatgt gctcacttcc
2701 ccacgccatt aagtgtcccc aagatggaca atctagctaa gagctgggaa gtagcgcaac
2761 agccgggcag tacacagagc aattcgattg aagatctggt tccttctgtc cccacatctt
2821 tgatgtcccc tctctcttct gctgccctcc ttgtctctcc ctctctgctt ggagcatcct
2881 gagcccatgg aaacctgatg cacagggaca ctgaactttg ttggttgtgc ctgtactgag
2941 ttcctgcctt gggagaatag ccttgttgga gcctggagta atggctatgt tttgttttgc
3001 tttgaatatg gctccttttc cccaccccca ccgcaccccc tatttggctt tcatttaaaa
3061 gcttatgata gctttgagga ctctgcaatg aggataactc tctagagacc cccaaagtag
3121 ggtctctctg agtctctcac atccacagat tcttcatcta catccctttc ctacttaagc
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3241 ctccctcccc tcttcctgga gttcctgctt gctcatacat gctaggattc ctactcagtc
3301 caaaagcgat gtccgtccta agagttctct gagccctcat tctcaaactg cagtctcttc
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3421 caggcttttc tatctcctaa cgttcgtcac atatcaggaa ggagtagggc ttgtccctgc
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3781 ccctagcagt ctggccagcc ccgttgttaa ctaatgttat tactgtagtt aatggaagaa
3841 aggctggaaa gatgtgtgtg cactgttctt gcaaggggcc caaattccgt tcccagcatc
3901 cttattaagg tgctcacaac aatctataac tctatctcca ggagagccgg tgccctcttc
3961 aggggcacct aaacttaatg cacatacctt cacacagaca catacatata aatacaaaat
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4141 agctatacag agaaaccctg tcttgcaaaa caaaaacaaa caaaccaaaa gagagttctt
4201 taaagacaaa agaaaagcta aaaatgagta ggcgataagt gtacatttgt ttatttctgc
4261 tgcattcctt gagaactgag taagaccagt tacctctgag gaggaagaca gctgagaggg
4321 aatcaggttc tgcctgccct tctgaacatc atggattgga accatgtgat catattgccc
4381 cgtcaaaata aataagtggg ctataaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
4441 aaaaaaaaaa aa

FIG. 1

siRNA no 1:

sense: 3'-U U U A A C A A U C G U C C A A G A C A G-5' (SEQ ID NO 2)
I I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P A U U G U U A G C A G G U U C U G U C U U -3' (SEQ ID NO 3)

siRNA no 2:

sense: 3'-U U A U A C A U C A G C A U U A A C U C G -5' (SEQ ID NO 4)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U A U G U A G U C G U A A U U G A G C U U -3' (SEQ ID NO 5)

siRNA no 3:

sense: 3'-U U A C C U U A A A C U G A A G A U A C C -5' (SEQ ID NO 6)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U G G A A U U U G A C U U C U A U G G U U -3' (SEQ ID NO 7)

siRNA no 4:

sense: 3'-U U A U U G G A G U G U C C G G C A C C G -5' (SEQ ID NO 8)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U A A C C U C A C A G G C C G U G G C U U -3' (SEQ ID NO9)

FIG. 2

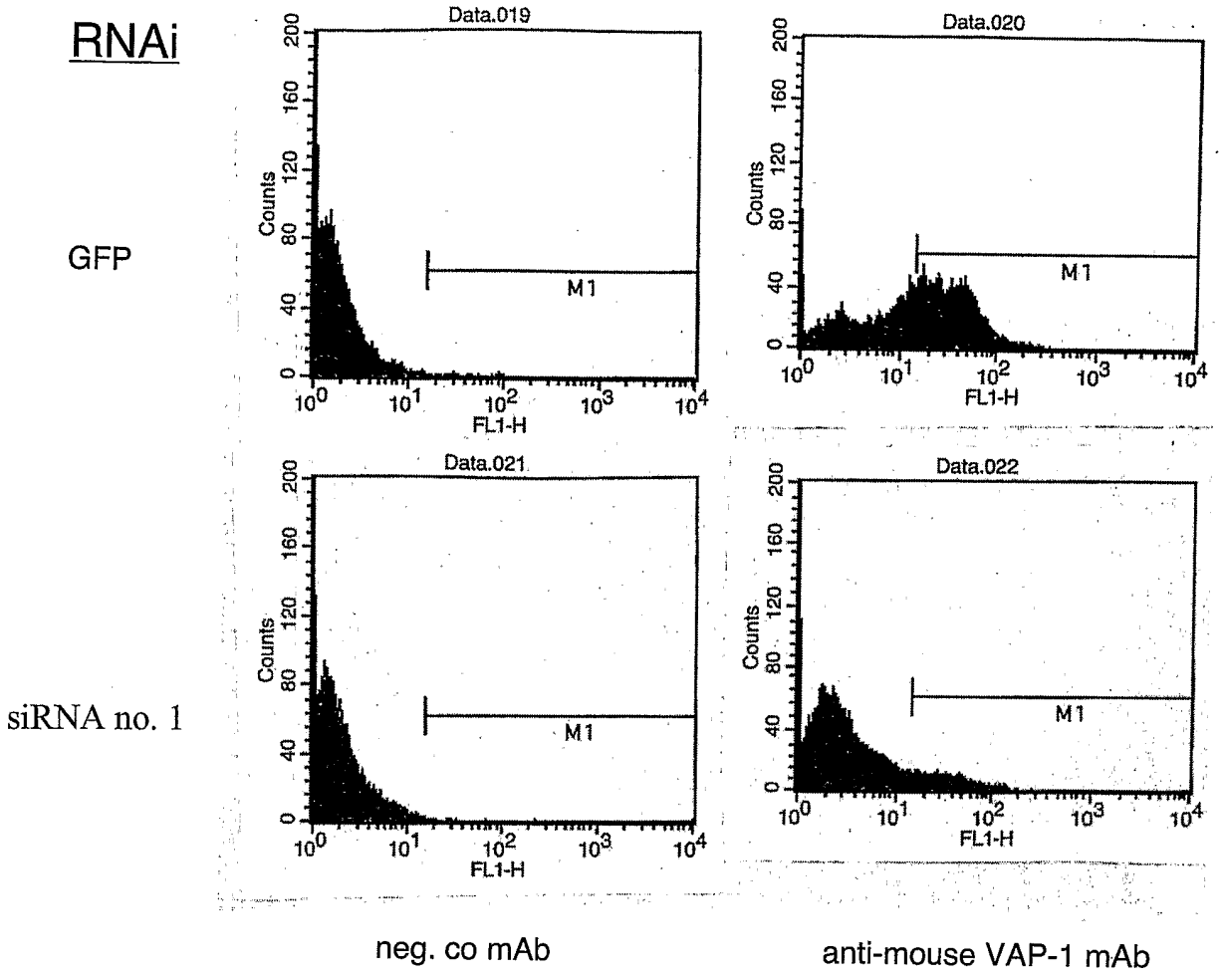
RNAi
GFP
siRNA no. 1
Data.019
Data.020
Data.021
Data.022
Counts
FL1-H
M1
neg. co mAb
anti-mouse VAP-1 mAb
IF staining

FIG. 3

Human VAP-1 mRNA (shown as cDNA); NM-003734

1 gtccttccca cccttagtcc caggcatctg actaccggga acctcagcca gagtccggga
61 gccccccacc ccgtccagga gccaacagag cccccgtctt gctggcgtga gaatacattg
121 ctctcctttg gttgaatcag ctgtccctct tcgtgggaaa atgaaccaga agacaatcct
181 cgtgctcctc attctggccg tcatcaccat ctttgccttg gtttgtgtcc tgctggtggg
241 caggggtgga gatgggggtg aacccagcca gcttccccat tgcccctctg tatctcccag
301 tgcccagcct tggacacacc ctggccagag ccagctgttt gcagacctga gccgagagga
361 gctgacggct gtgatgcgct ttctgaccca gcggctgggg ccagggctgg tggatgcagc
421 ccaggcccgg ccctcggaca actgtgtctt ctcagtggag ttgcagctgc ctcccaaggc
481 tgcagccctg gctcacttgg acaggggggag ccccccacct gcccgggagg cactggccat
541 cgtcttcttt ggcaggcaac cccagcccaa cgtgagtgag ctggtggtgg ggccactgcc
601 tcacccctcc tacatgcggg acgtgactgt ggagcgtcat ggaggccccc tgccctatca
661 ccgacgcccc gtgctgttcc aagagtacct ggacatagac cagatgatct tcaacagaga
721 gctgccccag gcttctgggc ttctccacca ctgttgcttc tacaagcacc ggggacggaa
781 cctggtgaca atgaccacgg ctccccgtgg tctgcaatca ggggaccggg ccacctggtt
841 tggcctctac tacaacatct cgggcgctgg gttcttcctg caccacgtgg gcttggagct
901 gctagtgaac cacaaggccc ttgaccctgc ccgctggact atccagaagg tgttctatca
961 aggccgctac tacgacagcc tggcccagct ggaggcccag tttgaggccg gcctggtgaa
1021 tgtggtgctg atcccagaca atggcacagg tgggtcctgg tccctgaagt cccctgtgcc
1081 cccgggtcca gctccccctc tacagttcta tccccaaggc ccccgcttca gtgtccaggg
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1321 aggctttggc atgggcaagt acaccacgcc cctgacccgt ggggtggact gcccctactt
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1441 tgccttttgt gtgtttgaac agaaccaggg cctccccctg cggcgacacc actcagatct
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1561 ***cttgctcaac tatga***ctatg tgtgggatac ggtcttccac cccagtgggg ccatagaaat
1621 acgattctat gccacgggct acatcagctc ggcattcctc tttggtgcta ctgggaagta
1681 cgggaaccaa gtgtcagagc acaccctggg cacggtccac acccacagcg cccacttcaa
1741 ggtggatctg gatgtagcag gactggagaa ctgggtctgg gccgaggata tggtctttgt
1801 ccccatggct gtgccctgga gccctgagca ccagctgcag aggctgcagg tgacccggaa
1861 gctgctggag atggaggagc aggccgcctt cctcgtggga agcgccaccc ctcgctacct
1921 gtacctggcc agcaaccaca gcaacaagtg gggtcacccc cggggctacc gcatccagat
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2161 ***gaccattgct ggaaaggat***t tggtggcctg ggtgacagct ggttttctgc atatcccaca
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2641 gacactgaac cttgttgatg ccagctgtac tgagttctca tccacagagg ccaggcatgg
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FIG. 4

cont.

cont.

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3181 ctgggggctgg gtgtgtttca ggacagctgc ttctgtgcat ttgtgtctgc ctgcctcatg
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3481 atgagctggg ccctgggtga ggtgggggtc tggcctagtg gggaggggcc tggcctgggt
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3961 tacattttga attttgaacc atgtgactgt attacctatt caaaataaac aataaatggg
4021 cccaaaaaaa aaaaaaaaaa
```

FIG. 4

siRNA no I:

sense: 3'-U U A G G G U C C U G G C G G A U C A A C-5' (SEQ ID NO 11)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U C C C A G G A C C G C C U A G U U G U U -3' (SEQ ID NO 12)

siRNA no II:

sense: 3'-U U A G U A U U U G A U C A G A A A G A G -5' (SEQ ID NO 13)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U C A U A A A C U A G U C U U U C U C U U -3' (SEQ ID NO 14)

siRNA no III:

sense: 3'-U U A G U A U C A A C U C G U U C C A C C -5' (SEQ ID NO 15)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P U C A U A G U U G A G C A A G G U G G U U -3' (SEQ ID NO 16)

siRNA no IV:

sense: 3'-U U U A G G A A A G G U C G U U A C C A G -5' (SEQ ID NO 17)
I I I I I I I I I I I I I I I I I I I I I (bond)
antisense: 5'-P A U C C U U U C C A G C A A U G G U C U U -3' (SEQ ID NO 18)

FIG. 5

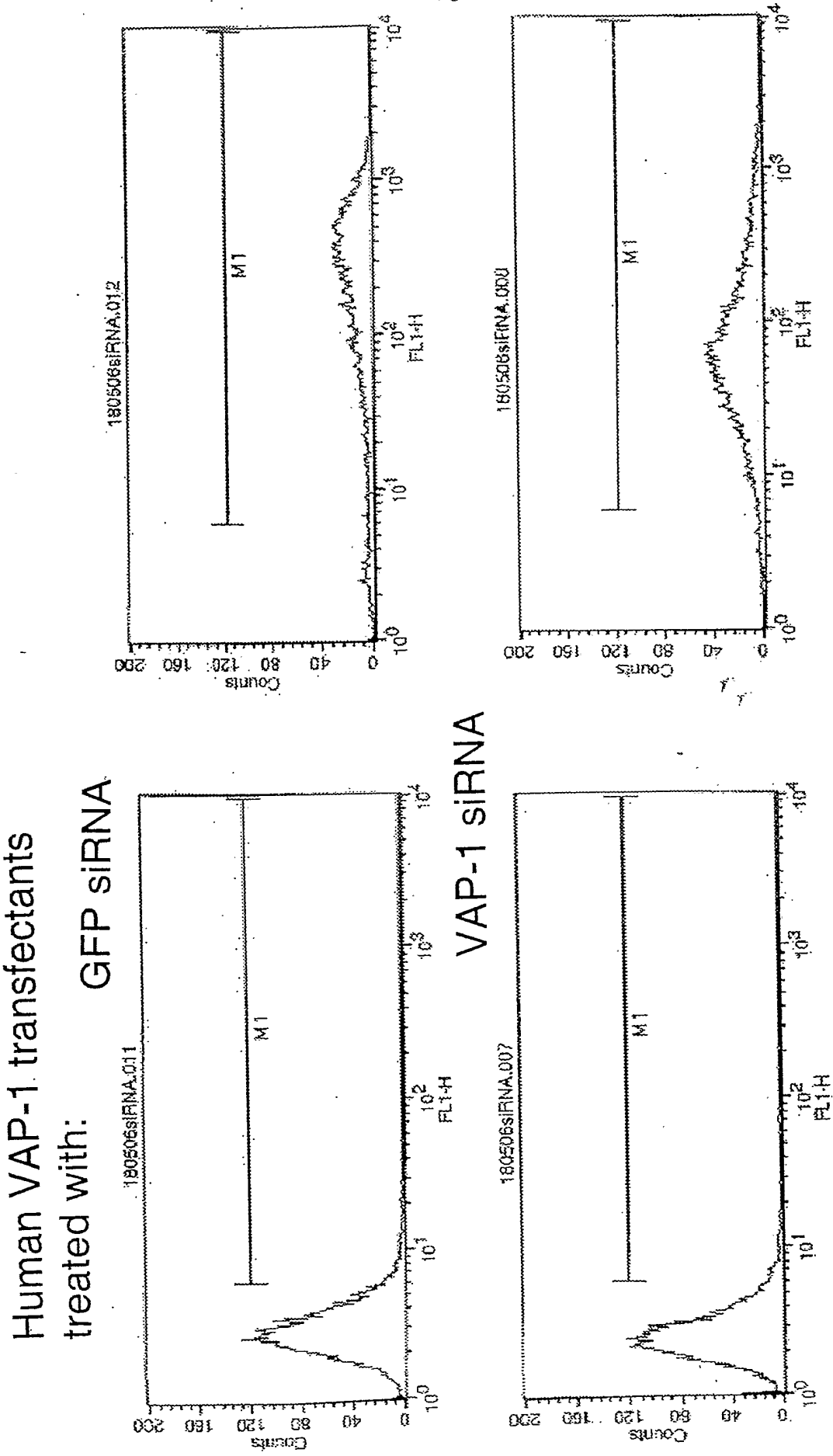
Human VAP-1 transfectants
treated with:
GFP siRNA
VAP-1 siRNA
Staining: neg. co
VAP-1
M1
Counts
FL1-H

FIG. 6

VaspirF1H1-b.ST25
SEQUENCE LISTING

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<120> Compounds for Treating or Preventing Amine Oxidase Related Diseases or Disorders

<130> Vapsir F1 H1

<150> FI 20050640
<151> 2005-06-16

<160> 18

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2006/000188

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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)

IPC 8: A61K, C07H, C12N

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

FI, SE, NO, DK

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

EPO-Internal, WPI, PAJ, BIOSIS, MEDLINE, CHEM. ABS., SEQUENCE SEARCHES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004045543 A2 (DHARMACON INC) 03 June 2004 (03.06.2004) page 52 lines 3-12; pages 73-75; Tables XII-XV mentioned on pages 74-75; claims	1-2, 4-13
A		3
X	WO 2004056961 A2 (CURAGEN CORP) 08 July 2004 (08.07.2004) page 11 Table 1; pages 22-27 and 105-126; claims	1-2, 4-13
A		3
A	WO 9853049 A1 (BIOTIE THERAPIES LTD) 26 November 1998 (26.11.1998) claim 1	1-13

☐ 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 another 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
19 September 2006 (19.09.2006)	09 October 2006 (09.10.2006)

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/FI2006/000188

Patent document cited in search report	Publication date	Patent family members(s)	Publication date
WO 2004045543 A2	03/06/2004	JP 2006507841T T	09/03/2006
		US 2005256525 A1	17/11/2005
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		RU 2204838 C2	20/05/2003
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		HU 0002234 A2	28/10/2000
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		AU 7531498 A	11/12/1998
		AU 742098B B2	20/12/2001

INTERNATIONAL SEARCH REPORT

International application No.
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CLASSIFICATION OF SUBJECT MATTER

Int.Cl.
A61K 31/7088 (2006.01)
C12N 15/11 (2006.01)
C07H 21/02 (2006.01)
C12N 9/06 (2006.01)