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(54) **METHODS FOR IDENTIFYING AGENTS THAT MODULATE GPR105 ACTIVITY**

(75) Inventors: **John Lamb**, Shoreline, WA (US); **Joseph Mancini**, Kirkland (CA); **Brian Kennedy**, Kirkland (CA)

Correspondence Address:
CHRISTENSEN, O'CONNOR, JOHNSON, KINDNESS, PLLC
1420 FIFTH AVENUE
SUITE 2800
SEATTLE, WA 98101-2347 (US)

(73) Assignees: **Rosetta Inpharmatics LLC**, Seattle, WA (US); **Merck and Co., Inc.**, Rahway, NJ (US)

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(57) **ABSTRACT**

In one aspect, the present invention provides methods for determining whether a chemical agent modulates the biological activity of a GPR105 protein. The methods of this aspect of the invention include the steps of: (a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity; (b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and (c) validating, in vivo, the modulating effect of the chemical agent on the biological activity of the GPR105 protein.

METHODS FOR IDENTIFYING AGENTS THAT MODULATE GPR105 ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/730,595, filed Oct. 26, 2005.

FIELD OF THE INVENTION

[0002] The present invention relates to methods for screening for new drug molecules.

BACKGROUND

[0003] Metabolic Syndrome is a disorder that includes obesity, dyslipidaemia, and hyperglycemia. Metabolic Syndrome has increased to epidemic proportions worldwide. The pathophysiology of this syndrome is attributed to central distributed obesity, decreased high density lipoprotein, elevated triglycerides, elevated blood pressure and hyperglycemia. People suffering from Metabolic Syndrome are at increased risk of type II diabetes, coronary heart disease, and other diseases related to plaque accumulation in artery walls (e.g., stroke and peripheral vascular disease). In two prospective European studies, Metabolic Syndrome was a predictor of increased cardiovascular disease and mortality. (Isomaa et al., "Cardiovascular Morbidity and Mortality Associated With the Metabolic Syndrome,"*Diabetes Care* 24:683-689, 2001; Lakka et al., "The Metabolic Syndrome and Total and Cardiovascular Disease Mortality in Middle Aged Men,"*JAMA* 288:2709-2716, 2002.)

[0004] The most significant underlying cause of Metabolic Syndrome is obesity. The genetic factors that also contribute to Metabolic Syndrome are not yet understood. Consequently, there is a need to identify genes that contribute to the development of Metabolic Syndrome. There is also a need for methods that permit the identification of chemical agents that modulate the activity of these genes or modulate the activity of the products (e.g., proteins) encoded by these genes. Such chemical agents may be useful, for example, as drugs to prevent Metabolic Syndrome or to ameliorate at least one symptom of Metabolic Syndrome.

SUMMARY

[0005] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[0006] In accordance with the foregoing, the present inventors have discovered that expression of GPR105 protein is correlated with weight gain and development of type II diabetes. Further, the present inventors have demonstrated that antisense inhibition of GPR105 expression in mice reduces the rate at which the mice gain weight in response to a high fat diet. The mice also have lower levels of insulin, suggesting a decreased level of insulin resistance in these mice. Accordingly, GPR105 is a target for drugs that prevent diabetes, obesity or Metabolic Syndrome, or that ameliorate at least one symptom of Metabolic Syndrome.

[0007] Thus, in one aspect, the present invention provides methods for determining whether a chemical agent modulates the biological activity of a GPR105 protein. The methods of this aspect of the invention include the steps of: (a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity; (b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and (c) validating, in vivo, the modulating effect of the chemical agent on the biological activity of the GPR105 protein.

[0008] In another aspect, the present invention provides methods for determining the effect of a chemical agent on GPR105 activity. The methods of this aspect of the invention each include the steps of: (a) observing a change in a GPR105-mediated response in a living cell, in vitro, in response to a chemical agent; and (b) confirming that the observed change in the GPR105-mediated response occurs in vivo.

[0009] The foregoing methods of the present invention are useful, for example, for identifying chemical agents that modulate the biological activity of a GPR105 protein in a living cell. These chemical agents are useful, for example, as drugs to prevent obesity or diabetes, or to ameliorate at least one symptom of Metabolic Syndrome, or to further characterize the mechanism of action of a GPR105 protein in a living cell. The foregoing methods of the present invention can be used, for example, as an initial screen to identify chemical compounds that can be further screened and analyzed to identify compounds that prevent obesity or type II diabetes or that ameliorate at least one symptom of Metabolic Syndrome.

DETAILED DESCRIPTION

[0010] The present invention provides methods for determining whether a chemical agent modulates the biological activity of a GPR105 protein. The methods comprise the steps of: (a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity; (b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and (c) validating, in vivo, the modulating effect of the chemical agent on the biological activity of the GPR105 protein.

[0011] As used herein, the term "chemical agent" encompasses any chemical molecule, or chemical element, or a combination of chemical molecules and/or chemical elements. For example, the term "chemical agent" encompasses proteins (comprising at least 100 covalently linked amino acid units) and peptides (comprising from 2 to 99 covalently linked amino acid units). Again, by way of example, the term "chemical agent" encompasses molecules that mimic the ligands for a GPR105 protein.

[0012] As used herein, the term "GPR105 protein" refers to a type of G-protein coupled receptor. The natural ligand for GPR105 protein is not known, but UDP-hexoses (e.g., UDP-glucose) binds the receptor with high affinities (100-500 nM). UDP-glucose is an activated form of glucose used for glycogen synthesis. The biological function of GPR105 is not known, but it may have a role in cellular chemotaxis and inflammation. Human body atlas data shows that GPR105 is predominantly expressed in the intestines and

subcutaneous white adipose tissue. Mouse data shows highest expression of GPR105 in spleen and pancreas, with only average expression in brain.

[0013] Some GPR105 proteins useful in the practice of the present invention are at least 79% identical (e.g., at least 80% identical, or at least 90% identical, or at least 95% identical, or at least 99% identical) to the human GPR105 protein having the amino acid sequence set forth in SEQ ID NO:1, and encoded by the transcript having GenBank accession number NM_014879.

[0014] The term "percent identity" or "percent identical," when used in connection with amino acid sequence relatedness between GPR105 proteins, is defined as the percentage of amino acid residues in a first GPR105 protein sequence that are identical with a second GPR105 protein sequence (such as the GPR105 amino acid sequence of SEQ ID NO: 1), after aligning the first and second GPR105 sequences to achieve the maximum percent identity. For example, percentage identity between two protein sequences can be determined by pairwise comparison of the two sequences using the b12seq interface at the Web site of the National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, Md. 20894, U.S.A. The b12seq interface permits sequence alignment using the BLAST tool described by A. Tatiana et al., "Blast 2 Sequences—A New Tool for Comparing Protein and Nucleotide Sequences,"*FEMS Microbiol Lett.* 174:247-250, 1999. The following alignment parameters are used: Matrix=BLOSUM62; Gap open penalty=11; Gap extension penalty=1; Gap x_dropff=50; Expect=10.0; Word size=3; and Filter=off.

[0015] As used herein, the term "biological activity" refers to an effect of a GPR105 protein on a biological process in a living cell, living tissue, living organ and/or living organism. Examples of biological processes include biochemical pathways, concentration of one or more chemical compounds within a living cell, physiological processes that contribute to the internal homeostasis of a living organism, developmental processes that contribute to the normal physical development of a living organism, and acute or chronic diseases.

[0016] Modulation of the biological activity of a GPR105 protein encompasses any change in a biological activity of a GPR105 protein. For example, the change can be a decrease in a biological activity of a GPR105 protein (e.g., complete, or substantially complete, inhibition of a biological activity of a GPR105 protein). Again by way of example, the change can be a reduction in the rate of a biological activity of a GPR105 protein. Again by way of example, the change can be an increase in the activity of a GPR105 protein.

[0017] In the practice of the invention, a living cell, more typically a population of living cells, such as a liquid culture of living cells, is/are contacted with a chemical agent. It is then determined whether the chemical agent modulates the biological activity of a GPR105 protein in the living cell. By way of example, modulation of the biological activity of GPR105 protein in a living cell can be identified using the method disclosed by P. Kunapuli et al., *Analytical Biochemistry* 314:16-29, 2003, which publication is incorporated herein by reference. In brief, a vector that includes a nucleic acid molecule encoding a GPR105 protein is stably introduced into cells, such as HEK cells or CHO cells, and the encoded GPR105 protein is expressed in the cells. Additionally, a nucleic acid molecule encoding β-lactamase is stably introduced into the cells expressing the GPR105 protein. Thereafter, the cells are contacted with a candidate agonist, or candidate antagonist, of GPR105 and the effect of the candidate agonist, or candidate antagonist, on the expression of β-lactamase in the cells is measured (e.g., to determine whether the candidate agonist or antagonist causes an increase or decrease of expression of β-lactamase in the cells). Examples of methods for measuring β-lactamase activity in living cells are set forth, for example, in J. K. Chambers et al., "AG Protein-Coupled Receptor for UDP-Glucose,"*J. Biol. Chem.* 275(15):10767-10771, 2000; and P. Kunapuli et al., supra. Alternatively, the effect of the candidate agonist, or candidate antagonist, on GPR105 activity can be assessed by measuring changes in intracellular calcium levels in response to the action of the candidate agonist or candidate antagonist on GPR105.

[0018] By way of example, the cDNA molecule disclosed in SEQ ID NO:2 encodes a chimpanzee GPR105 protein (SEQ ID NO:3) that is useful in the practice of the present invention. As described more fully in Example 2, the nucleic acid molecule shown in SEQ ID NO:2 was modified and cloned into a pcDEF3 expression vector and co-transfected with a vector containing Gqi5 into HEK-293 cells so that the transfected cells expressed GPR105 protein (SEQ ID NO:3). The cells were maintained for several days and then plated into a 384 well format and challenged with various concentrations of UDP-glucose. Measurement of Ca^{2+}stimulation in these HEK cells was performed using FLIPR (Molecular Devices, CA, USA) as previously described (K. Freeman et al., "Cloning, Pharmacology, and Tissue Distribution of G-Protein-Coupled Receptor GPR105 (KIAA0001) Rodent Orthologs,"*Genomics* 78:124-128, 2001).

[0019] An assay for assessing the effect of a chemical agent on a biological activity of a GPR105 protein typically includes at least one experimental treatment wherein a living cell (or population of living cells), expressing a GPR105 protein, is/are contacted with a chemical agent; and a control treatment wherein the same type of cell(s) expressing the GPR105 protein (e.g., an aliquot of the same preparation of cells used in the experimental treatment) is/are treated identically to the cell(s) used in the experimental treatment, except that the cell(s) used in the control treatment is/are not contacted with the chemical agent. Comparison of the GPR105 biological activity in the experimental treatment(s) with the GPR105 biological activity in the control treatment(s) permits determination of whether the chemical agent modulates GPR105 biological activity. For example, a level of GPR105 biological activity that is significantly lower in the experimental treatment(s) compared to the control treatment(s) indicates that the chemical agent inhibits GPR105 biological activity. Again by way of example, a level of GPR105 biological activity that is significantly higher in the experimental treatment(s) compared to the control treatment(s) indicates that the chemical agent stimulates GPR105 biological activity.

[0020] Numerous assays (e.g., hundreds or thousands of assays) for assessing the effect of a chemical agent on GPR105 biological activity can be automated and conducted simultaneously. For example, the assays can be automated in

a high-throughput sequence format as described, for example, by O. Kornienko et al., *J. Biomol. Screen* 9(3):186-195, 2004.

[0021] In the practice of the claimed methods, the modulating effect of a chemical agent on GPR105 biological activity is validated in vivo. The validation step shows that a chemical agent that modulates the biological activity of GPR105, in vitro, also causes a significant improvement in a phenotype, in vivo, associated with type II diabetes and/or obesity (e.g., the chemical agent causes one or more of the following changes: lowers LDL cholesterol, raises HDL cholesterol, lowers body weight, decreases the rate of body weight gain in response to a diet high in fat, decreases insulin resistance, increases glucose tolerance and/or decreases fat pad mass (in rats or mice) in response to a high fat diet).

[0022] Animal models can be used to validate the efficacy of a chemical agent that modulates a biological activity of a GPR105 protein in vitro. For example, the effect of a chemical agent on blood pressure can be directly determined using, for example, a radiotelemetry technique (see, e.g., P. A. Mills et al., "A New Method for Measurement of Blood Pressure, Heart Rate, and Activity in the Mouse by Radiotelemetry,"*J. Appl. Physiol.* 88(5):1537-1544, 2000). Again by way of example, the effect of a chemical agent on blood pressure can be indirectly determined using, for example, a tail-cuff technique (see, e.g., B. N. Van Vliet et al., "Direct and Indirect Methods Used to Study Arterial Blood Pressure, "*J. Pharmacol. Toxicol. Methods* 44(2):361-373, 2000).

[0023] Again by way of example, the effect of a chemical agent on body weight can be determined using an obesity model wherein obesity is induced by a high fat diet or by using an obese mutant mouse model (e.g., ob/ob mice) (see, e.g., M. Tschop and M. L. Heiman, "Rodent Obesity Models: An Overview,"*Exp. Clin. Endocrinol. Diabetes,* 109(6):307-319, 2001).

[0024] By way of further example, the effect of a chemical agent on a component of type II diabetes can be measured using a streptozotocin-induced diabetic model, or, for example, by using a spontaneous mutant model such as the obese Zucker rats (fa/fa rats) or the db/db mice (see, e.g., D. Methe, "Dyslipidemia and Diabetes: Animal Models,"*Diabetes Metab.* 21(2): 106-111, 1995).

[0025] In another aspect, the present invention provides methods for determining the effect of a chemical agent on GPR105 activity. The methods of this aspect of the invention each include the steps of: (a) observing a change in a GPR105-mediated response in a living cell, in vitro, in response to a chemical agent; and (b) confirming that the observed change in the GPR105-mediated response occurs in vivo.

[0026] In the practice of this aspect of the invention, a change in a GPR105-mediated response can be observed using, for example, any of the techniques described herein for determining whether a chemical agent modulates the biological activity of a GPR105 protein in a living cell. An observed change in a GPR105-mediated response can be confirmed, in vivo, using any of the methods described herein for validating, in vivo, the modulating effect of a chemical agent on GPR105 biological activity.

[0027] In a further aspect, the present invention provides methods for identifying a chemical agent that ameliorates a symptom of type II diabetes or obesity. The methods of this aspect of the invention include the steps of: (a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity; (b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and (c) determining, in vivo, whether the chemical agent ameliorates a symptom of type II diabetes or obesity. Examples of symptoms of type II diabetes and/or obesity include increased insulin resistance, increased body mass, and a decreased rate of glucose clearance from the blood stream.

[0028] In a further aspect, the present invention provides an isolated nucleic acid molecule (SEQ ID NO:2) that encodes a chimpanzee GPR105 protein (SEQ ID NO:3). The present invention also provides an isolated GPR105 protein (SEQ ID NO:3) that can be obtained, for example, by expressing the cDNA molecule disclosed in SEQ ID NO:2 in living cells. The present invention also provides a further isolated nucleic acid molecule (SEQ ID NO:4) that encodes the GPR105 protein having the amino acid sequence set forth in SEQ ID NO:3.

[0029] The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention.

EXAMPLE 1

[0030] This Example shows that an antisense oligonucleotide directed against GPR105 reduced the expression of GPR105 in transgenic mice expressing the antisense oligonucleotide, and reduced the extent of phenotypes associated with Metabolic Syndrome.

[0031] Several large mouse crosses were performed, including crosses between C57BL/6 ApoE −/− x C3H/HEJ ApoE −/− crosses. The F2 mice resulting from this cross were analyzed for various genetic as well as phenotype traits relating to metabolic disorders and these traits were correlated with gene expression analysis. Central to the genetic analysis was a likelihood-based test for causality that takes into account genotypic, RNA, and clinical data in a segregating population to identify genes in the trait-specific transcriptional network that are under the control of multiple Quantitative Trait Loci (QTLs) for the trait of interest, but still upstream of the trait. The mouse crosses, and the gene analysis methodology are described by E. E. Schadt et al., *Nature Genetics* 37(7):710-717, 2005, which publication is incorporated herein by reference.

[0032] GPR105 was identified as a candidate gene that may cause adiposity. Several antisense oligonucleotides (ASOs) were evaluated, in vitro, in NIH-3T3 cells, and one oligonucleotide (referred to as the "effective GPR105 antisense oligonucleotide") caused greater than 90% reduction of GPR105 expression.

[0033] The effective GPR105 antisense oligonucleotide was then used to reduce GPR105 expression in vivo in diet induced obesity (DIO) mice. The experiment used a saline control (referred to as the "vehicle"), a scrambled antisense oligonucleotide as a negative control, and an SCD-1 antisense oligonucleotide as a positive control. This experiment was conducted for four weeks, a timeframe in which the positive control demonstrates efficacy. The results are shown

in Table 1. The following abbreviations are used in Table 1: GPR105, the effective GPR105 antisense oligonucleotide; SEM, standard error of the mean.

TABLE 1

	% Weight gain							
	Vehicle		GPR105		SCD-1		Scrambled	
Week	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	8.35	1.2	5.08	1.61	12.13	1.06	11.87	1.49
2	18.47	2.38	10.47	1.49	18.67	2.52	22.78	2.44
3	27.58	3.28	15.08	2.25	23.48	3.42	34.22	3.18
4	37.92	1.83	18.2	2.49	25.55	3.35	38.93	2.52

[0034] As shown in Table 1, the mice treated with the SCD-1 antisense oligonucleotide, and the effective GPR105 antisense oligonucleotide, were significantly resistant to weight gain induced by a high-fat diet. After four weeks of treatment, the mice treated with the effective GPR105 antisense oligonucleotide had less than half the weight of the animals treated with the scrambled oligonucleotide or saline control, with no significant difference in food intake.

[0035] In order to confirm that a reduction of GPR105 mRNA was caused by the effective GPR105 antisense oligonucleotide, quantitative PCR analysis was performed on RNA isolated from liver, adipose, and kidney. In animals treated with the effective GPR105 antisense oligonucleotide, greater than 85% reduction of GPR105 MRNA levels was observed in liver and kidney, whereas adipose tissue showed a lesser reduction of 37%. Similar reduced levels of GPR105 mRNA were also observed in animals treated with the SCD-1 antisense oligonucleotide. The results of this experiment are shown in Table 2.

TABLE 2

	Liver		Adipose Tissue		Kidney	
Group	GPR105 relative exp	SCD-1 relative exp	GPR105 relative exp	SCD-1 relative exp	GPR105 relative exp	SCD-1 relative exp
Vehicle	1.00	1.00	1.00	1.00	1.00	1.00
Vehicle	1.83	0.81	0.66	1.60	0.81	0.49
Vehicle	0.53	0.33	0.96	1.39	0.55	0.72
Vehicle	0.68	1.21	0.67	0.70	0.48	1.05
Vehicle	0.43	0.60	0.89	0.52	0.46	
Vehicle (Mean ± SEM)	0.89 ± 0.25	0.79 ± 0.15	0.84 ± 0.07	1.04 ± 0.2	0.66 ± 0.11	0.82 ± 0.13
Antisense	0.02	0.12	0.65	0.86	0.10	0.35
Antisense	0.01	0.15	0.40	0.82	0.09	0.89
Antisense	0.01	0.19	0.44	0.83	0.11	0.48
Antisense	0.27	0.10	0.69	0.30	0.09	0.23
Antisense	0.07	0.09	0.49	0.27	0.07	0.30
Antisense (Mean ± SEM)	0.08 ± 0.05	0.13 ± 0.02	0.53 ± 0.06	0.62 ± 0.14	0.09 ± 0.01	0.45 ± 0.12
Scrambled Oligo	0.96	0.37	1.22	1.08	0.45	0.47
Scrambled Oligo	1.09	1.43	1.19	1.66	1.01	0.96
Scrambled Oligo	0.63	0.44	1.38	0.98	0.57	0.62
Scrambled Oligo	0.89	0.50	1.49	0.98	0.46	0.49
Scrambled Oligo	0.57	0.54	1.60	0.88	0.35	
Scrambled Oligo (Mean ± SEM)	0.83 ± 0.1	0.66 ± 0.2	1.38 ± 0.08	1.12 ± 0.14	0.57 ± 0.12	0.63 ± 0.11

[0036] Since significant resistance to weight gain was achieved with the effective GPR105 antisense oligonucleotide, insulin levels were also measured. In this experiment, insulin levels were measured from mouse plasma at the end of the 4 week antisense oligonucleotide experiment described in connection with Table 1. Samples were analyzed in duplicate using an ELISA assay (the ELISA kit was purchased from CrystalChem, IL, USA, Catalog No. 90060) and the corresponding mean, and the mean of the group of animals, is reported.

[0037] Significant increases in insulin levels were observed in mice treated with the vehicle and scrambled antisense oligonucleotides as compared to mice treated with the effective GPR105 antisense oligonucleotide and the SCD-1 antisense oligonucleotide ($p<0.005$ and 0.01, respectively) for similar glucose levels. The results of this experiment are shown in Table 3.

TABLE 3

	Insulin Measurement ng/ml					
Treatment	Mouse #	Dupl #1	Dupl #2	Mean	Group Mean	SEM
Vehicle	1	2.9	3.2	3.0	3.4	0.6
Vehicle	2	2.4	2.5	2.4		
Vehicle	3	3.1	3.0	3.1		
Vehicle	4	2.8	2.5	2.6		
Vehicle	5	5.7	5.6	5.6		
GPR105ASO	6	0.7	0.8	0.7	1.1	0.2
GPR105ASO	7	1.0	0.7	0.8		
GPR105ASO	8	1.3	1.2	1.3		
GPR105ASO	9	1.8	1.8	1.8		
GPR105ASO	10	0.7	0.7	0.7		
SCD-1ASO	11	0.8	0.7	0.8		
SCD-1ASO	12	1.8	1.6	1.7	1.5	0.2

TABLE 3-continued

Treatment	Insulin Measurement ng/ml Mouse #	Dupl #1	Dupl #2	Mean	Group Mean	SEM
SCD-1ASO	13	2.0	1.7	1.8		
SCD-1ASO	14	1.3	1.2	1.2		
SCD-1ASO	15	2.1	1.9	2.0		
Scrambled ASO	16	1.4	1.3	1.4	2.4	0.3
Scrambled ASO	17	1.9	2.2	2.1		
Scrambled ASO	18	2.3	2.8	2.6		
Scrambled ASO	19	3.5	3.3	3.4		
Scrambled ASO	20	3.0	2.5	2.8		

[0038] The data shown in Table 3 suggest that the obese mice were becoming insulin resistant in relation to the mice treated with the effective GPR105 antisense oligonucleotide or the SCD-1 antisense oligonucleotide.

[0039] White adipose tissue (WAT) was isolated (and weighed) from the mice treated with the effective GPR105 antisense oligonucleotide, the vehicle, and the scrambled antisense oligonucleotide negative control. The weight of the epididymal fat pads is shown in Table 4.

TABLE 4

	Weight (g)	Mean	SEM
Vehicle	0.26	0.44	0.16
Vehicle	0.57		
Vehicle	1.02		
GPR105 ASO	0.17	0.16	0.02
GPR105 ASO	0.17		
GPR105 ASO	0.11		
GPR105 ASO	0.17		
SCD-1 ASO	0.37	0.29	0.04
SCD-1 ASO	0.39		
SCD-1 ASO	0.29		
SCD-1 ASO	0.19		
SCD-1 ASO	0.23		
Scrambled ASO	0.15	0.46	0.17
Scrambled ASO	0.15		
Scrambled ASO	0.25		
Scrambled ASO	0.95		
Scrambled ASO	0.81		

[0040] The weight of the epididymal fat pads in the animals treated with the effective GPR105 antisense oligonucleotide were significantly reduced compared to the vehicle and scrambled antisense oligonucleotide controls ($p<0.05$). This data demonstrates that a significant amount of the weight loss observed in the DIO study was due to decreases in fat pad weight.

[0041] Since the adipose tissue weight was significantly decreased in the mice treated with the effective GPR105 antisense oligonucleotide, adipose tissues was also sectioned for histological analysis. WAT sections from both inguinal and epididymal regions were obtained. The sections were 5μm thick and they were fixed and stained using heamatoxylin and Eosin. The researcher was blinded to the tissue samples and a microscope eye piece with plain cross-hairs was used. A 20× objective was used and a field was chosen where both the horizontal and vertical lines were overlaying the cross section. Each time one of the cross hairs intersected the adipocyte wall provides a quantitative measure of adipocyte size. This was repeated for 10 different regions throughout the histology section. The value that was obtained is an Lm measurement, where Lm=nL/I, n equals the number of lines superimposed, L is the length of the line and I is the total number of intercepts (W. M. Thurlbeck, "Measurement of Pulmonary Emphysema,"*American Review of Respiratory Disease* 95:752-764, 1967). Therefore, the higher the value of Lm the larger the adipocyte. The results of this analysis for inguinal and epididymal WAT are depicted below in Table 5.

TABLE 5

	Inguinal WAT Lm (μM)			Epididymal WAT Lm (μM)		
Group	n	Mean	SEM	n	Mean	SEM
Vehicle	4	91.0	9.7	3	109.5	13.7
GPR105 ASO	3	44.6	5.5	4	68.2	7.4
SCD-1 ASO	5	73.4	7	5	103.7	10
Scrambled ASO	5	73.2	7	5	91.8	8.9

[0042] As shown in Table 5, the mice treated with the effective GPR105 antisense oligonucleotide had both a significant decrease in adipose tissue weight and size. In fact, the size of the adipocytes in the inguinal WAT from the mice treated with the effective GPR105 antisense oligonucleotide was only 60% ($p<0.01$) of either the scrambled ASO or vehicle control animals. In the epididymal WAT the adipocyte size of the mice treated with the effective GPR105 antisense oligonucleotide was 74% of the size of the scrambled ASO or vehicle control animals (difference with vehicle $p<0.05$, difference with scrambled, $p=0.063$). These data clearly demonstrate a substantial effect on adipose cell size in mice placed on a high fat/high carbohydrate diet when treated with the effective GPR105 antisense oligonucleotide.

EXAMPLE 2

[0043] This Example describes the development of a cell-based functional assay to identify modulators (e.g., agonists or antagonists) of GPR105 activity.

[0044] A region of the chimpanzee genome was identified which was predicted to encode a simian GPR105 protein. The human P2RY14 protein sequence (Genbank RefSeq NP_055694.2) was used to perform a TBLASTN search against the Ensembl chimpanzee (Pan troglodytes) Ab-initio/SNAP DNA database using the default configuration parameters for TBLASTN, with the exception that the query sequence was not filtered to remove regions of low complexity.

[0045] A near-perfect match (99.1 percent identity) corresponding to the complete 338 amino acid residues of the human P2RY14 protein sequence was identified as GENSCAN00000068233. The nucleic acid sequence of the 1017 bp predicted cDNA is shown in SEQ ID NO:2, and encodes the predicted GPR105 protein having the amino acid sequence shown in SEQ ID NO:3. The 1017 bp predicted cDNA (SEQ ID NO:2) is located on chimpanzee chromosome 2, with genomic coordinates 155619963 to 155620976 on the negative strand.

[0046] The chimpanzee GPR105 sequence (SEQ ID NO:2) was chemically synthesized (BIO S & T, Montreal,

PQ, Canada), modified by the addition of restriction sites at the 5' and 3' ends to produce the cDNA having the sequence shown in SEQ ID NO:4, and cloned into the pcDEF3 expression vector (Goldman et al., "Modifications of Vectors pEF-BOS, pcDNA1 and pcDNA3 Result in Improved Convenience and Expression,"*Biotechniques* 21:1013-1015, 1996). A clonal cell line was selected using limited dilution cloning and testing for Ca++ release in HEK cells co-expressing simial GPR105 and Gqi5 by FLIPR, as previously described (K. Freeman et al., *Genomics* 78:124-128, 2001). This cell-based functional assay can be utilized to identify modulators (e.g., agonists or antagonists) of GPR105 activity.

[**0047**] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 5

<210> SEQ ID NO 1
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Met Ile Asn Ser Thr Ser Thr Gln Pro Pro Asp Glu Ser Cys Ser Gln
1               5                   10                  15

Asn Leu Leu Ile Thr Gln Gln Ile Ile Pro Val Leu Tyr Cys Met Val
            20                  25                  30

Phe Ile Ala Gly Ile Leu Leu Asn Gly Val Ser Gly Trp Ile Phe Phe
        35                  40                  45

Tyr Val Pro Ser Ser Glu Ser Phe Ile Ile Tyr Leu Lys Asn Ile Val
    50                  55                  60

Ile Ala Asp Phe Val Met Ser Leu Thr Phe Pro Phe Lys Ile Leu Gly
65                  70                  75                  80

Asp Ser Gly Leu Gly Pro Trp Gln Leu Asn Val Phe Val Cys Arg Val
                85                  90                  95

Ser Ala Val Leu Phe Tyr Val Asn Met Tyr Val Ser Ile Val Phe Phe
            100                 105                 110

Gly Leu Ile Ser Phe Asp Arg Tyr Tyr Lys Ile Val Lys Pro Leu Trp
        115                 120                 125

Thr Ser Phe Ile Gln Ser Val Ser Tyr Ser Lys Leu Leu Ser Val Ile
    130                 135                 140

Val Trp Met Leu Met Leu Leu Leu Ala Val Pro Asn Ile Ile Leu Thr
145                 150                 155                 160

Asn Gln Ser Val Arg Glu Val Thr Gln Ile Lys Cys Ile Glu Leu Lys
                165                 170                 175

Ser Glu Leu Gly Arg Lys Trp His Lys Ala Ser Asn Tyr Ile Phe Val
            180                 185                 190

Ala Ile Phe Trp Ile Val Phe Leu Leu Leu Ile Val Phe Tyr Thr Ala
        195                 200                 205

Ile Thr Lys Lys Ile Phe Lys Ser His Leu Lys Ser Ser Arg Asn Ser
    210                 215                 220

Thr Ser Val Lys Lys Lys Ser Ser Arg Asn Ile Phe Ser Ile Val Phe
225                 230                 235                 240

Val Phe Phe Val Cys Phe Val Pro Tyr His Ile Ala Arg Ile Pro Tyr
                245                 250                 255

Thr Lys Ser Gln Thr Glu Ala His Tyr Ser Cys Gln Ser Lys Glu Ile
            260                 265                 270

Leu Arg Tyr Met Lys Glu Phe Thr Leu Leu Leu Ser Ala Ala Asn Val
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-continued

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        275                 280                 285

Cys Leu Asp Pro Ile Ile Tyr Phe Phe Leu Cys Gln Pro Phe Arg Glu
    290                 295                 300

Ile Leu Cys Lys Lys Leu His Ile Pro Leu Lys Ala Gln Asn Asp Leu
305                 310                 315                 320

Asp Ile Ser Arg Ile Lys Arg Gly Asn Thr Thr Leu Glu Ser Thr Asp
                325                 330                 335

Thr Leu


<210> SEQ ID NO 2
<211> LENGTH: 1017
<212> TYPE: DNA
<213> ORGANISM: Chimpanzee
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1017)

<400> SEQUENCE: 2

atg atc aat tca acc tcc aca cag cct cca gat gaa tcc tgc tct cag       48
Met Ile Asn Ser Thr Ser Thr Gln Pro Pro Asp Glu Ser Cys Ser Gln
1               5                   10                  15

aac ctc ctg atc act cag cag atc att cct gtg ctg tac tgt gtg gtc       96
Asn Leu Leu Ile Thr Gln Gln Ile Ile Pro Val Leu Tyr Cys Val Val
            20                  25                  30

ttc att gcg gga atc ctc ctc aat gga gtg tca gga tgg ata ttc ttt      144
Phe Ile Ala Gly Ile Leu Leu Asn Gly Val Ser Gly Trp Ile Phe Phe
        35                  40                  45

tac gtg ccc agc tct aag agt ttc att gtc tat ctc aag aac att gtt      192
Tyr Val Pro Ser Ser Lys Ser Phe Ile Val Tyr Leu Lys Asn Ile Val
    50                  55                  60

att gct gac ttt gtg atg agc ctg act ttt cct ttc aag atc ctt ggt      240
Ile Ala Asp Phe Val Met Ser Leu Thr Phe Pro Phe Lys Ile Leu Gly
65                  70                  75                  80

gac tca ggc ctt ggt ccc tgg cag ctg aac gtg ttt gtg tgc agg gtc      288
Asp Ser Gly Leu Gly Pro Trp Gln Leu Asn Val Phe Val Cys Arg Val
                85                  90                  95

tct gcc gtg ctc ttc tac gtc aac atg tac gtc agc att gtg ttc ttt      336
Ser Ala Val Leu Phe Tyr Val Asn Met Tyr Val Ser Ile Val Phe Phe
            100                 105                 110

ggg ctc atc agc ttt gat aga tat tat aaa att gta aag cct ctt tgg      384
Gly Leu Ile Ser Phe Asp Arg Tyr Tyr Lys Ile Val Lys Pro Leu Trp
        115                 120                 125

act tct ttc atc cag tca gtg agt tac agc aaa ctc ctg tca gtg ata      432
Thr Ser Phe Ile Gln Ser Val Ser Tyr Ser Lys Leu Leu Ser Val Ile
    130                 135                 140

gta tgg atg ctc atg ctg ctc ctt gct gtt cca aat att att ctc acc      480
Val Trp Met Leu Met Leu Leu Leu Ala Val Pro Asn Ile Ile Leu Thr
145                 150                 155                 160

aac cag agt gtt agg gag gtt aca caa ata aaa tgt ata gaa ctg aaa      528
Asn Gln Ser Val Arg Glu Val Thr Gln Ile Lys Cys Ile Glu Leu Lys
                165                 170                 175

agt gaa ctg gga cgg aag tgg cac aaa gca tca aac tac atc ttc gtg      576
Ser Glu Leu Gly Arg Lys Trp His Lys Ala Ser Asn Tyr Ile Phe Val
            180                 185                 190

gcc atc ttc tgg att gtg ttt ctt ttg tta atc gtt ttc tat act gct      624
Ala Ile Phe Trp Ile Val Phe Leu Leu Leu Ile Val Phe Tyr Thr Ala
        195                 200                 205
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atc aca aag aaa atc ttt aag tcc cac ctt aag tca agt cgg aat tcc      672
Ile Thr Lys Lys Ile Phe Lys Ser His Leu Lys Ser Ser Arg Asn Ser
    210                 215                 220

act tcg gtc aaa aag aaa tct agc cgc aac ata ttc agc att gtg ttc      720
Thr Ser Val Lys Lys Lys Ser Ser Arg Asn Ile Phe Ser Ile Val Phe
225                 230                 235                 240

gtg ttt ttt atc tgt ttt gta cct tac cat att gcc aga atc ccc tac      768
Val Phe Phe Ile Cys Phe Val Pro Tyr His Ile Ala Arg Ile Pro Tyr
                245                 250                 255

aca aag agt cag acc gaa gct cat tac agc tgc cag tca aaa gaa atc      816
Thr Lys Ser Gln Thr Glu Ala His Tyr Ser Cys Gln Ser Lys Glu Ile
            260                 265                 270

ttg cgg tat atg aaa gaa ttc act ctg cta cta tct gct gca aat gta      864
Leu Arg Tyr Met Lys Glu Phe Thr Leu Leu Leu Ser Ala Ala Asn Val
        275                 280                 285

tgc ttg gac cct att att tat ttc ttt cta tgc cag cca ttt agg gaa      912
Cys Leu Asp Pro Ile Ile Tyr Phe Phe Leu Cys Gln Pro Phe Arg Glu
    290                 295                 300

atc tta tgt aag aaa ttg cac att cca tta aaa gct cag aat gac cta      960
Ile Leu Cys Lys Lys Leu His Ile Pro Leu Lys Ala Gln Asn Asp Leu
305                 310                 315                 320

gac att tcc aga atc aaa aga gga aat aca aca ctt gaa agc aca gat     1008
Asp Ile Ser Arg Ile Lys Arg Gly Asn Thr Thr Leu Glu Ser Thr Asp
                325                 330                 335

act ttg tga                                                        1017
Thr Leu
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<210> SEQ ID NO 3
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Chimpanzee

<400> SEQUENCE: 3

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Met Ile Asn Ser Thr Ser Thr Gln Pro Pro Asp Glu Ser Cys Ser Gln
1               5                   10                  15

Asn Leu Leu Ile Thr Gln Gln Ile Ile Pro Val Leu Tyr Cys Val Val
            20                  25                  30

Phe Ile Ala Gly Ile Leu Leu Asn Gly Val Ser Gly Trp Ile Phe Phe
        35                  40                  45

Tyr Val Pro Ser Ser Lys Ser Phe Ile Val Tyr Leu Lys Asn Ile Val
    50                  55                  60

Ile Ala Asp Phe Val Met Ser Leu Thr Phe Pro Phe Lys Ile Leu Gly
65                  70                  75                  80

Asp Ser Gly Leu Gly Pro Trp Gln Leu Asn Val Phe Val Cys Arg Val
                85                  90                  95

Ser Ala Val Leu Phe Tyr Val Asn Met Tyr Val Ser Ile Val Phe Phe
            100                 105                 110

Gly Leu Ile Ser Phe Asp Arg Tyr Tyr Lys Ile Val Lys Pro Leu Trp
        115                 120                 125

Thr Ser Phe Ile Gln Ser Val Ser Tyr Ser Lys Leu Leu Ser Val Ile
    130                 135                 140

Val Trp Met Leu Met Leu Leu Leu Ala Val Pro Asn Ile Ile Leu Thr
145                 150                 155                 160

Asn Gln Ser Val Arg Glu Val Thr Gln Ile Lys Cys Ile Glu Leu Lys
                165                 170                 175
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Ser Glu Leu Gly Arg Lys Trp His Lys Ala Ser Asn Tyr Ile Phe Val
            180                 185                 190

Ala Ile Phe Trp Ile Val Phe Leu Leu Leu Ile Val Phe Tyr Thr Ala
        195                 200                 205

Ile Thr Lys Lys Ile Phe Lys Ser His Leu Lys Ser Ser Arg Asn Ser
    210                 215                 220

Thr Ser Val Lys Lys Lys Ser Ser Arg Asn Ile Phe Ser Ile Val Phe
225                 230                 235                 240

Val Phe Phe Ile Cys Phe Val Pro Tyr His Ile Ala Arg Ile Pro Tyr
                245                 250                 255

Thr Lys Ser Gln Thr Glu Ala His Tyr Ser Cys Gln Ser Lys Glu Ile
            260                 265                 270

Leu Arg Tyr Met Lys Glu Phe Thr Leu Leu Leu Ser Ala Ala Asn Val
        275                 280                 285

Cys Leu Asp Pro Ile Ile Tyr Phe Phe Leu Cys Gln Pro Phe Arg Glu
    290                 295                 300

Ile Leu Cys Lys Lys Leu His Ile Pro Leu Lys Ala Gln Asn Asp Leu
305                 310                 315                 320

Asp Ile Ser Arg Ile Lys Arg Gly Asn Thr Thr Leu Glu Ser Thr Asp
                325                 330                 335

Thr Leu


<210> SEQ ID NO 4
<211> LENGTH: 1037
<212> TYPE: DNA
<213> ORGANISM: Chimpanzee
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (10)..(1029)

<400> SEQUENCE: 4

ggtaccacc acc atg atc aat tca acc tcc aca cag cct cca gat gaa tcc     51
          Thr Met Ile Asn Ser Thr Ser Thr Gln Pro Pro Asp Glu Ser
          1               5                  10

tgc tct cag aac ctc ctg atc act cag cag atc att cct gtg ctg tac       99
Cys Ser Gln Asn Leu Leu Ile Thr Gln Gln Ile Ile Pro Val Leu Tyr
15                  20                  25                  30

tgt gtg gtc ttc att gcg gga atc ctc ctc aat gga gtg tca gga tgg      147
Cys Val Val Phe Ile Ala Gly Ile Leu Leu Asn Gly Val Ser Gly Trp
                35                  40                  45

ata ttc ttt tac gtg ccc agc tct aag agt ttc att gtc tat ctc aag      195
Ile Phe Phe Tyr Val Pro Ser Ser Lys Ser Phe Ile Val Tyr Leu Lys
            50                  55                  60

aac att gtt att gct gac ttt gtg atg agc ctg act ttt cct ttc aag      243
Asn Ile Val Ile Ala Asp Phe Val Met Ser Leu Thr Phe Pro Phe Lys
        65                  70                  75

atc ctt ggt gac tca ggc ctt ggt ccc tgg cag ctg aac gtg ttt gtg      291
Ile Leu Gly Asp Ser Gly Leu Gly Pro Trp Gln Leu Asn Val Phe Val
    80                  85                  90

tgc agg gtc tct gcc gtg ctc ttc tac gtc aac atg tac gtc agc att      339
Cys Arg Val Ser Ala Val Leu Phe Tyr Val Asn Met Tyr Val Ser Ile
95                  100                 105                 110

gtg ttc ttt ggg ctc atc agc ttt gat aga tat tat aaa att gta aag      387
Val Phe Phe Gly Leu Ile Ser Phe Asp Arg Tyr Tyr Lys Ile Val Lys
                115                 120                 125

cct ctt tgg act tct ttc atc cag tca gtg agt tac agc aaa ctc ctg      435
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Pro Leu Trp Thr Ser Phe Ile Gln Ser Val Ser Tyr Ser Lys Leu Leu
            130                 135                 140

tca gtg ata gta tgg atg ctc atg ctg ctc ctt gct gtt cca aat att      483
Ser Val Ile Val Trp Met Leu Met Leu Leu Leu Ala Val Pro Asn Ile
        145                 150                 155

att ctc acc aac cag agt gtt agg gag gtt aca caa ata aaa tgt ata      531
Ile Leu Thr Asn Gln Ser Val Arg Glu Val Thr Gln Ile Lys Cys Ile
    160                 165                 170

gaa ctg aaa agt gaa ctg gga cgg aag tgg cac aaa gca tca aac tac      579
Glu Leu Lys Ser Glu Leu Gly Arg Lys Trp His Lys Ala Ser Asn Tyr
175                 180                 185                 190

atc ttc gtg gcc atc ttc tgg att gtg ttt ctt ttg tta atc gtt ttc      627
Ile Phe Val Ala Ile Phe Trp Ile Val Phe Leu Leu Leu Ile Val Phe
                195                 200                 205

tat act gct atc aca aag aaa atc ttt aag tcc cac ctt aag tca agt      675
Tyr Thr Ala Ile Thr Lys Lys Ile Phe Lys Ser His Leu Lys Ser Ser
            210                 215                 220

cgg aat tcc act tcg gtc aaa aag aaa tct agc cgc aac ata ttc agc      723
Arg Asn Ser Thr Ser Val Lys Lys Lys Ser Ser Arg Asn Ile Phe Ser
        225                 230                 235

att gtg ttc gtg ttt ttt atc tgt ttt gta cct tac cat att gcc aga      771
Ile Val Phe Val Phe Phe Ile Cys Phe Val Pro Tyr His Ile Ala Arg
    240                 245                 250

atc ccc tac aca aag agt cag acc gaa gct cat tac agc tgc cag tca      819
Ile Pro Tyr Thr Lys Ser Gln Thr Glu Ala His Tyr Ser Cys Gln Ser
255                 260                 265                 270

aaa gaa atc ttg cgg tat atg aaa gaa ttc act ctg cta cta tct gct      867
Lys Glu Ile Leu Arg Tyr Met Lys Glu Phe Thr Leu Leu Leu Ser Ala
                275                 280                 285

gca aat gta tgc ttg gac cct att att tat ttc ttt cta tgc cag cca      915
Ala Asn Val Cys Leu Asp Pro Ile Ile Tyr Phe Phe Leu Cys Gln Pro
            290                 295                 300

ttt agg gaa atc tta tgt aag aaa ttg cac att cca tta aaa gct cag      963
Phe Arg Glu Ile Leu Cys Lys Lys Leu His Ile Pro Leu Lys Ala Gln
        305                 310                 315

aat gac cta gac att tcc aga atc aaa aga gga aat aca aca ctt gaa     1011
Asn Asp Leu Asp Ile Ser Arg Ile Lys Arg Gly Asn Thr Thr Leu Glu
    320                 325                 330

agc aca gat act ttg tga gcggccgc                                   1037
Ser Thr Asp Thr Leu
335


<210> SEQ ID NO 5
<211> LENGTH: 339
<212> TYPE: PRT
<213> ORGANISM: Chimpanzee

<400> SEQUENCE: 5

Thr Met Ile Asn Ser Thr Ser Thr Gln Pro Pro Asp Glu Ser Cys Ser
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Gln Asn Leu Leu Ile Thr Gln Gln Ile Ile Pro Val Leu Tyr Cys Val
            20                  25                  30

Val Phe Ile Ala Gly Ile Leu Leu Asn Gly Val Ser Gly Trp Ile Phe
        35                  40                  45

Phe Tyr Val Pro Ser Ser Lys Ser Phe Ile Val Tyr Leu Lys Asn Ile
    50                  55                  60

Val Ile Ala Asp Phe Val Met Ser Leu Thr Phe Pro Phe Lys Ile Leu
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65                  70                  75                  80

Gly Asp Ser Gly Leu Gly Pro Trp Gln Leu Asn Val Phe Val Cys Arg
                85                  90                  95

Val Ser Ala Val Leu Phe Tyr Val Asn Met Tyr Val Ser Ile Val Phe
            100                 105                 110

Phe Gly Leu Ile Ser Phe Asp Arg Tyr Tyr Lys Ile Val Lys Pro Leu
        115                 120                 125

Trp Thr Ser Phe Ile Gln Ser Val Ser Tyr Ser Lys Leu Leu Ser Val
    130                 135                 140

Ile Val Trp Met Leu Met Leu Leu Leu Ala Val Pro Asn Ile Ile Leu
145                 150                 155                 160

Thr Asn Gln Ser Val Arg Glu Val Thr Gln Ile Lys Cys Ile Glu Leu
                165                 170                 175

Lys Ser Glu Leu Gly Arg Lys Trp His Lys Ala Ser Asn Tyr Ile Phe
            180                 185                 190

Val Ala Ile Phe Trp Ile Val Phe Leu Leu Leu Ile Val Phe Tyr Thr
        195                 200                 205

Ala Ile Thr Lys Lys Ile Phe Lys Ser His Leu Lys Ser Ser Arg Asn
    210                 215                 220

Ser Thr Ser Val Lys Lys Lys Ser Ser Arg Asn Ile Phe Ser Ile Val
225                 230                 235                 240

Phe Val Phe Phe Ile Cys Phe Val Pro Tyr His Ile Ala Arg Ile Pro
                245                 250                 255

Tyr Thr Lys Ser Gln Thr Glu Ala His Tyr Ser Cys Gln Ser Lys Glu
            260                 265                 270

Ile Leu Arg Tyr Met Lys Glu Phe Thr Leu Leu Leu Ser Ala Ala Asn
        275                 280                 285

Val Cys Leu Asp Pro Ile Ile Tyr Phe Phe Leu Cys Gln Pro Phe Arg
    290                 295                 300

Glu Ile Leu Cys Lys Lys Leu His Ile Pro Leu Lys Ala Gln Asn Asp
305                 310                 315                 320

Leu Asp Ile Ser Arg Ile Lys Arg Gly Asn Thr Thr Leu Glu Ser Thr
                325                 330                 335

Asp Thr Leu
```

1. A method for determining whether a chemical agent modulates the biological activity of a GPR105 protein, the method comprising the steps of:

(a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity;

(b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and

(c) validating, in vivo, the modulating effect of the chemical agent on the biological activity of the GPR105 protein.

2. A method of claim 1, wherein the chemical agent consists essentially of a chemical compound.

3. A method of claim 1, wherein the chemical agent consists essentially of a protein.

4. A method of claim 1, comprising the step of determining whether the agent decreases the biological activity of the GPR105 protein.

5. A method of claim 1, comprising the step of determining whether the agent increases the biological activity of the GPR105 protein.

6. A method of claim 1, wherein the cell is selected from the group consisting of a CHO cell and an HTK cell.

7. A method of claim 1, wherein the GPR105 protein is at least 70% identical to a GPR105 protein consisting of the amino acid sequence set forth in SEQ ID NO:1.

8. A method of claim 1, wherein the GPR105 protein is at least 80% identical to a GPR105 protein consisting of the amino acid sequence set forth in SEQ ID NO:1.

9. A method of claim 1, wherein the GPR105 protein is at least 90% identical to a GPR105 protein consisting of the amino acid sequence set forth in SEQ ID NO:1.

10. A method of claim 1, wherein the GPR105 protein is at least 95% identical to a GPR105 protein consisting of the amino acid sequence set forth in SEQ ID NO:1.

11. A method of claim 1, wherein the GPR105 protein is at least 99% identical to a GPR105 protein consisting of the amino acid sequence set forth in SEQ ID NO:1.

12. A method of claim 1, wherein the modulating effect of the chemical agent on the GPR105 is validated by measuring the effect of the chemical agent on a physiological response selected from the group consisting of the concentration of LDL cholesterol in blood plasma, the concentration of HDL cholesterol in blood plasma, body weight, the rate of body weight gain in response to a high fat diet, insulin resistance, and glucose clearance from the blood stream.

13. A method of claim 1, wherein a population of living cells are contacted, in vitro, with a chemical agent, wherein all, or substantially all, of the living cells express a GPR105 protein having a biological activity.

14. An automated method of claim 1.

15. A method for determining the effect of a chemical agent on GPR105 activity in a living cell, the method comprising the steps of:

(a) observing a change in a GPR105-mediated response in a living cell, in vitro, in response to a chemical agent; and

(b) confirming that the observed change in the GPR105-mediated response occurs in vivo.

16. A method of claim 15, wherein the GPR105-mediated response is selected from the group consisting of the concentration of LDL cholesterol in blood plasma, the concentration of HDL cholesterol in blood plasma, body weight, the rate of body weight gain in response to a high fat diet, insulin resistance, and glucose clearance from the blood stream.

17. A method of claim 15, wherein the chemical agent consists essentially of a chemical compound.

18. A method of claim 1, wherein the chemical agent consists essentially of a protein.

19. A method of claim 15, wherein the change in the GPR105-mediated response is an increase in the GPR105-mediated response.

20. A method of claim 15, wherein the change in the GPR105-mediated response is a decrease in the GPR105-mediated response.

21. A method for identifying a chemical agent that ameliorates a symptom of type II diabetes or obesity, the method comprising the steps of:

(a) contacting a living cell, in vitro, with a chemical agent, wherein the living cell expresses a GPR105 protein having a biological activity;

(b) determining whether the chemical agent modulates the biological activity of the GPR105 protein in the living cell; and

(c) determining, in vivo, whether the chemical agent ameliorates a symptom of type II diabetes or obesity.

22. A method of claim 21, wherein the symptom of type II diabetes or obesity is selected from the group consisting of insulin resistance, body mass, and glucose clearance from the blood stream.

23. An isolated nucleic acid molecule that encodes a GPR105 protein, wherein the isolated nucleic acid molecule comprises the nucleic acid sequence set forth in SEQ ID NO:2.

24. An isolated nucleic acid molecule of claim 23, consisting of the nucleic acid sequence set forth in SEQ ID NO:2.

25. An isolated GPR105 protein comprising the amino acid sequence set forth in SEQ ID NO:3.

26. An isolated GPR105 protein of claim 25, consisting of the amino acid sequence set forth in SEQ ID NO:3.

* * * * *

专利名称(译)	鉴定调节GPR105活性的药剂的方法		
公开(公告)号	US20070092913A1	公开(公告)日	2007-04-26
申请号	US11/584424	申请日	2006-10-19
[标]申请(专利权)人(译)	罗斯塔英法美蒂克斯有限责任公司		
申请(专利权)人(译)	ROSETTA Inpharmatics LLC公司在美克有限公司		
当前申请(专利权)人(译)	ROSETTA Inpharmatics LLC公司在美克有限公司		
[标]发明人	LAMB JOHN MANCINI JOSEPH KENNEDY BRIAN		
发明人	LAMB, JOHN MANCINI, JOSEPH KENNEDY, BRIAN		
IPC分类号	G01N33/53 C07H21/04 C12P21/06 C07K14/705		
CPC分类号	C07K14/705 C12N15/1138 C12N2310/11 G01N33/566 G01N2333/726 G01N2800/042 G01N2800/044		
优先权	60/730595 2005-10-26 US		
外部链接	Espacenet USPTO		

摘要(译)

在一个方面，本发明提供了用于确定化学试剂是否调节GPR105蛋白的生物活性的方法。本发明该方面的方法包括以下步骤：（a）使活细胞在体外与化学试剂接触，其中活细胞表达具有生物活性的GPR105蛋白质；（b）确定化学试剂是否调节活细胞中GPR105蛋白的生物活性；（c）体内验证化学试剂对GPR105蛋白生物活性的调节作用。

TABLE 2

	Liver		Adipose Tissue		Kidney	
Group	GPR105 relative exp	SCD-1	GPR105 relative exp	SCD-1	GPR105 relative exp	SCD-1
Vehicle	1.00	1.00	1.00	1.00	1.00	1.00
Vehicle	1.83	0.81	0.66	1.60	0.81	0.49
Vehicle	0.53	0.33	0.96	1.39	0.55	0.72
Vehicle	0.68	1.21	0.67	0.70	0.48	1.05
Vehicle	0.43	0.60	0.89	0.52	0.46	
Vehicle (Mean ± SEM)	0.89 ± 0.25	0.79 ± 0.15	0.84 ± 0.07	1.04 ± 0.2	0.66 ± 0.11	0.82 ± 0.13
Antisense	0.02	0.12	0.65	0.86	0.10	0.35
Antisense	0.01	0.15	0.40	0.82	0.09	0.89
Antisense	0.01	0.19	0.44	0.83	0.11	0.48
Antisense	0.27	0.10	0.69	0.30	0.09	0.23
Antisense	0.07	0.09	0.49	0.27	0.07	0.30
Antisense (Mean ± SEM)	0.08 ± 0.05	0.13 ± 0.02	0.53 ± 0.06	0.62 ± 0.14	0.09 ± 0.01	0.45 ± 0.12
Scrambled Oligo	0.96	0.37	1.22	1.08	0.45	0.47
Scrambled Oligo	1.09	1.43	1.19	1.66	1.01	0.96
Scrambled Oligo	0.63	0.44	1.38	0.98	0.57	0.62
Scrambled Oligo	0.89	0.50	1.49	0.98	0.46	0.49
Scrambled Oligo	0.57	0.54	1.60	0.88	0.35	
Scrambled Oligo (Mean ± SEM)	0.83 ± 0.1	0.66 ± 0.2	1.38 ± 0.08	1.12 ± 0.14	0.57 ± 0.12	0.63 ± 0.11