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(57) Abstract

The present invention refers to a method which allows the selection of molecules capable of mimicking, inhibiting or potentiating the effects of interaction between leptin and cells which display on the membrane the receptor thereof. The selection is consequent to the detection of the variation of neuropeptide Y production, or choline acetyltransferase enzymatic activity, following the bond of a molecule of interest with the leptin receptor displayed on target cells, optionally engineered, which can be neuronal but also cells of any type. This method allows the pre–selection of molecules potentially suitable in medical treatment of diseases such as obesity, anorexia, diabetes, cachexia and certain cases of sterility.

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METHOD FOR IDENTIFYING MODIFIERS OF THE LEPTIN: LEPTIN RECEPTOR INTERACTION

DESCRIPTION

Subject of the present invention is a method to determine whether a certain molecule is capable of binding the leptin receptor and, in relation to the biological effects thereof, of mimicking, inhibiting or potentiating the biological action of leptin itself on the cells used as targets.

This makes it possible to determine firstly whether such molecules are involved in pathologies in which a direct or indirect implication of leptin has been demonstrated, with particular reference to obesity, diabetes, certain cases of sterility, as well as cases of anorexia and cachexia.

Moreover, based on the analysis of effects on the target cell biology in accordance with the method subject of the present invention, it is possible to select naturally produced or chemically modified molecules which may be used to produce drugs capable of treating the aforesaid pathologies, on account of the molecules' proven capacity of inhibiting, potentiating or mimicking leptin's biological action.

Leptin is a protein belonging to the cytokine family, produced by adipocytes, and its involvement in body weight homeostasis has been clearly demonstrated.

Obesity is the result of a positive energy balance determined in the organism by an increase in the caloric intake/energy consumption ratio. It is known that over 30% of the adult population in the industrial world suffers from obesity. This disease represents one of the most important public health problems, given that it is associated with type II diabetes, hypertension, hyperlipidemia and an increase in mortality rate. Leptin is a protein of 16 Kilodalton whose levels in blood are

correlated with body weight index in rodents and in humans (Maffei et al., 1995). In particular, the existence of a direct correlation between the quantity of leptin produced and adiposity seems quite certain and was determined as a consequence of studies on healthy and obese subjects of human and other animal species (Frederich et al., 1995; Maffei et al., 1995).

Leptin therefore carries out the function of an adipose tissue sensor by acting directly upon the brain, and in particular as mentioned above, upon the centres governing the feeling of satiety and the energy consumption (Spiegelman et al, 1996; Caro et al., 1996).

These conclusions indicate that leptin regulates body weight (Harris, 1990), and are supported by experiments carried out on mutant ob/ob mice which lack functional leptin (Zhang et al, 1994), and db/db mice,which lack a functional leptin receptor. Both the ob and db mutations determine an obese phenotype(Zhang et al, 1994; Tartaglia et al., 1995; Lee et al., 1996).

Further proof for the direct involvement of leptin in obesity is provided by the observation that hyperphagia and subsequent obesity in ob/ob mice which do not produce leptin but express the wild type leptin receptor can be treated by systemic administration of leptin (Halaas et al., 1995; Pelleymounter et al., 1995; Campfield et al., 1995).

However, it seems that human obesity is not due to insufficient expression of leptin, as it has been proved that the mRNA of leptin and the levels of the protein in the blood are higher in obese subjects than in normal ones (Maffei et al., 1995). Thus, obese humans seem insensitive to the lipostatic effect of leptin, probably due to a defect in the leptin transport mechanism, in leptin receptor activity or in post-receptor signalling (Bray, 1996).

Hence the necessity in this specific sector for new pharmacological agents capable or correcting obesity in

people who are leptin-resistant.

The weight- and appetite-reducing actions of leptin are mediated by the long-form splice variant of leptin receptor (OB-Rb) (Lee et al., 1996; Pelleymounter et al., 1995), which is predominantly expressed in hypothalamic nuclei involved in the regulation of energy balance (Mercer et al., 1996_; Schwartz et al., 1996). OB-Rb is a protein related to the gp130-family of cytokine receptors (Tartaglia et al., 1995), which stimulate gene expression by activating cytosolic STAT transcription activators (Kishimoto et al., 1995). The recent finding that leptin activates Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice (which lack functional OB-Rb) (Vaisse et al., 1996) strongly argues for a direct action of leptin on the hypothalamus.

Therefore, there is a close correlation between leptin's mechanism of action and the activation of STAT factors.

Regarding this, it must underlined that WO96EP2291 supplies some information concerning the possibility of selecting compounds which modulate leptin's effects using a transduction signal and a STAT-responsive DNA element for the transcription of a reporter gene.

Several hypothalamic neuropeptides have been implicated as leptin-sensitive regulators of energy balance (Schwartz et al., 1996; Stephens et al., 1995; Qu et al., 1996). Strong evidence exists for the involvement of neuropeptide Y (NPY) as a mediator of the hyperphagia, hypometabolism and endocrine alterations resulting from chronic leptin deficiency. Injection of NPY into the brain increases food intake and body weight, and the expression of NPY by neurons of the hypothalamic arcuate nucleus is elevated in obese rodents (reviewed in refs. Qu et al., 1996; Sahu et al., 1993). Leptin inhibits the expression of NPY mRNA in the arcuate nucleus (Schwartz et al., 1996; Schindler et al., 1995) and the release of NPY immunoreactive material from isolated hypothalamus

(Stephens et al., 1995). Finally, the obesity syndrome of ob/ob mice is attenuated by NPY deficiency (Erickson et al., 1996).

Therefore, it is possible to say that leptin's anti-obesity activity is mediated at least in part by the inhibition of NPY expression and release.

The object of the present invention is the reconstitution of the said effect characterizing the action of leptin in vivo in a cellular system in vitro. Such reconstitution is carried out firstly by using cells which express the leptin receptor naturally, or through genetical engineering. Subsequently, by means of a series of operations which are known per se but whose combination had never before been considered, it is possible to detect variations in NPY production or in choline acetyltransferase activity, two of the nodal points in the signal cascade through which leptin exercises its biological action within the cell.

In this element lies a further aspect of the invention's novelty: the parameters considered in fact refer to neuropeptide Y and to choline acetyltransferase, namely, two molecules of proteinic nature. Although the induction of reporter genes whose expression is controlled by means of leptin-responsive regulative elements has already been described (Baumann et al., 1996; Rosenblum et al., 1996), direct effects of leptin on the production of endogenous neuronal peptides or proteins, in particular NPY or choline acetyl transferase has not been described before.

The method subject of the invention thus proves capable of assaying biological activity of all compounds which show they are capable of binding the leptin receptor.

Indeed, by means of said method it is possible to consider in particular the anti-obesity potential activity of compounds capable of activating the leptin receptor, or of mimicking or potentiating its action; and

viceversa, the potential capacity of stimulating food intake of compounds capable of inhibiting or antagonizing leptin's action.

Moreover, as this assay is based on the detection of leptin's capacity of inducing the production in neuronal cell lines of a neuronal protein (NPY) whose involvement in the brain's neuro-transmissive mechanism has been demonstrated, it can also be used to determine in a detailed the neuronal signalling mechanism involved in the action of leptin.

The invention will be further clarified with the aid of the annexed figures.

Figure 1 is a graph showing that in SN-56 cells stably transfected with an expression vector for human leptin receptor leptin induces the activation of cellular STAT transcription factors. Cells were not treated (-) or treated for 15 min. with leptin at the indicated concentrations. The arrows denote DNA binding of STAT3 homodimers, STAT1:STAT3 heterodimers, and STAT1 homodimers (Zhong et al., 1994).

Figure 2, is a graph showing that SN-56 cells stably transfected with an expression vector for human leptin receptor SN-56.L64 (IRBM-1) deposited on June 5th 1997 at the Advanced Biotechnology Center (ABC), with access number PD97001, respond to the addition of leptin by decreasing the production of neuropeptide Y. In addition, the graph shows that native SN-56 cells do not respond to leptin, and that ciliary neurotrophic factor (CNTF), which shares anti-obesity effects with leptin, also down-regulates the production of neuropeptide Y. Abbreviations: D, experiments performed in the presence of 10 μM dexamethasone. C, 10 ng/ml CNTF; L, 100 ng/ml leptin.

Figure 3 is a graph showing that SN-56 cells stably transfected with an expression vector for human leptin receptor (SN-56/OB-Rb) respond to the addition of leptin by increasing the cellular enzymatic activity of choline

acetyltransferase. In addition, the graph shows that native SN-56 do not respond to leptin, and that CNTF, which shares anti-obesity effects with leptin, also increases the activitity of choline acetyltransferase.

Subject of the present invention is a method to determine whether a certain molecule is capable of binding leptin's receptor and, in relation to the subsequent biological effects thereby, of mimicking, inhibiting or potentiating the biological action of leptin itself on cells used as targets.

By means of a series of combined operations the method in question makes it possible to detect NPY production and enzymatic activity of choline acetyltransferase (parameters associated with leptin's biological action), once the molecule of interest binds with the receptor of leptin itself.

This implies that given a certain molecule of interest, this cell assay can supply a wide range of data referred to leptin.

First of all it is possible to determine whether such molecule of interest is capable of binding with leptin's receptor and of transducting consequently a biological signal within the cell. Secondly, by means of the variation of one of the aforementioned parameters (i.e., NPY production, choline acetyltransferase activity) it is possible to determine also the type of biological effect resulting from the link of said molecule with the receptor of leptin itself.

In particular, all those potential ligands which stimulate the choline acetyltransferase activity or determine a decrease in NPY production in target cells, only in presence of the leptin receptor are individuated as specific agonists thereof. This means they bind the leptin receptor, and are consequently capable of mimicking its action or of producing a cellular response which is at least equivalent to, or greater than, the response to leptin itself. Thus, should this data be

supported by adequate proof in vivo, such compounds could be used in those cases in which leptin insufficiency (for instance in ob/ob mice) or resistance to leptin causes obesity, diabetes or sterility.

Since the anti-obesity effects of leptin are mediated, at least in part, by a reduction of neuropeptide Y levels in the hypothalamus, compounds that can reduce neuropeptide Y in the assay of this invention can be expected to have anti-obesity potential. Thus, it has been found, in accordance with the invention, that ciliary neurotrophic factor (CNTF), a cytokine that shares anti-obesity effects with leptin (Laufer et al., Italian Patent application RM96A000790), reduces NPY levels in this assay. (see Example 2).

Likewise it is possible to identify leptin antagonists in all compounds considered which can bind the leptin receptor but cannot produce any biological response in target cells. A compound of this kind may prove useful in preparing drugs suitable in the treatment of anorexia and cachexia.

Focusing on the method separate steps it must be stated beforehand that the execution of single operations, whose combination constitutes the method, can by carried out through a series of conventional techniques. It is however possible to highlight some of them.

Concerning the detection of alterations in NPY production, which results from the interaction of any molecule or of leptin with its cellular receptor, measurement of NPY cellular immunoreactivity is preferably performed by radioimmunassay (RIA). Other methods described in the art and likewise employed to assess NPY cellular levels may be for instance immunologic and immunoenzymatic methods, or else, if the quantity of messenger RNA must be detected, Northern Blot, RNA-ase protection, or reverse transcription by polymerase chain reaction (RT-PCR).

The release of neuropeptide Y in a cell culture can be measured however by other methods known in the art.

In fact, the regions of leptin-sensitive DNA controlling the gene coding for neuropeptide Y can be associated to a reporter gene, such as the one coding for chloroamphenicol acetyl trasferase or luciferase, and the effect of the compounds which activate leptin receptor can be measured by meanso of the alterations in levels of the reporter gene product, using methods known in the art for these techniques.

Regarding host cells, there are no particular limitations as they can be of any type or line cultivated in the most convenient way. In general eucharіotic mammal cells, and particularly murine neuronal cell lines (SN-56, GT-1-7) and human neuronal cells or a neuroblast cell line, as a non-limiting example of the invention's potential scope. The host cell however must show abundant leptin receptor expression on its surface membrane so that if necessary cells can be engineered to produce a sufficient quantity of receptors.

In particular, it must be underlined that in the particular case of cell line SN-56.L64 (IRBM-1), obtained by engineering of a cell line (SN-56) derived from septal neurons, although it contains no neurons from the hypothalamus (i.e. a region in which leptin acts physiologically and in which the relevant receptor OB-Rb is physiologically expressed) the cell line also constitutes surprisingly enough a reference system for the method's actuation. This confirms the remarkable relative importance of the possibility of causing the receptor's stable expression in target cells for the analysis of the biological response to leptin and the comprehension of its mechanism of action.

Any natural or modified leptin receptor can be used for the invention's purpose, although the human rather than the rodent type is preferable. The structural sequences coding for human leptin receptors have been

described in the art (Tartaglia et al., 1995 Cell 83:1263-1271). Genes for mouse receptors can also be used, particularly the one in GenBank data bank, containing the sequence from nucleotide 27 to nucleotide 2775. The sequence of the rat receptor also described in the art (Phillips et al., 1996, Nature Gen. 13:18-19) may also be used.

If cell engineering is necessary, it is preferable to use an expression vector containing a cDNA for human leptin receptor, nucleotides 141-3770, containing the sequence coding for the whole receptor form OB-Rb. In the present invention pCMV4 vector (Andersson, 1989) is preferably used but any other expression vector compatible with the cells on which the assay must be carried out can be used and with the necessity of obtaining a stable expression of leptin receptor.

In an assay preferably used in the invention it is possible to evaluate the compounds considered ligands capable of binding the leptin receptor. The cells expressing the (either natural or recombinant) leptin receptor are placed in contact with the presumed ligand, then levels of neuropeptide Y and choline acetyltransferase are measured.

These results can be compared with those obtained by placing the same cells in contact with leptin. Moreover, one advantage of this type of assay lies in the fact that the entity of response depends on dosage, the biologic effect measured increases with the quantity of ligand administered, so that it allows the quantitative detection of the leptin receptor's binding activity.

A counterscreen may be constituted by a second cell of the same type which has not been transfected by the leptin receptor.

In another preferred embodiment, cells capable of expressing the (either natural or recombinant) leptin receptor are placed simultaneously in contact with the compounds of interest and leptin, before related NPY

production or choline acetyltransferase variation are measured. The result is compared with that obtained placing an identical cell in contact with leptin alone. It is thus possible to determine whether a certain compound is capable of inhibiting or potentiating leptin's natural action, which may be useful to produce drugs for pathologies in which there is not a total lack of leptin, but rather a deficit of, or a resistance to, leptin at cellular level.

This is a further datum which may be obtained with the method subject of the present invention, shows a certain compound's capacity of interfering with leptin's action, independently from the effects it can produce if used alone.

With reference to the above, the subject of the present invention is a method for the selection of molecules capable of mimicking, potentiating or inhibiting the effects of interaction between leptin and cells displaying on the membrane the receptor thereof, comprising the following operations

a) contacting said molecules with said cells;and

b) detecting the variation in said cells of the neuropeptide Y production, or of the enzymatic activity of choline acetyltransferase, caused by the binding of said molecule to said receptor.

The cells can be also engineered. Engineering can be carried out by a series of operations in combination on any cell line, extensively described in the example 1 below, including the following essential operations:

- transferaction of the cell line with an expression vector;
- selection of transfected clones; and
- selection of the clones capable of stably expressing leptin receptor by the assay of STAT factor activity.

If the cell line concerned is SN-56 the result is the line SN-56.L64 (IRBM-1), also subject of the present

invention.

With particular reference to the method's two possible variants, it is however possible to find in both cases preferred embodiments which in turn imply the performing of a combination of operations known per se. In a preferred embodiment the detection of NPY production is based on the following operations:

- treatment of culture cells with an NPY production inducer in the cells used, leptin, or no factor;
- preparation of cell extracts;
- assay for the qualitative and/or quantitative NPY assessment by conventional methods.

In this embodiment the qualitative and/or quantitative NPY assay can be carried out with immunoenzymatic methods.

In the other preferred embodiment the analysis of choline acetyltransferase enzymatic activity is based on the following operations:

- incubation of the cells in presence of leptin or other effectors;
- lysis of the cells;
- assay for choline acetyltransferase activity by modified Fonnum method.

With regard to this last series of operations, a remarkably interesting case is that in which the assay for cell response to the biological activity of molecules under analysis is carried out by measuring cellular enzymatic activity.

Other cases of particular interest are those in which the leptin receptor used is recombinant, those in which receptor is of either human or rodent type, and finally the case in which a cell of neuronal origin is used. Moreover a case of specific interest is the application according to which the leptin receptor is OB-Rb and the cell line is SN-56.

So far a general description has been given of the present invention. With the aid of the following

examples, a more detailed description of specific embodiments will now be given, in order to give a better understanding of the objects, characteristics, advantages and operating methods of the invention. Such examples serve merely to illustrate and do not limit the scope of the present invention, which is defined in the annexed claims.

DEPOSITS

Cell line SN-56.L64(IRBM-1), obtained from cell line SN-56 by a cell engineering process extensively described in example 1, in which the stable expression of leptin receptor has been guaranteed, was deposited on June 5th 1997 at the Advanced Biotechnology Center, ABC in Genoa, with access number PD97001.

EXAMPLE 1

SN-56.L64(IRBM-1); engineering of cells SN-56 for the stable expression of leptin receptor

A leptin receptor expression vector was constructed by subcloning a fragment (nucleotides 141-3770 of the sequence deposited in Gen Bank; accession number U43168) of the human leptin receptor (OB-Rb) cDNA into the vector pCMV4 (Andersson, 1989). The mouse septal neuron-neuroblastoma hybrid cell line SN-56 (Lee et al., 1990) (a kind gift of Dr. J. Blusztajn, Boston University) was maintained in complete culture medium (Dulbecco's modified Eagle medium, 10% fetal calf serum, 50 µg/ml streptomycin, 50 U/ml penicillin, 1 mM sodium pyruvate) at 37°C in an atmosphere of 95% air, 5% CO_2. $4x10^6$ cells were grown on a 150 mm cell culture dish.

The expression vector for leptin receptor (20mg) and plasmid pcDNA3 (2mg) which carries the neomycin-resistance gene (Invitrogen), was introduced in cells by using Lipofectamine™ (Gibco/BRL) according to manufacturer's instructions.

24 hours after the transfection the medium was replaced by a fresh one, and 48 hours after the transfection cells SN-56.L64(IRBM-1) were selected adding

1 mg/ml of G418 to the culture medium. Resistant clones were isolated and subcloned and one of these was identified as capable of stably expressing the leptin receptor OB-Rb. This was identified evaluating leptin activity of inducing the binding of STAT factors to DNA (as described below). The clone thus obtained was maintained in culture in the medium and in the conditions described above, except for the addition of 0.2 mg/ml of G418.

Assaying of STAT factor activity was carried out on 10^6 cells grown on a 100 mm cell culture dish. Within 24 hours the culture reached semiconfluence. Cells were left to incubate for 15 mins in presence or absence of leptin, and were washed with cold PBS containing 50mM NaF. Cells were then COLLECTED for centrifugation and frozen in liquid nitrogen. Total cell extracts were obtained BY resuspending the cell pellet in 5 volumes of 10 mM Hepes pH 7,9; 0.4 M NaCl; 0.1 mM EGTA, 5% glycerol, 50 mM NaF, 10mM $Na_4P_2O_7$, 0.5 MM dithiotreitol, 0.5 mM phenilmethilsulphonylfluoride, 10 µg/ml trasylol, 2 µg/ml leupeptin. Cell lysate was centrifugated at 100,000 x revolutions for 10 min, and an aliquot of the supernatant (10 µg of protein) was used for the determination of the DNA binding activity of STAT factors by assaying electrophoretic mobility shift, in accordance with the procedure described by Sadowsky and Gilman (Sadowsky et al., 1993), using the high affinity SIE m67 oligonucleotide (Wagner et al., 1990). The probe was labelled by filling in 5' protruding end with Klenow enzyme in presence of (a^{32}P)dATP and (a^{32}PdCTP) (3000 Ci/mmol). Complexes were resolved on 5% polycrylamide gel - 2.5% glycerol - (45mM Tris-borate; 0.5mM EDTA, pH 7.8) gel 0.5 times, which was then dried and subjected to autoradiography.

EXAMPLE 2

Assay on cells SN-56.L64(IRBM-1) to determine the biological activity of leptin based on neuropeptide Y

(NPY).

The cells (in the example, which are provided only for illustrative purposes and do not limit the scope of the present invention we refer as described to wild type SN-56 cells and to the same cells transfected with an expression vector for the leptin receptor) have been plated on 100 mm dishes ($1.5x10^6$ cells per dish) containing the culture medium as described in the general information section. The cells were treated for three days with dexametasone (10μM), human leptin (100 ng/ml) or CNTF (10 ng/ml) or with no factor. Cells were washed with PDS and centrifugated. Cells were extracted with 200 μl of 0.1 N HCl and cell extracts were centrifugated for 10 min at 14000 x rpm. The pellet was resuspended in 1 N NaOH and 0.1 volumes of a 0.5 M sodium phosphate buffer, pH 8.2. Aliquots in duplicate of cell extracts were subjected to radioimmunoassay to assess the presence of NPY, using rabbit anti-serum anti-neuropeptide Y (Peninsula Laboratories, Inc.), 125I-NPY (Amersham) and human NPY (Boehringer Mannheim) as standard, according to the protocol supplied by the anti-serum manufacturer.

As shown in figure 2, stabilized SN-56 cells for the expression of recombinant human leptin receptor respond to leptin addition by decreasing neuropeptide Y production. Dexametasone described as an inducer of NPY production in neuronal cell lines (Higuchi et al., 1988), doubles NPY immunoreactivity in cells SN-56. In cells which express Ob-Rb, leptin inhibits NPY production in presence of dexametasone. Moreover, the diagram in figure 2 shows that wild type cells SN-56 do not respond to leptin, and that the neurotrophic ciliary factor which has anti-obesity effects similar to those of leptin, negatively regulates neuropeptide Y production.

EXAMPLE 3

Assay on cells SN-56.L64(IRBM-1) for the assessment of biological activity of leptin based on the activity of choline acetyltransferase.

Cells (SN-56 and SN-56.L64(IRBM-1) were seeded on a 24-well culture dish (3 x 10^4 cells per well) containing 0.5 ml of Opti-MEM ® (GibcoBRL) supplemented with penicillin and streptomycin. Three days after incubation in presence or in absence of various effectors, the culture medium was removed by aspiration and the cells lyzed in 250 µl of 20 mM Tris-Hcl, 0.1% Triton X-100, pH 7.5. Lysates were clarified by centrifugation (10 min at 10000 x g) and aliquots in duplicates were tested for choline acetyltransferase activity with the Fonnum modified method (Fonnum, 1975). The reaction mix, containing 15 µl of cell extract and 50 µl of the reaction buffer (50 mM Tris-Hcl pH 7.5; 400 mM NaCl; 10 mM choline chloride; 0.1 mM eserine hemisulphate; 10mM EDTA; 1 mg/ml of bovine serum albumin; 5 µM acetyl-coenzyme A; 25 nCi [^{14}C] acetyl-coenzyme A) were incubated for 1h at 37 °C, and subsequently the labelled acetylcholine was identified by separation according to procedures known in the art (31). Finally results were normalized to the content of total cellular proteins.

As shown in figure 3, cells SN-56.L64(IRBM-1) stably transfected with an expression vector for the human leptin receptor respond to leptin addition with an increase of cell enzymatic activity in choline acetyltransferase. The diagram shows wild type SN-56 cells which are insensitive to leptin and that CNTF, which has an anti-obesity effect similar to that of leptin, causes the increase of the intracellular activity of choline acetyltransferase.

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CLAIMS

1. A method for the selection of molecules capable of mimicking, potentiating, or inhibiting the effects of interaction between leptin and cells displaying on the membrane the receptor thereof, comprising the following operations

a) contacting said molecules with said cells;and

b) detecting the variation in said cells of the neuropeptide Y production, or of the enzymatic activity of choline acetyltransferase, caused by the binding of said molecule to said receptor.

2. The method according to claim 1, wherein the detection of neuropeptide Y production of the operation b) is carried out by the combination of the following operations:

- treatment of culture cells with an NPY production inducer in cells used, leptin or no factor;

- preparation of the cell extracts;

- assay for the qualitative and/or quantitative detection of NPY by conventional methods.

3. The method according to claim 2, wherein said assay is carried out by immunoenzymatic methods.

4. The method according to claim 1, wherein the detection of choline acetyltransferase enzymatic activity of the operation b) is performed by the combination of the following operations:

- incubation of cells in presence of leptin or of other effectors

- cell lysis;

- assay of choline acetyltransferase activity by means of modified Fonnum method.

5. The method according to claim 4, wherein said assay is performed by measuring cellular enzymatic activity.

6. The method according to any of the claim 1 to 5, wherein the cell line used is of neuronal type.

7. The method according to claim 7 wherein the cell

line used is SN-56 and the leptin receptor is the human type Ob-Rb.

8. The method according to any of the claim 1 to 7, wherein said cells are engineered to produce the leptin receptor.

9. The method according to claim 8, wherein the cells engineering is carried out by the combination of the following operations:

- transfection of said cells with an expression vector;

- selection of transfected clones; and

- selection of clones capable of stably expressing the leptin receptor by assaying STAT factor activity.

10. The method according to any of the claim 1 to 9, wherein said leptin receptor is recombinant.

11. The method according to any of the claim 1 to 10, wherein said leptin receptor is human or rodent.

12. A cell line named SN-56.L64(IRBM-1), deposited on June 5th 1997 at the Advanced Biotechnology Center, ABC, in Genoa, with access number PD97001 obtained from cell line SN-56, by means of the engineering procedure of claim 9.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IT 98/00195

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/573 G01N33/68 C12Q1/48

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 20158 A (BEELEY LEE JAMES ;SMITHKLINE BEECHAM PLC (GB)) 14 May 1998 see claims 8-11 see page 1, line 32 - page 2, line 26 see page 5, line 23 - line 42	1-12
P,X	WO 97 26523 A (PROGENITOR INC) 24 July 1997 see claims 11,12 see page 9, line 9 - line 23	1-12
X	WO 97 21731 A (NEW ENGLAND MEDICAL CENTER INC) 19 June 1997 see claims 1-15 see page 6, line 18 see page 10, line 2 see page 32, line 28	1-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 October 1998	24/11/1998

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IT 98/00195

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 96 38586 A (SMITHKLINE BEECHAM PLC ;BEELEY LEE JAMES (GB); SMITH RICHARD ANTHO) 5 December 1996 cited in the application see claims see page 1, line 29 - line 38 see page 4, line 4 - line 44	1-12
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		PL 323638 A	14-04-1998