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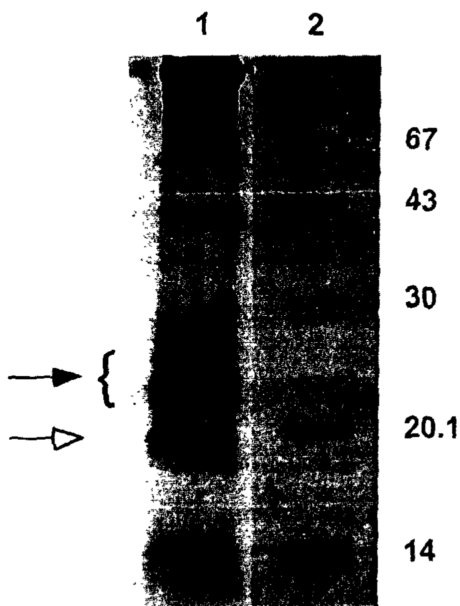
(54) Title: RESISTIN BINDING PROTEINS, THEIR PREPARATION AND USE



(57) Abstract: The invention relates to the use of a Resistin Binding Protein for use in treating diseases requiring modulation or blocking of resistin activity.

WO 03/099863 A2

Resistin Binding Proteins, their Preparation and Use.

Field of the Invention

5 The present invention relates to resistin binding proteins, their preparation and their use.

Background of the Invention

Non-insulin-dependent diabetes mellitus (NIDDM) is a common, chronic disease that is a major cause of morbidity and mortality in industrialized societies. NIDDM affects 95% of 10 diabetics and afflicts more than 5% of the world's population. NIDDM has a strong genetic component and is tightly-linked to obesity. The disorder is characterized by severe tissue resistance to the effects of insulin. Although impaired insulin secretion contributes to NIDDM, insulin levels are often increased in the early course of the disease. This peripheral insulin resistance is a major difference between NIDDM and insulin-dependent diabetes (Olefsky 1985, 15 Olefsky et al. 1985). The resistance occurs despite qualitatively and quantitatively normal insulin receptors, thus implicating one or more defective steps in the insulin signaling pathway downstream from insulin binding to its receptor.

Nevertheless, although NIDDM is characterized by insulin resistance, the only available pharmacological treatments for NIDDM until only recently were insulin or agents that increase 20 insulin secretion. New pharmacological approaches to treating NIDDM have been developed that target other metabolic abnormalities (Larkins 1997). For instance, the thiazolidinediones (TZDs) are a new class of orally active drugs that decrease insulin resistance by enhancing the actions of insulin at a level distal to the insulin receptor (Henry 1997). TZDs, which include troglitazone, pioglitazone, and rosaglitazone, are thought to sensitize target tissues to the action of insulin. 25 These compounds are ineffective at lowering serum glucose levels in the absence of insulin. In animal models of NIDDM, TZDs lower plasma glucose levels, and decrease insulin and triglycerides to near normal levels (Fujita et al 1983 and Fujiwara et al. 1988).

TZDs are generally well tolerated by patients although troglitazone therapy has been associated with hepatic dysfunction, which has been fatal in a few cases (Watkins et al. 1998). 30 Thus, liver function tests should be monitored frequently during treatment, although the causal relationship and mechanism of TZD-mediated liver toxicity have not been established. Hence,

there is still a need to find and develop other anti-diabetic compounds that will be safe and efficient.

Gestational Diabetes Mellitus (GDM) is diagnosed during pregnancy. In most cases, it
5 disappears after birth. About two to five percent of pregnant women are diagnosed with GDM- about 180,000 women per year in the United States. It develops during the second trimester (about 24 to 26 weeks of pregnancy) and is manifested by insulin resistance. The mother's high blood sugar stimulates the fetus to make more insulin, absorb more glucose and gain extra weight. If unregulated, these changes can have serious and harmful effects on both mother and
10 child.

Polycystic ovary syndrome (PCOS) is characterized by anovulation (irregular or absent menstrual periods) and hyperandrogenism (elevated serum testosterone and androstenedione). Other changes associated with this condition include enlarged ovaries with an increased number of small (6-10mm) follicles around the periphery (Polycystic Appearing Ovaries or PAO). PCOS is
15 estimated to affect 6-10% of women. One of the major biochemical features of polycystic ovary syndrome is insulin resistance accompanied by compensatory hyperinsulinemia. There is increasing data that hyperinsulinemia produces the hyperandrogenism of polycystic ovary syndrome by increasing ovarian androgen production, particularly testosterone and androstenedione and by decreasing the serum sex hormone binding globulin concentration. The
20 high levels of androgenic hormones interfere with the pituitary ovarian axis, leading to increased LH levels, anovulation, amenorrhea, and infertility. Hyperinsulinemia has also been associated high blood pressure and increased clot formation and appears to be a major risk factor for the development of heart disease, stroke and type II diabetes.

25 Recently, a protein named resistin was identified, isolated and cloned (WO00/64920). According to this publication, resistin is secreted by adipocytes and administration of recombinant resistin to mice impairs glucose tolerance and insulin action. Importantly, TZDs inhibit resistin expression, suggesting at least one of their therapeutic mechanisms in diabetes. According to WO00/64920, resistin is at least one of the factors that cause insulin resistance in
30 obesity. Furthermore, antibodies to resistin enhanced insulin action in vitro, as measured by glucose uptake by adipocytes. However, therapeutic use of such antibodies to resistin may be problematic because such antibodies may act as antigens to elicit secondary immune responses.

Cytokine binding proteins (soluble cytokine receptors) correspond to the extracellular ligand binding domains of their respective cell surface cytokine receptors. They are derived either by alternative splicing of a pre-mRNA, common to the cell surface receptor, or by proteolytic cleavage of the cell surface receptor. Such soluble receptors have been described in the past, including among others, the soluble receptors of IL-6 and IFN-gamma (Novick et al. 1989), TNF (Engelmann et al. 1989 and 1990), IL-1 and IL-4 (Maliszewski et al. 1990), IFN-alpha/beta (Novick et al. 1994 and 1992) and others. One cytokine-binding protein, named osteoprotegerin (OPG, also known as osteoclast inhibitory factor - OCIF), a member of the TNFR/Fas family, appears to be the first example of a soluble receptor that exists only as a secreted protein (Anderson et al. 1997, Simonet et al 1997, Yasuda et al. 1998). Interleukin-18-binding protein (IL-18BP) (Novick et al. 1999) represents a second example of such a soluble receptor, which exists only as a secreted protein.

Peptidoglycan recognition-protein (PGRP) was disclosed in US 6,034,217 by Ashida et al. Kang et al. (1998) reported that the gene encoding PGRP was found to be bacteria-induced in insects. Excluding its putative signal sequence PGRP is a 19 kDa protein. It binds strongly to Gram-positive bacteria. The corresponding cDNA was cloned from mouse and human.
This protein is ubiquitous i.e. is present in vertebrates and conserved. In humans, bone marrow is the major site of expression, but weak expression is also seen in kidney, liver, small intestine, spleen thymus, peripheral leukocyte, lung, and fetal spleen. The expression profile is consistent with an immunity function of PGRP also in mammals.

Summary of the Invention

The invention relates to the use of a resistin binding protein (ResBP), a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof (e.g. PEG-conjugated or fused to another protein), capable of at least one of the following:
(i) binding to resistin,
(ii) modulating the activity of resistin, or
(iii) blocking the activity of resistin,
in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases, which require modulation or blocking of resistin activity.

In one embodiment ResBP is a peptidoglycan recognition-protein (PGRP), in another embodiment ResBP is cell associated and has an apparent molecular weight of about 30 kD and Kd to resistin of about 10^{-10}M.

5 In one aspect, the invention relates to the use of an expression vector comprising the coding sequence of said ResBP, a mutein, variant, fusion protein, circularly permutated derivative, or fragment thereof, in the manufacture of a medicament for the treatment and/or prevention of disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.
10 In addition, the invention provides the use of a vector for inducing and/or enhancing the endogenous production of said ResBP in the manufacture of a medicament for the treatment and/or prevention of disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity. Alternatively, the invention provides a vector for negative regulation of endogenous
15 resistin expression in the manufacture of a medicament for the treatment and/or prevention of disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

In another aspect, the invention relates to the use of a cell that has been genetically
20 modified to overexpress said ResBP in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

In addition, the invention provides a pharmaceutical composition for treating or
25 preventing a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity comprising said ResBP, a vector encoding the sequence of said ResBP or an antibody capable of binding ResBP (e.g. polyclonal antibody, monoclonal antibody, anti-idiotypic antibody, humanized antibody, a mutein, variant, fusion protein, circularly permutated derivative, active
30 fraction, or fragment thereof) and a pharmaceutically acceptable carrier for treating or preventing disease which require modulation or blocking of resistin activity.

In addition, the invention provides a pharmaceutical composition comprising a vector including regulatory sequences functional in the cells for inducing and/or enhancing the endogenous production of ResBP in a cell, in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

Alternatively, the invention provides a pharmaceutical composition comprising a vector including regulatory sequences functional in the cells for negative regulation of endogenous resistin expression in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

In another embodiment, the invention provides a pharmaceutical composition comprising a cell that has been genetically modified to overexpress said ResBP, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

Also, the invention provides a method for treating or preventing a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity, comprising administering to a subject in which such treatment or prevention is desired, said pharmaceutical composition in an amount sufficient to treat or prevent said disease in the subject.

In another aspect, the invention provides a ResBP, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof, except PGRP (SEQ ID No: 1), capable of at least the following:

(i) binding to resistin,
(ii) modulating the activity of resistin, or

(iii) blocking the activity of resistin,

More specifically the invention relates to a cell associated ResBP having an apparent molecular weight of about 30 kD and Kd to resistin of about 10-10M. In one embodiment, such a
5 cell associated protein is the resistin receptor.

The invention provides a nucleic acid molecule comprising a nucleic acid sequence encoding said ResBP, selected from genomic or cDNA and provides vectors including such nucleic acid sequences and methods for production and isolation of the ResBP by introducing
10 such vectors in prokaryotic or eukaryoptic cells such mammalian e.g. CHO cells.

In addition, the invention provides an antibody prepared using said ResBP, e.g. polyclonal antibody, monoclonal antibody, anti-idiotypic antibody, chimeric antibody, humanized antibody a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment
15 thereof and salt thereof

In another aspect, the invention provides a process for the isolation or purification of said ResBP, comprising:
passing a sample through a chromatographic column to which resistin is coupled,
20 washing the column to remove unbound protein in the sample, and
recovering the bound ResBP. Thus, the invention provides ResBP obtainable by the process.

The invention further provides a process for the purification of resistin comprising:
Passing a sample trough a chromatographic column to which ResBP e.g. PGRP or a fragment,
25 isoform, mutein, or functional derivative thereof is coupled, washing the column to remove unbound protein in the sample, and recovering the bound resistin.

Description of the Figures

30

Figure 1 shows silver-stained SDS-PAGE (12% acrylamide; non-reducing conditions) affinity purified fraction (Elution 2) containing enriched urinary ResBPs. Lane 1 is the Elution 2 fraction.

The full arrow indicates specific resistin binding proteins of molecular mass 21-25 kD. The empty arrow represents human resistin dimer that leaked from the affinity resin. Lane 2 shows the molecular mass markers, indicated on the right side (in kD).

5 **Figure 2** shows an autoradiogram of SDS-PAGE (10 % acrylamide; reducing conditions) of complexes consisting of ^{125}I-resistin (apparent molecular weight 10 kD), cross-linked as follows: Lane 1: to itself. Lane 2: to Elution 2 of the resistin affinity column described in Example 1. Lane 3: to Elution 2 in the presence of an excess of unlabeled resistin. Lane 4: to Wash (unbound fraction) of the resistin affinity column. The bands corresponding to cross-linked products with 10 resistin in lane 2 are indicated by arrows. The molecular weight in the middle of the lower band corresponds to 29 KD and the band extends from molecular weight 26 KD to 32 KD.

Figure 3 shows an autoradiogram of SDS-PAGE (10 % acrylamide; reducing conditions) of complexes consisting of ^{125}I-resistin (apparent molecular weight 10 kD), cross-linked to itself or 15 to different cells. Following cross-linking to cells, the cells were washed, solubilized and immuno-precipitated with polyclonal anti resistin anti serum: Lane 1: Cross-linking of ^{125}I-resistin to murine 3T3 F442 cells grown in medium containing fetal bovine serum. Lane 2: Cross-linking of ^{125}I-resistin to murine 3T3 F442 cells grown in medium containing bovine serum. Lanes 3 and 7: molecular mass markers (indicated on the right sides of the gels in kD). 20 Lanes 4 and 8: cross-linking of ^{125}I-resistin to itself. Lane 5: Cross-linking of ^{125}I-resistin to human HeLa cells. Lane 6: Cross linking of ^{125}I-resistin to human foreskin fibroblasts. The specific cross-linked complexes of both human and mouse cells had a molecular mass of about 40 kD (indicated by arrows on the left sides of the gels).

25 **Figure 4** shows a saturation-binding curve of ^{125}I-resistin to its receptor on murine 3T3 F442 fibroblasts.

Detailed Description of the Invention

The present invention relates to the use of a resistin binding protein (ResBP) such as 30 peptidoglycan recognizing protein (PGRP) for the treatment or prevention of insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases or conditions which

require modulation or blocking of resistin activity or in cases of complications due to exogenously administered resistin. "ResBP" of the invention includes proteins which may be variants or isoforms of each other, possibly due to alternative splicing, proteins comprising signal sequences, muteins of ResBPs, circularly permuted derivatives of ResBPs, truncated forms or
5 ResBPs fragments, active fractions of ResBPs, fusion proteins of ResBPs and salts thereof, but exclude antibodies specific to resistin.
The present invention relates to pharmaceutical compositions prepared for administration of ResBP by mixing a ResBP, or a mutein, variant, fusion protein, circularly permutated derivative, active fraction, fragment thereof, their salts, or vectors for expressing it with physiologically
10 acceptable carriers, and/or stabilizers and/or excipients, and prepared in dosage form, *e.g.*, by lyophilization in dosage vials.

The present invention further relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a ResBP of the invention, or a mutein, variant, fusion
15 protein, circularly permutated derivative, active fraction, fragment and salt thereof, in any disease in which endogenous production or exogenous administration of resistin induces the disease or aggravates the situation of the patient.

The pharmaceutical compositions may comprise a pharmaceutically acceptable carrier and e.g., a
20 viral vector such as any one of said AAV-based viral vectors or another vector expressing an ResBP, or a mutein, variant, fusion protein, circularly permutated derivative, or fragment thereof and suitable for administration to humans and other mammals for the purpose of attaining expression in vivo of ResBP or a mutein, variant, fusion protein, circularly permutated derivative, and fragment thereof, i.e. for use in gene therapy.

25

Accordingly, ResBP a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof, or vectors expressing same in vivo are indicated for the treatment of insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases or conditions which require modulation or blocking of resistin activity or in
30 cases of complications due to exogenously administered resistin.

The method of administration can be via any of the accepted modes of administration for similar agents and will depend on the condition to be treated, *e.g.*, intravenously, intramuscularly, and subcutaneously, by local injection or topical application, or continuously by infusion, etc. The amount of active compound to be administered will depend on the route of administration, the disease to be treated and the condition of the patient. Local injection, for instance, will require a lower amount of the protein on a body weight basis than will intravenous infusion.

ResBPs or a mutein, variant, fusion protein, circularly permutated derivative, active fraction, fragment and salt thereof. and vectors for expressing such ResBPs, in humans and other mammals may be employed in the treatment, alleviation or prevention of conditions in which resistin is involved or caused by an excess of exogenously administered or endogenously produced resistin. Such conditions are, e.g., insulin resistance, gestational diabetes, polycystic ovary syndrome and NIDDM.

As used herein, the expression "modulating the activity of resistin" means the capability of ResBP to modulate any resistin activity other than blocking, e.g. partial inhibition, enhancement, or the like.

As used herein, the expression "blocking the activity of resistin" refers to any biological activity of resistin. The biological activity of ResBP is measured by adding it at different concentrations to a biological assay for resistin. For example, inhibition of insulin-induced adypocyte differentiation by resistin is one possible biological assay for resistin. The resistin blocking activity of ResBP is exemplified by the ability of ResBP to block the resistin associated inhibition of insulin-induced increase of glucose uptake by rat adipocytes.

The terms "treating" and "preventing", as used herein, should be understood as partially or totally preventing, inhibiting, attenuating, ameliorating or reversing one or more symptoms or cause(s) of the disease. When "treating" with the substances according to the invention are given after the onset of the disease, "prevention" relates to administration of the substances before signs of disease can be noted in the patient.

In addition to the use of a ResBP in modulating the activity of resistin, they can, of course, also be employed for the purification of resistin itself. For this purpose, ResBP is coupled to an

affinity column and crude resistin is passed through. The resistin can then be recovered from the column by, *e.g.*, elution at low pH.

Isolation and enrichment of ResBP was carried out in accordance with the invention by passing a sample, e.g. human fluid through a chromatographic column to which resistin was coupled, washing the column to remove unbound protein in the sample, and eluting the bound ResBP. The sample may be selected from supernatant of cell cultures, cell lysates and body fluids, such as urine.

According to the present invention a ResBP can be isolated from a body fluid, e.g. normal human urine and human plasma by ligand-affinity chromatography or from conditioned medium of recombinant cells secreting ResBP. In one embodiment of the invention, a preparation of crude human urinary proteins concentrated from 500 L of normal human urine was loaded on a column consisting of human resistin bound to agarose. The column was washed and bound proteins were eluted at low pH. Eluted fractions were neutralized and aliquots were analyzed by SDS-PAGE (12% acrylamide) under non-reducing conditions and silver staining. ResBP was identified as a broad protein band of 21-25 kD that was specifically obtained in the eluted fractions.

The ResBP was further characterized by mass spectrometry (MS). For this purpose, the eluted protein was subjected to SDS-PAGE followed by silver staining. Stained protein bands were excised, the protein was electroeluted from the gel, subjected to digestion with trypsin and the resulting peptide mixture was analyzed by liquid chromatography (LC) coupled to tandem MS-MS. By this method one ResBP was identified and found to be identical with a previously cloned protein termed human peptidoglycan recognition protein (PGRP), Gene bank Accession No. AF076483 (http://www.ncbi.nlm.nih.gov).

The affinity-purified urinary ResBP retained the ability to bind its labeled ligand (^{125}I-resistin), and following covalent cross-linking, a complex of resistin (10 kD) and ResBP of molecular mass in a range of 26-32 kD was apparent by SDS-PAGE and autoradiography The calculated molecular weight of the resistin binding protein, obtained by subtracting the molecular weigh of resistin (10 kD), is in the range of 16-22 kD.

Affinity-purified ResBP in accordance to the present invention, may block the biological activity of resistin. Inhibition of the biological activity of resistin by a ResBP obtainable according to the invention is exemplified below and can be assayed as follows: ResBP is added to a culture of pre-adipocytes and blockage of resistin inhibition of insulin-induced differentiation
5 into adipocyte-like cells is measured.

Studies of cross-linking of ^{125}I-resistin to mouse and human cells revealed a specific cross-linked complex of apparent molecular mass of about 40 kD comprising resistin (10 kD) and a cell associated protein (. The cell associated ResBP was shown to have high affinity to resistin
10 (Kd of about 10^{-10}M). This ResBP may be the receptor for resistin. The calculated molecular weight of the resistin binding protein, obtained by subtracting the molecular weigh of resistin (10 kD), is about 30 kD.

As used herein, the expression "binding to resistin" means the capability of ResBP to bind
15 resistin, e.g. as evidenced by its binding to labeled resistin when affinity-purified e.g. as in Example 4 herein.

Cell associated ResBP may be obtained by affinity purification e.g. by passing a cell lysate trough a resistin column as exemplified below. The column-bound ResBP may be subjected to
20 partial protein sequence and the sequence obtained can be used to search a protein or ESTs data bank or for synthesis of degenerated primers for the isolation of the corresponding gene or cDNA. For recombinant expression, the gene or cDNA encoding the whole cell associated ResBP or a fragment thereof lacking the transmembrane domain, optionally with an appropriated signal peptide sequence can be inserted in an expression vector, preferably in a vector in which the cell
25 associated ResBP will be produced as a fusion protein with a specific tag, such as Histidine x6, to facilitate its purification. Such a cell associated ResBP may be the resistin receptor.

Thus, ResBPs according to the invention comprise soluble or cell associated proteins obtainable by employing affinity chromatography of cell lysate or body using resistin . In one
30 embodiment soluble ResBP in the range of 16-22 kD such as PGRP were found. And in another embodiment of the invention, a cell associated ResBP in the range of 28-32 kD with affinity of about 10-^{10}M to resistin has been found.

Thus, the invention relates also to a 30 kD cell associated ResBP with an affinity of about 10^{-10}M to resistin, or a mutein or their fragments, fused to another polypeptide, functional derivatives or active fractions.

5

The present invention also encompasses variants of said proteins of the invention. Preferred variants are the ones having at least 80% amino acid sequence identity, a more preferred variant is one having at least 90% identity and a most preferred variant is one having at least 95% identity to the amino acid sequence of said proteins of the invention.

10 The term "sequence identity" as used herein means that the amino acid sequences are compared by alignment according to Hanks and Quinn (1991) with a refinement of low homology regions using the Clustal-X program, which is the Windows interface for the ClustalW multiple sequence alignment program (Thompson et al., 1994). The Clustal-X program is available over the internet at ftp://ftp-igbmc.u-strasbg.fr/pub/clustalx/. Of course, it should be understood that if this link 15 becomes inactive, those of ordinary skill in the art could find versions of this program at other links using standard internet search techniques without undue experimentation. Unless otherwise specified, the most recent version of any program referred herein, as of the effective filing date of the present application, is the one, which is used in order to practice the present invention.

Another method for determining "sequence identity" is he following. The sequences are aligned 20 using Version 9 of the Genetic Computing Group's GDAP (global alignment program), using the default (BLOSUM62) matrix (values -4 to +11) with a gap open penalty of -12 (for the first null of a gap) and a gap extension penalty of -4 (per each additional consecutive null in the gap). After alignment, percentage identity is calculated by expressing the number of matches as a percentage of the number of amino acids in the claimed sequence.

25 Muteins in accordance with the present invention include those encoded by a nucleic acid, such as DNA or RNA, which hybridizes to DNA or RNA under stringent conditions and which encode said protein in accordance with the present invention, comprising essentially all of the naturally-occurring sequences encoding for example ResBP and fragments thereof comprising regions responsible for binding resistin.

30

The term "hybridization" as used herein shall include any process by which a strand of nucleic acid joins with complementary strand through a base pairing (Coombs J, 1994, Dictionary

of Biotechnology, stokton Press, New York NY). "Amplification" is defined as the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction technologies well known in the art (Dieffenbach and Dveksler, 1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY).

5 "Stringency" typically occurs in a range from about Tm-5°C (5°C below the melting temperature of the probe) to about 20°C to 25°C below Tm.

The term "stringent conditions" refers to hybridization and subsequent washing conditions, which those of ordinary skill in the art conventionally refer to as "stringent". See Ausubel et al., Current Protocols in Molecular Biology, Greene Publications and Wiley Interscience, New York, NY, 10 1987-1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.

As used herein, stringency conditions are a function of the temperature used in the hybridization experiment, the molarity of the monovalent cations and the percentage of formamide in the hybridization solution. To determine the degree of stringency involved with any given set of 15 conditions, one first uses the equation of Meinkoth et al. (1984) for determining the stability of hybrids of 100% identity expressed as melting temperature Tm of the DNA-DNA hybrid:

$$Tm = 81.5\ C + 16.6\ (\log M) + 0.41\ (\%GC) - 0.61\ (\%\ form) - 500/L$$

where M is the molarity of monovalent cations, %GC is the percentage of G and C nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the 20 length of the hybrid in base pairs. For each 1 C that the Tm is reduced from that calculated for a 100% identity hybrid, the amount of mismatch permitted is increased by about 1%. Thus, if the Tm used for any given hybridization experiment at the specified salt and formamide concentrations is 10 C below the Tm calculated for a 100% hybrid according to the equation of Meinkoth, hybridization will occur even if there is up to about 10% mismatch.

25 As used herein, "highly stringent conditions" are those which provide a Tm which is not more than 10 C below the Tm that would exist for a perfect duplex with the target sequence, either as calculated by the above formula or as actually measured. "Moderately stringent conditions" are those, which provide a Tm, which is not more than 20 C below the Tm that would exist for a perfect duplex with the target sequence, either as calculated by the above formula or as actually 30 measured. Without limitation, examples of highly stringent (5-10 C below the calculated or measured Tm of the hybrid) and moderately stringent (15-20 C below the calculated or measured Tm of the hybrid) conditions use a wash solution of 2 X SSC (standard saline citrate) and 0.5%

SDS (sodium dodecyl sulfate) at the appropriate temperature below the calculated Tm of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those, which allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 X SSC (or 6 X SSPE (standard saline-phosphate-EDTA), 5 X Denhardt's reagent, 0.5% SDS, 100 µ g/ml denatured, fragmented salmon sperm DNA at a temperature approximately 20 to 25 C below the Tm. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC (Ausubel, 1987, 1999).

As used herein the term "muteins" refers to analogs of an ResBP, in which one or more of the amino acid residues of a natural ResBP are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the natural sequence of an ResBP without changing considerably the capability of the resulting products as compared with the wild type ResBP to bind to resistin. These muteins are prepared by known synthesis and/or by site-directed mutagenesis techniques, or any other known technique suitable thereof.

Any such mutein preferably has a sequence of amino acids sufficiently duplicative of that of a ResBP such as to have substantially similar activity to ResBP. One activity of ResBP is its capability of binding resistin. As long as the mutein has substantial binding activity to resistin, it can be used in the purification of resistin, such as by means of affinity chromatography, and thus can be considered to have substantially similar activity to ResBP. Thus, it can be determined whether any given mutein has substantially the same activity as ResBP by means of routine experimentation comprising subjecting such a mutein, *e.g.,* to a simple sandwich competition assay to determine whether or not it binds to an appropriately labeled resistin, such as radioimmunoassay or ELISA assay.

In a preferred embodiment, any such mutein has at least 40% identity or similarity with the amino acid sequence of ResBP. More preferably, it has at least 40%, at least 60%, at least 70%, at least 80% or, most preferably, at least 90% identity or similarity thereto.

Muteins of ResBP polypeptides or muteins of which can be used in accordance with the present invention, or nucleic acid coding therefore, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely

obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz, G.E. et al., Principles of Protein Structure, Springer-Verlag, New York, 1978; and Creighton, T.E., Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al, *supra*, at §§ A.1.1-A.1.24, and Sambrook et al, *supra*, at Appendices C and D.

Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of ResBP polypeptides or proteins may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule, Grantham, Science, Vol. 185, pp. 862-864 (1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, *e.g.*, under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, *e.g.*, cysteine residues, Anfinsen, "Principles That Govern The Folding of Protein Chains", Science, Vol. 181, pp. 223-230 (1973). Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

However, cysteine residues which are not required for biological activity may be replaced with other residues, e.g. in order to avoid the formation of undesired intramolecular or intermolecular disulfide bridges which may cause a reduction in the activity of the ResBPs.

Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

TABLE I

Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu

Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

TABLE II

More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	His, Lys, Arg
Leu	Leu, Ile, Phe, Met
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Val, Met, Ile

Gly	Gly
Ile	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr	Phe, Tyr
Cys	Cys, Ser
His	His, Gln, Arg
Gln	Glu, Gln, His
Asn	Asp, Asn
Lys	Lys, Arg
Asp	Asp, Asn
Glu	Glu, Gln
Met	Met, Phe, Ile, Val, Leu
Trp	Trp

TABLE III

Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
Leu	Leu, Ile, Met
Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
Gly	Gly
Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Met, Ile, Leu
Trp	Trp

Examples of production of amino acid substitutions in proteins which can be used for obtaining muteins of ResBP polypeptides or proteins, or muteins of for use in the present invention include any known method steps, such as presented in US patents RE 33,653, 4,959,314, 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al., 4,965,195 to Namen et al; 4,879,111 to Chong et al; and 5,017,691 to Lee et al; and lysine substituted proteins presented in US patent No. 4,904,584 (Shaw et al).

In another preferred embodiment of the present invention, any mutein of a ResBP has an amino acid sequence essentially corresponding to that of a ResBP. The term "essentially corresponding

to" is intended to comprehend proteins with minor changes to the sequence of the natural protein which do not affect the basic characteristics of the natural proteins, particularly insofar as their ability to bind resistin. The type of changes which are generally considered to fall within the "essentially corresponding to" language are those which would result from conventional mutagenesis techniques of the DNA encoding these proteins, resulting in a few minor modifications, and screening for the desired activity in the manner discussed above. In addition to binding resistin, the muteins may also modulate and/or block resistin activity.

Muteins in accordance with the present invention comprise proteins encoded by a nucleic acid, such as DNA or RNA, which hybridizes to DNA or RNA, which encodes a ResBP in accordance with the present invention, under highly stringent conditions. The invention also includes such nucleic acid, which is also useful as a probe in identification and purification of the desired nucleic acid. Furthermore, such nucleic acid would be a prime candidate to determine whether it encodes a polypeptide, which retains the functional activity of a ResBP of the present invention. The term "highly stringent conditions" refers to hybridization and subsequent washing conditions, which those of ordinary skill in the art conventionally refer to as "highly stringent". See Ausubel et al., Current Protocols in Molecular Biology, supra, Interscience, N.Y., §§6.3 and 6.4 (1987, 1992), and Sambrook et al., supra. Without limitation, examples of highly stringent conditions include washing conditions 12-20°C below the calculated Tm of the hybrid under study in, *e.g.*, 2 x SSC and 0.5% SDS for 5 minutes, 2 x SSC and 0.1% SDS for 15 minutes; 0.1 x SSC and 0.5% SDS at 37°C for 30-60 minutes and then, a 0.1 x SSC and 0.5% SDS at 68°C for 30-60 minutes. Those of ordinary skill in this art understand that stringency conditions also depend on the length of the DNA sequences, oligonucleotide probes (such as 10-40 bases) or mixed oligonucleotide probes. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC. See Ausubel, supra.

The term "circularly permuted" as used herein refers to a linear molecule in which the termini have been joined together, either directly or through a linker, to produce a circular molecule, and then the circular molecule is opened at another location to produce a new linear molecule with termini different from the termini in the original molecule. Circular permutations include those molecules whose structure is equivalent to a molecule that has been circularized and then opened. Thus, a circularly permuted molecule may be synthesized de novo as a linear molecule and never go through a circularization and opening step. The particular circular permutation of a molecule is

designated by brackets containing the amino acid residues between which the peptide bond is eliminated. Circularly permuted molecules, which may include DNA, RNA and protein, are single-chain molecules, which have their normal termini fused, often with a linker, and contain new termini at another position. See Goldenberg, et al. J. Mol. Biol., 165: 407-413 (1983) and
5 Pan et al. Gene 125: 111-114 (1993), both incorporated by reference herein. Circular permutation is functionally equivalent to taking a straight-chain molecule, fusing the ends to form a circular molecule, and then cutting the circular molecule at a different location to form a new straight chain molecule with different termini. Circular permutation thus has the effect of essentially preserving the sequence and identity of the amino acids of a protein while generating new termini
10 at different locations.

The term “fragment” refers to fragments of ResBP capable of binding resistin or capable of modulating its activity, e.g. an extracellular domain of a cell associated ResBP, obtainable by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or
15 recombinant techniques.

The term "fused protein" refers to a polypeptide comprising a ResBP or a mutein thereof, fused with another protein, which, *e.g.*, has an extended residence time in body fluids. A ResBP may thus be fused to another protein, polypeptide or the like, *e.g.*, an immunoglobulin or a
20 fragment thereof. It may also be fused to a high molecular weight polymer, such as polyethylene glycol (PEG) in order to prolong residence time.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of a ResBP, muteins, or fused proteins thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium,
25 ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid. Of course, any such salts must have substantially similar activity to ResBP.

30 "Functional derivatives" as used herein cover derivatives of ResBPs and its muteins and fused proteins, which may be prepared e.g. from the functional groups which occur as side chains on the residues, or by adding groups to the N- or C-terminus, by any means known in the art, and

are included in the invention as long as they remain pharmaceutically acceptable, *i.e.* they do not destroy the activity of the protein which is substantially similar to the activity of ResBP and do not confer toxic properties on compositions containing it.

The above derivatives may, for example, include polyethylene glycol side-chains (PEG-conjugated), which may mask antigenic sites and extend the residence of a ResBP in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (*e.g.* alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fractions" of an ResBP, muteins and fused proteins, the present invention covers any fragment or precursors of the polypeptide chain of the protein molecule alone or together with associated molecules or residues linked thereto, *e.g.*, sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said active fraction substantially retains the capability of binding resistin.

Various recombinant cells such as prokaryotic cells, e.g., E. coli, or eukaryotic cells, such as CHO, yeast or insect cells can produce ResBPs. Methods for constructing appropriate vectors, carrying DNA that codes for an ResBP and suitable for transforming (*e.g.*, E. coli, mammalian cells and yeast cells), or infecting insect cells in order to produce a recombinant ResBP are well known in the art. See, for example, Ausubel et al., eds. "Current Protocols in Molecular Biology" Current Protocols, 1993; and Sambrook et al., eds. "Molecular Cloning: A Laboratory Manual", 2nd ed., Cold Spring Harbor Press, 1989.

The invention relates to a cell that has been genetically modified to produce recombinant ResBP. For this purpose, DNA encoding a ResBP or a mutein, variant, fusion protein, circularly permutated derivative, and fragment thereof, and the operably linked transcriptional and translational regulatory signals, are inserted into vectors which are capable of integrating the desired gene sequences into the host cell chromosome. In order to be able to select the cells, which have stably integrated the introduced DNA into their chromosomes, one or more markers which allow for selection of host cells which contain the expression vector is used. The marker may provide for prototrophy to an auxotropic host, biocide resistance, *e.g.*, antibiotics, or

resistance to heavy metals, such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by cotransfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals.

Said DNA molecule to be introduced into the cells of choice will preferably be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Such plasmids and vectors are well known in the art (Bollon, D. P., et al. 1980 J. Clin. Hematol. Oncol. 10:39-48, Botstein, D., et al. 1982 Miami Wint. Symp. 19:265-274, Broach, J. R., in "The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 445-470 (1981), Broach, J. R., 1982. Cell 28:203-204. and Maniatis, T., in "Cell Biology: A Comprehensive Treatise, Vol. 3: Gene Expression", Academic Press, NY, pp. 563-608 (1980).). Once the vector or DNA sequence containing the construct(s) has been prepared for expression, the expression vector may be introduced into an appropriate host cell by any of a variety of suitable means, such as transformation, transfection, lipofection, conjugation, protoplast fusion, electroporation, calcium phosphate precipitation, direct microinjection, etc.

Host cells to be used in this invention may be either prokaryotic or eukaryotic. Prokaryotic hosts include bacteria such as *E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia,* etc. Bacterial hosts which may be used include *E. coli* K12 strain 294 (ATCC 31446), *E. coli* X1776 (ATCC 31537), *E. coli* W3110 (F^-, lambda$^-$, phototropic (ATCC 27325). Under such conditions, the protein will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.
Regarding eukaryotic hosts, mammalian cells, *e.g.*, human, monkey, mouse and Chinese hamster ovary (CHO) cells, provide post-translational modifications to protein molecules including correct folding, correct disulfide bond formation, as well as glycosylation at correct sites. Also yeast cells and insect cells can carry out post-translational peptide modifications including high mannose glycosylation.

A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids, which can be utilized for production of the desired proteins in yeast and in insect cells. Yeast and insect cells recognize leader sequences on cloned

mammalian gene products and secrete mature ResBP. After the introduction of the vector, the host cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of a ResBP, fusion proteins, or muteins or fragments thereof. The expressed proteins are then isolated and purified by any conventional procedure involving extraction, precipitation, chromatography, electrophoresis, or the like, or by affinity chromatography, using, e.g., an anti-ResBP monoclonal antibodies immobilized on a gel matrix contained within a column. Crude preparations containing said recombinant ResBP are passed through the column whereby ResBP will be bound to the column by the specific antibody, while the impurities will pass through. After washing, the protein is eluted from the gel under conditions usually employed for this purpose, *i.e.* at a high or a low pH, *e.g.* pH 11 or pH 2 respectively.

The invention further relates to vectors useful for expression of a ResBP, or their derivatives in mammals and more specifically in humans e.g. for gene therapy. Vectors for short and long-term expression of genes in mammals are well known in the literature. Studies have shown that gene delivery to e.g., skeletal muscle, vascular smooth muscle and liver result in systemic levels of therapeutic proteins. Skeletal muscle is a useful target because of its large mass, vascularity and accessibility. However, other targets and particularly bone marrow precursors of immune cells have been used successfully. Currently available vectors for expression of proteins in e.g., muscle include plasmid DNA, liposomes, protein-DNA conjugates and vectors based on adenovirus, adeno-associated virus and herpes virus. Of these, vectors based on adeno-associated virus (AAV) have been most successful with respect to duration and levels of gene expression and with respect to safety considerations (Kessler, P.D. 1996, Proc. Natl. Acad. Sci. USA 93, 14082-14087).

Procedures for construction of an AAV-based vector have been described in detail (Snyder et al, 1996, Current Protocols in Human Genetics, Chapters 12.1.1-12.1.17, John Wiley & Sons) and are incorporated into this patent by reference. Briefly plasmid psub201, containing the wild-type AAV genome is cut with the restriction enzyme Xba I and ligated with a construct consisting of an efficient eukaryotic promoter, e.g., the cytomegalovirus promoter, a Kozak consensus sequence, a DNA sequence coding for an ResBP or its muteins or fusion proteins or fragments thereof, a suitable 3' untranslated region and a polyadenylation signal, e.g., the polyadenylation signal of simian virus 40. The resulting recombinant plasmid is cotransfected

with a helper AAV plasmid e.g., pAAV/Ad into mammalian cells e.g., human T293 cells. The cultures are then infected with adenovirus as a helper virus and culture supernatants are collected after 48-60 hours. The supernatants are fractionated by ammonium sulfate precipitation, purified on a CsCl density gradient, dialyzed and then heated at 56°C to destroy any adenovirus, whereas the resulting recombinant AAV, capable of expressing ResBP or its muteins or fusion proteins remains stable at this step.

The use of a vector for inducing and/or enhancing the endogenous production of ResBP, e.g. PGRP, in a cell normally silent for expression of a ResBP, or expressing amounts of ResBP which are not sufficient, are also contemplated according to the invention. The vector may comprise regulatory sequences functional in the cells desired to express the ResBP. Such regulatory sequences comprise promoters or enhancers. The regulatory sequence is then introduced into the right locus of the genome by homologous recombination, thus operably linking the regulatory sequence with the gene, the expression of which is required to be induced or enhanced. The technology is usually referred to as "endogenous gene activation" (EGA), and it is described e.g. in WO 91/09955.

The invention also contemplates the use of a pharmaceutical composition comprising a cell that has been genetically modified to overexpress ResBP or a mutein, variant, fusion protein, circularly permutated derivative, and fragment thereof an isoform, a mutein, fused protein, or functional derivative thereof, in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

It will be understood by the person skilled in the art that, if desired, it is also possible to shut down resistin expression using the same technique, i.e. by introducing a negative regulation element, like e.g. a silencing element, into the gene locus of resistin, which will result in down-regulation or prevention of resistin expression. The person skilled in the art will understand that such down-regulation or silencing of resistin expression has the same effect as the use of a resistin inhibitor in order to prevent and/or treat disease.

So far, the physiological role of the soluble cytokine receptors has not been established. The soluble receptors bind their specific ligands and in most cases inhibit their biological activity, as is shown, e.g., in the TNF system (Engelmann, H et al. 1989J. Biol. Chem. 264:11974-11980.

Engelmann, Het al. 1990 J. Biol. Chem. 265:1531-1536). In very few cases, e.g., IL-6, the soluble receptor enhances the biological activity. The recombinant soluble TNF receptor, also known as TBP (TNF binding protein) is found to prevent septic shock in animal models, while soluble forms of IL-1 receptor are found to have profound inhibitory effects on the development
5 of in vivo alloreactivity in mouse allograft recipients.

Similarly, the ResBPs of the present invention e.g. PGRP or the cell associated ResBP lacking the transmembrane domain, may find use as modulators of resistin activity, *e.g.* in insulin resistance, gestational diabetes, polycystic ovary syndrome, and NIDDM. They may thus be used, *e.g.* in any disease in which endogenous production or exogenous administration of resistin
10 induces the disease or aggravates the situation of the patient.

The invention also includes antibodies against a ResBP, as well as against its muteins, fused proteins, salts, functional derivatives and active fractions. The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (MAbs), chimeric antibodies, anti-idiotypic
15 (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, and humanized antibodies as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques. A specific ELISA may be developed using a pair of antibodies obtained from different species e.g. rabbit and mouse antibodies.

20

The invention will now be illustrated by the following non-limiting examples:

25

30

Examples

Example 1: Isolation of a resistin binding protein.

5 A human resistin having an extra lysine at its N-terminus (2 mg, Peprotech Inc. Rocky Hills NJ) was coupled to Affigel-10 (1 ml, BioRad), according to the manufacturer's instructions and packed into a column. A 1000-fold concentrate of crude urinary proteins (500 ml) was loaded onto the column at a flow rate of 0.25 ml/min. The column was washed with 250 ml 0.5M NaCl in phosphate buffered saline (PBS). Bound proteins were then eluted with 25 mM citric acid, pH 10 2.2 containing benzamidine (1 mM), and immediately neutralized by 1M Na_2CO_3. Fractions of 1 ml were collected. The second fraction, "elution 2", was collected and analyzed together with the wash fraction by SDS-PAGE (12%) under non reducing conditions and the protein bands were visualized by silver staining. A specific broad band (21-25kD) of a resistin binding protein was observed in the elution 2 fraction. This band was not seen in the wash fraction, which represents 15 crude urinary proteins (Fig. 1).

The results show that a fraction of urinary proteins enriched with resistin binding proteins is obtained by affinity chromatography.

Example 2: Molecular Characterization of a resistin binding protein by Mass Spectrometry.

20

The specific protein bands obtained in Example 1 (21-25 kD) were excised from the gel, the proteins were electroeluted and digested with trypsin. The resulting tryptic digest was subjected to liquid-chromatography and tandem mass spectrometry (LC-MS/MS). Two tryptic peptides provided unequivocal sequences: AAQGLLACGVAQGALR (SEQ ID NO: 2) and 25 ALASECAQHLSLPLR (SEQ ID NO: 3). A computerized search in the NCBI protein database using these two sequences with the Blast program has identified resistin binding protein to be the human peptidoglycan recognition protein (PGRP) (Accession No AF076483 SEQ ID NO: 1). An additional protein corresponding to human immunoglobulin JC kappa chain was also identified in the same protein band but it probably represents a non-specific component.

30 The results show that one of the resistin binding protein present in elution 2 fraction is a peptidoglycan recognition protein (Accession No AF076483 MW 19 kD, SEQ ID NO: 1).

Example 3: Preparation of a radio-iodinated human resistin derivative.

A mammalian expression vector for human resistin, to which the tag KYWSHPQFEK (SEQ ID
5 NO: 4) (comprising the Strep-tag II, which is a streptavidine binding sequence) is added at the C-terminus, was prepared. The resulting resistin derivative was expressed in HEK293 cells and purified on a Streptavidin-agarose column. It gave a single band of 10 Kd as determined by SDS-PAGE (12% acrylamide) under reducing conditions. This preparation was found to be biologically active (see below) and was used for radioiodination by the following modification of
10 the Chloramine T method. The whole procedure was performed at 4°C. Resistin (20 μg in 50 ml PBS containing 20% glycerol) was mixed with an equal volume of 0.5 M phosphate buffer pH 7.5 [A]. Chloramine T solution (50 μl, 1 mg/ml in H_2O) was mixed with 1 mCi of [^{125}I] NaI in 10 μl for 20 sec [B]. Mix [A] was added to Mix [B] and incubated for 20 sec. The reaction was stopped by the addition of $NaHSO_3$ (5 mg/ml, 50 μl) and KI (5 mg/ml, 50 μl) for 2 min. Free
15 iodine was separated from the radio-iodinated resistin by a size exclusion column (Sephadex G-25, PD-10 column, Amersham-Pharmacia) pre-equilibrated with 25 ml PBS containing 0.25% gelatine and 0.02% NaN_3. The eluted fractions (1 ml each) were monitored by a gamma-counter counter. Peak fractions had a specific activity of $3X10^{17}$ cpm/mol.

20 **Example 4: Cross-linking of affinity-purified resistin-BP to radioiodinated resistin.**

Samples (50 μl) from the ligand affinity purification step were incubated (overnight at 4°C) with ^{125}I-resistin (1,800,000 cpm). Disuccinimidyl suberate (DSS), dissolved in dimethyl sulfoxide (Me_2SO, 20 mM), was then added to a final concentration of 2 mM and the mixture was left for
25 20 min. at 4°C. The reaction was stopped by the addition of 1M Tris-HCl pH 7.5, and 1M NaCl to a final concentration of 100 mM. A sample buffer containing Dithiothreitol (DTT, 25 mM final) was added and the mixtures were analyzed by SDS-PAGE (7.5 % acrylamide) followed by autoradiography (Fig. 2).

A specific broad band having an apparent molecular mass of about 29 kD calculated from
30 the middle of the band or of molecular mass range of 26-32 kD if calculated from the bottom and top of the band respectively, was obtained in fractions eluted from the resisitn affinity column

(lanes 2) but not in the column wash (lane 4), containing all other crude urinary proteins. The band was not seen when ^{125}I-resistin was cross-linked to itself (lane 1). This band probably consisted of a 16-22 kD resistin binding protein cross-linked to the 10 kD ^{125}I-resistin. The band was not observed if an excess of unlabeled resistin was added together with ^{125}I-resistin (lane 3).
5 An additional band of 37 kD calculated from the middle of the band or of molecular mass range of 36-38 kD if calculated from the bottom and top of the band respectively, was observed in the elution fraction and was partially abolished by the addition of excess unlabeled resistin. One may speculate that this band is a cross linked complex comprising a dimer of ^{125}I-resistin (20 kD) and a resistin binding protein (16-22 kD).
10 The cross-linking studies show that resistin binds specifically a 16-22 kD urinary protein from the elution 2 fraction.

Example 5: Identification of a cell-associated resistin binding protein by cross-linking to radioiodinated resistin and immunoprecipitation.

15 Cross-linking studies of human ^{125}I-resistin were carried out with mouse cells. Murine 3T3 F442 fibroblasts (Green and Kehindle 1975 Cell 5 19-27 and Cell 7 105-113) can be differentiated into mature adipocytes following treatment with insulin. Since fetal bovine serum (FBS), in unlike bovine serum (BS), contains insulin, a change in medium supplementation to the cells from BS to FBS will trigger differentiation into adypocytes.
20 Murine 3T3 F442 fibroblasts (0.5-2x10^8 cells) grown in either FBS or BS were washed with PBS and then incubated with ^{125}I-resistin (12x10^6 cpm) for 1.5 hr at 4°C. DSS (final concentration of 2 mM) was added for 20 min. The cells were then washed with excess PBS containing 0.1% Triton X 100 and solubilized for 1 hr at 4°C by a Lysis Buffer (1% Triton X 100 in 10 mM CHAPS, 20 mM Tris HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM
25 Benzamidine, 1 mM PMSF, 6 μg/ml aprotinin). The lysate was spun (15,000xg, 4°C for min) and the clarified supernatant was immunoprecipitated with anti human resistin rabbit antibodies (Example 8) bound to Protein-G agarose beads overnight at 4°C. The beads were then washed with 0.1% Triton X 100 in PBS and boiled with SDS-PAGE-Sample Buffer containing β - mercaptoethanol. The supernatant was analyzed by SDS-PAGE (10% acrylamide) followed by
30 autoradiography. A specific band corresponding to a molecular weight of about 40 kD if calculated from the middle of the band or of 38-42 kD if calculated from the bottom or top of the

band respectively was found. This band probably consists of a cell surface protein of about 30 kD or to a cell surface protein in a range of 28-32 kD cross-linked to the 10 kD ^{125}I-resistin (Fig. 3).

5 In similar manner, Human HeLa cells ($4x10^8$) and human foreskin fibroblasts (cells $6x10^7$) were washed with PBS, cross-linked with human ^{125}I-resistin ($33x10^6$ cpm), washed, solubilized, immunoprecipitated and analyzed by SDS-PAGE to investigate whether a cell-associated ResBP exists. A band of similar molecular was observed (Fig. 3).

10 The cross-linking studies carried with whole cells show that a cell surface ResBP of apparent molecular weight of about 30 kD or in the range of 28-32 kD is present in both human and mouse cells.

Example 6: Determination of the affinity of human^{125}I-resistin to its cell-associated
15 **binding sites.**

Murine 3T3 F422 cells ($5X10^4$) in DMEM containing 10% bovine serum (BS) were seeded in a 24 well plates. On the next day, the cells were washed with DMEM containing 2% BS and 0.1% sodium azide (diluent) and incubated with the diluent for 20 minutes at room temperature.
20 Medium was discarded and a series of dilutions of ^{125}I-resistin ($3x10^{17}$ dpm/mol) in the diluent ($12x10^6$-$3x10^3$ cpm, 0.5 ml) were added to the wells and incubated for 90 min at room temperature with occasional shaking. Wells were quickly washed twice with the diluent and once with PBS, cells were collected by trypsin and the radioactivity was counted by a gamma counter. The binding affinity was determined by Scatchard analysis using the Ligand program. A
25 dissociation constant (Kd) of 10^{-10}M was obtained. The saturation binding curve is shown in Fig. 4.

The results show that cell associated ResBP has high affinity to resistin (Kd of about 10^{-10}M).

30

Example 7: measuring the biological activity of resistin and ResBP.

The biological activity of resistin (Peprotech) was measured by its ability to inhibit the differentiation of murine pre-adipocytes (3T3 F422) into adipocytes induced by switching the
5 supplementation of culture growth medium from bovine serum (BS) to fetal bovine serum (FBS). Addition of ResBP to the resisitin may be used to study its effect on resistin activity.
For the bioassay, murine 3T3 F442 cells were grown in 24 well plates ($5x10^4$/well) in DMEM containing 10% BS. When cells reached confluency the BS was replaced with 10% FBS. At day 8 differentiation into adipocytes was observed by the appearance of oil droplets in the cytoplasm.
10 Cells were observed microscopically for 7 more days and then stained with Oil Red, which highlights the oil droplets. Resistin was assayed for its ability to inhibit this differentiation. Upon switching of the cultures to medium containing FBS, a resisitin stock (1 μg/ml) in DMEM containing 10% FBS was serially diluted down to 7.5 pg/ml (in two-fold dilutions) and the dilutions were added to the cells. It was found that resistin at a concentration of 70 ng/ml
15 completely inhibited the differentiation of 3T3 F442 cells into adipocytes.
The biological activity of ResBP is measured by adding it at different concentrations together with resistin and checking its effect on the biological activity of resistin.

Example 8: Preparation of polyclonal antibodies to resistin.

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Rabbits were injected subcutaneously with 12 μg of a pure resistin preparation, emulsified in complete Freund's adjuvant. Three weeks later, they were injected again subcutaneously with 12 μg of the resistin preparation in incomplete Freund's adjuvant. Three additional injections of resistin as solution in PBS were given at 10-day intervals. The rabbits were bled 10 days after the
25 last immunization. The development of resistin specific antibody was followed by a radioimmunoassay. ^{125}I-labeled resistin (100,000 cpm) was mixed with various dilutions (1:200, 1:1000, 1:5,000 1:25,000 and 1:125,000) of the rabbit serum. A suspension of protein-G agarose beads (20 μl, Pharmacia) was added in a total volume of 200 μl. The mixture was left for 1 and a half hour at room temperature, the beads were then washed 3 times and bound radioactivity was
30 counted. Preimmune serum was used as a negative control. The titer of the resistin antiserum was >1:125,000.

Example 9: Preparation of monoclonal antibodies to ResBP.

5 Female Balb/C mice (3 months old) are first injected with 2 μg purified ResBP in an emulsion of complete Freund's adjuvant, and three weeks later, subcutaneously in incomplete Freund's adjuvant. Three additional injections of 2μg each are given at 10-day intervals, subcutaneously in PBS. Final boosts of 2μg are given intraperitoneally, 4 and 3 days before the fusion, to the mouse showing the highest binding titer as determined by IRIA (see below). Fusion is performed using 10 NSO/1 myeloma cell line and lymphocytes extracted from both the spleen and lymph nodes of the animal as fusion partners. The fused cells are distributed into microculture plates and the hybridomas are selected in DMEM supplemented with HAT and 15% horse serum. Hybridomas that are found to produce antibodies to ResBP are subcloned by the limiting dilution method and injected into Balb/C mice that had been primed with pristane for the production of ascites. The 15 isotypes of the antibodies are defined with the use of a commercially available ELISA kit (Amersham, UK).

The screening of hybridomas producing anti-ResBP monoclonal antibodies is performed as follows: Hybridoma supernatants are tested for the presence of anti-ResBP antibodies by an inverted solid phase radioimmunoassay (IRIA). 96 well microtiter flexible plates (Dynatech 20 Laboratories, Alexandria, VA) are coated with homogenous ResBP (10 μg/ml, 100 μl/well). Following overnight incubation at 4°C, the plates are washed twice with PBS containing BSA (0.5%) and Tween 20 (0.05%) and blocked in washing solution for at least 2 hrs at 37°C. Hybridoma culture supernatants (100 μl/well) are added and the plates are incubated for 4 hrs at 37°C. The plates are washed 3 times and a ^{125}I-goat-anti-mouse (100,000 cpm/well) is added for 4 25 hrs at room temperature. The plates are washed 3 times, wells are cut and counted in a gamma counter.

Alternatively the hybridomas can be screened as follows: 96-well microtiter plates are coated with goat anti-mouse antibodies and blocked as above. Hybridoma supernatants are added and the plates incubated for 4 hrs at 37°C. The plates are washed 3 times and a ^{125}I-ResBP (100,000 30 cpm/well) is added for 4 hrs at room temperature. The plates are washed 3 times, wells are cut and counted in a gamma counter.

Example 10: Purification of ResBP by affinity chromatography with monoclonal antibodies.

Antibodies against ResBP are utilized for the purification of ResBP by affinity chromatography. Ascitic fluid containing the monoclonal antibody secreted by the hybridoma is purified by ammonium sulfate precipitation at 50% saturation followed by extensive dialysis against PBS. About 10 mg of immunoglobulins are bound to 1 ml Affigel 10 (BioRad USA), as specified by the manufacturer.

250 ml of human urinary proteins (equivalent to 250 l of crude urine) are loaded on 0.5 ml of the anti ResBP antibody column at 4°C at a flow rate of 0.25 ml/min. The column is washed with PBS until no protein is detected in the washings. ResBP is eluted by 25 mM citric acid buffer, pH 2.2 (8 x 1 column volume fractions) and immediately neutralized by 1 M Na_2CO_3. Further purification of this preparation is obtained by size exclusion chromatography.

Example 11: ELISA test.

Microtiter plates (Dynatech or Maxisorb, by Nunc) are coated with anti-ResBP monoclonal antibody (serum free hybridoma supernatant or ascitic fluid immunoglobulins) overnight at 4°C. The plates are washed with PBS containing BSA (0.5%) and Tween 20 (0.05%) and blocked in the same solution for at least 2 hrs at 37°C. The tested samples are diluted in the blocking solution and added to the wells (100 μl/well) for 4 hrs at 37°C. The plates are then washed 3 times with PBS containing Tween 20 (0.05%) followed by the addition of rabbit anti-ResBP serum (1:1000, 100 μl/well) for further incubation overnight at 4°C. The plates are washed 3 times and a conjugate of goat-anti-rabbit horseradish peroxidase (HRP, Jackson Labs, 1:10,000, 100 μl/well) is added for 2 hrs at room temperature. The plates are washed 4 times and the color is developed by ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid, Sigma) with H_2O_2 as a substrate. The plates are read by an automatic ELISA reader.

The foregoing description of the specific embodiments reveal the general nature of the invention so that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and,

therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

5

Alternatively, a sandwich ELISA test is developed as above, but instead of the polyclonal antibody another monoclonal antibody is used. The monoclonal antibody used for detection is biotynilated and detected by HRP-streptavidin.

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CLAIMS:

1. The use of a resistin binding protein (ResBP), a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof, capable of at least one of the following:
(i) binding to resistin,
(ii) modulating the activity of resistin, or
(iii) blocking the activity of resistin,
in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases, which require modulation or blocking of resistin activity.

2. The use according to claim 1, wherein the ResBP is a peptidoglycan recognition-protein (PGRP), a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment and salt thereof.

3. The use according to claim 1, wherein the ResBP is cell associated and has an apparent molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment and salt thereof.

4. The use according to anyone of claims 1 to 3, wherein the ResBP binding protein is PEG-conjugated.

5. The use according to anyone of claims 1 and 4, wherein the ResBP is a fusion protein.

6. The use of an expression vector comprising the coding sequence of ResBP, a mutein, variant, fusion protein, circularly permutated derivative or fragment thereof, in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

7. The use according to claim 6, wherein the ResBP is PGRP.

8. The use according to claim 6, wherein the ResBP is a cell associated ResBP with an apparent
5 molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M, or a mutein, variant, fusion protein, circularly permutated derivative, active fraction, fragment and salt thereof.

9. The use of a vector for inducing and/or enhancing the endogenous production of ResBP in the manufacture of a medicament for the treatment and/or prevention of a disease selected from
10 insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

10. The use according to claim 9, wherein the ResBP is PGRP.

15 11. The use according to claim 9, wherein the ResBP is cell associated and has an apparent molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M.

12. The use of a vector for negative regulation of endogenous resistin expression in the manufacture of a medicament for the treatment and/or prevention of disease selected from insulin
20 resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

13. The use of a cell that has been genetically modified to overexpress ResBP, a mutein, variant,
25 fusion protein, circularly permutated derivative, active fraction, or fragment thereof in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

30 14. The use according to claim 13, wherein the ResBP is PGRP, a mutein, variant, fusion protein, circularly permutated derivative, or fragment thereof.

15. The use according to claim 13, wherein the ResBP is cell associated and has an apparent molecular weight of about 30 kD and a Kd for resistin of about 10^{-10}M, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof.

5 16. A pharmaceutical composition for treating or preventing a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity comprising a resistin binding protein (ResBP), a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof and mixtures thereof capable of at least one of the following:

10 (i) binding to resistin,

(ii) modulating the activity of resistin, or

(iii) blocking the activity of resistin.

17. A pharmaceutical composition according to claim 16, wherein ResBP is cell associated and

15 has an apparent molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment and salt thereof.

18. A pharmaceutical composition comprising an antibody that immunospecifically binds to

20 ResBP and a pharmaceutically acceptable carrier for treating or preventing disease, which require modulation or blocking of resistin activity.

19. A pharmaceutical composition according to claim 18, wherein the antibody is a polyclonal antibody, monoclonal antibody, anti-idiotypic antibody, humanized antibody or a mutein, variant,

25 fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof.

20. A pharmaceutical composition comprising an expression vector encoding the sequence of ResBP, a mutein, variant, fusion protein, circularly permutated derivative, or fragment thereof,

30 in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

21. The pharmaceutical composition according to claim 20, wherein the ResBP is PGRP.

22. The pharmaceutical composition according to claim 20, wherein the ResBP is a cell
5 associated ResBP with an apparent molecular weight of about 30 kD and Kd to resistin of about 10^{-10}M.

23. A pharmaceutical composition comprising a vector including regulatory sequences functional in the cells for negative regulation of endogenous resistin expression in the manufacture of a
10 medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

24. A pharmaceutical composition comprising a vector including regulatory sequences functional
15 in the cells for inducing and/or enhancing the endogenous production of ResBP in a cell, in the manufacture of a medicament for the treatment and/or prevention of a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

20 25. The pharmaceutical composition according to claim 24, wherein the ResBP is PGRP.

26. A pharmaceutical composition comprising a cell that has been genetically modified to overexpress ResBP or a mutein, variant, fusion protein, circularly permutated derivative, and fragment thereof, in the manufacture of a medicament for the treatment and/or prevention of a
25 disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity.

27. The pharmaceutical composition according to claim 26, wherein the ResBP is PGRP a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment
30 thereof.

28. The pharmaceutical composition according to claim 26, wherein the ResBP is cell associated and has an apparent molecular weight of about 30 kD and a Kd to resistin of about 10^{-10}M, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment and salt thereof.

5

29. A method for treating or preventing a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity, comprising administering to a subject in which such treatment or prevention is desired, the pharmaceutical composition according to anyone of claims 16 to 28 in
10 an amount sufficient to treat or prevent said disease in the subject.

30. A method for treating or preventing a disease selected from insulin resistance, gestational diabetes, polycystic ovary syndrome, NIDDM and other diseases which require modulation or blocking of resistin activity, comprising administering to a subject in which such treatment or
15 prevention is desired, comprising administration of PGRP a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof.

31. A ResBP, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof, except PGRP (SEQ ID No: 1), and salt thereof, capable of at least the
20 following:

(i) binding to resistin,
(ii) modulating the activity of resistin, or
(iii) blocking the activity of resistin,

25

32. A protein according to claim 31 wherein the ResBP is cell associated and has an apparent molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M, a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof.

30 33. A protein according to claims 31 or 32, wherein the protein is the resistin receptor.

34. A nucleic acid molecule comprising a nucleic acid sequence encoding a ResBP a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof, according to anyone of claims 31 to 33.

5 35. A nucleic acid molecule according to claim 34, being a cDNA sequence.

36. A nucleic acid molecule according to claim 34, being a genomic DNA sequence.

37. A vector comprising a nucleic acid molecule according to anyone of claims 34 to 36.

10

38. A vector according to claim 37, further comprising a promoter operably linked to the nucleic acid molecule.

39. A cell that has been genetically modified to produce a protein according to anyone of claims
15 31 to 33.

40. A cell according to claim 39, wherein the cell has been genetically modified to contain a vector according to claims 37 or 38.

20 41. A cell according to claims 39 or 40, wherein the cell is eukaryotic

42. A cell according to claim 41, wherein the cell is mammalian.

43. A cell according to claim 42, wherein the cell is CHO.

25

44. A cell according to claim 40, wherein the cell is prokaryotic.

45. A method for the production of a protein according to any one of claims 31 to 33, comprising culturing a cell according to anyone of claims 39 to 44 and isolating the protein so produced.

30

46. An antibody capable of binding a ResBP protein according to anyone of claims 31 to 33.

47. An antibody according to claim 46, wherein the antibody is a polyclonal antibody, monoclonal antibody, anti-idiotypic antibody, chimeric antibody, humanized antibody a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof.

5 48. A process for the isolation or purification of ResBP comprising:
passing a sample through a chromatographic column to which resistin is coupled, washing the column to remove unbound protein in the sample, and recovering the bound ResBP.

49. A process for isolation of ResBP according to claim 48, wherein the sample comprises cell
10 lysate.

50. A process for isolation of ResBP according to claim 48, wherein the sample comprises a body fluid.

15 51. A process for isolation of ResBP according to claim 50, wherein the sample comprises urine.

52. A process for isolation of ResBP according to claim 48, wherein the sample comprises conditioned medium of cells.

20 53. A process for the purification of resistin comprising:
Passing a sample trough a chromatographic column to which ResBP or a fragment, isoform, mutein, or functional derivative thereof is coupled, washing the column to remove unbound protein in the sample, and recovering the bound resistin.

25 54. A process according to claim 53, wherein ResBP is PGRP a mutein, variant, fusion protein, circularly permutated derivative, active fraction, or fragment thereof and salt thereof

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55. A process according to claim 53, wherein ResBP is cell associated and has an apparent molecular weight of about 30 kD and Kd for resistin of about 10^{-10}M, or a mutein, variant, fusion protein, circularly permutated derivative, active fraction, fragment and salt thereof.

5 56. A ResBP protein obtainable by the process according to anyone of claims 48 to 53.

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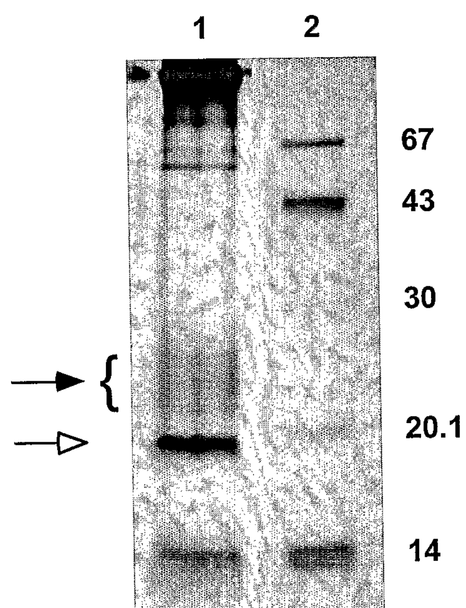

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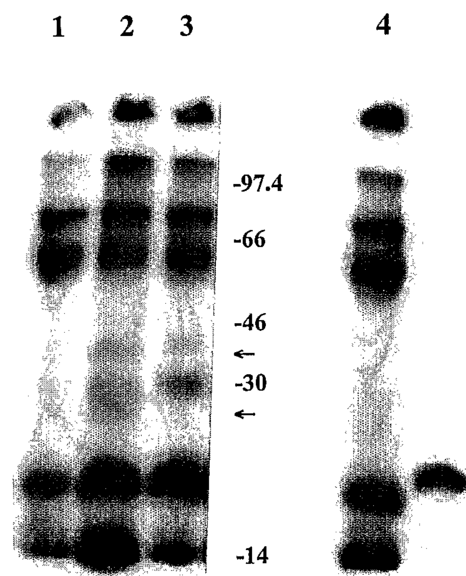
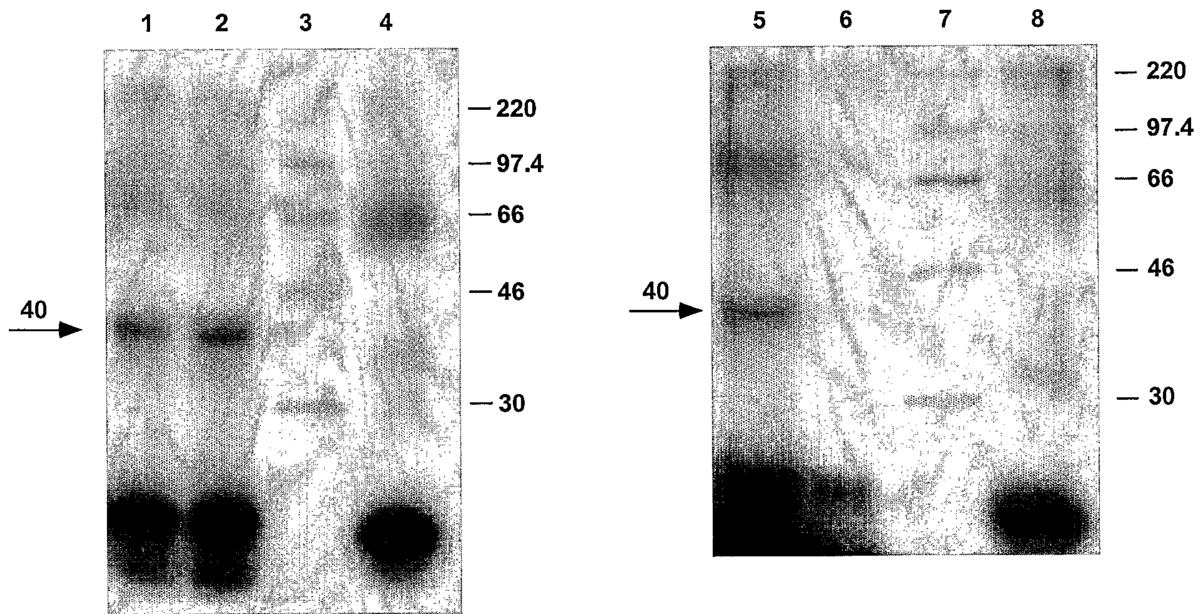


Figure 2



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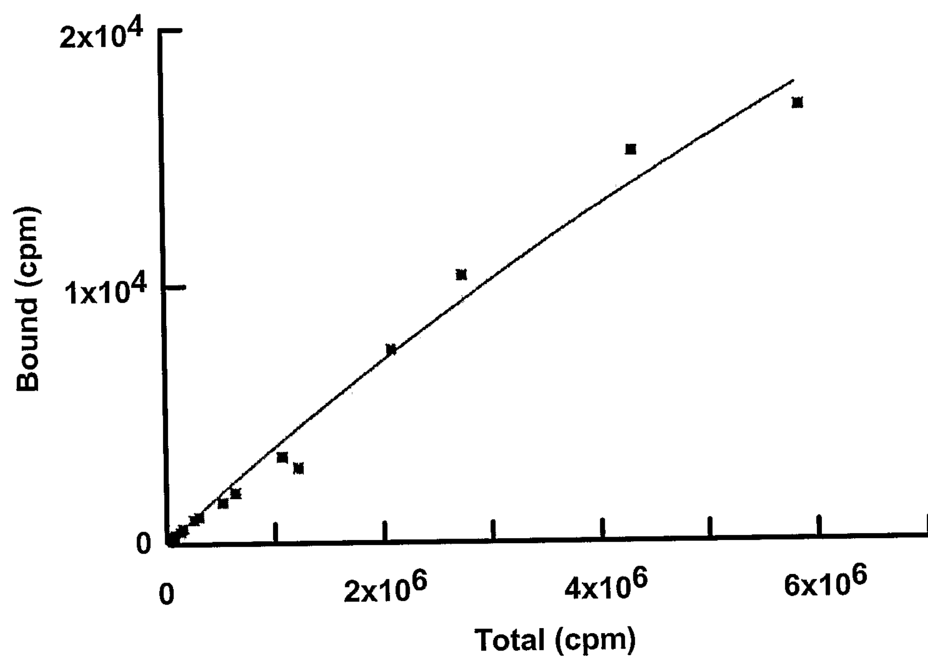

Fig. 4

476.ST25

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