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(54) Title: ISOLATED GENETICALLY MODIFIED CELL POPULATION

(57) Abstract: The present invention concerns an isolated population of cells characterized by the fact that (I) they comprise at least one exogenous RNA, (II) have the PHD-2 gene inducible silenced, and / or (IIIa) have the UQCRB gene inducible silenced or (IIIb) have the SIRT3 gene inducible overexpressed or (IIIC) have one or more of the following systems modulated by the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1 -3), Nrf2 / keep11 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (perox-iredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx); optionally (IV) they include at least one suicide gene, and / or (V) have the gene HIF-1 alpha silenced. This isolated population of cells is useful in the treatment of tissue damage, degenerative diseases, inflammatory and immune disorders, bacterial infections, in gene therapy, in the treatment of viral diseases and tumors. A method for producing said isolated population of cells is a further object of the invention.



ISOLATED GENETICALLY MODIFIED CELL POPULATION

DESCRIPTION**FIELD OF INVENTION**

5 The present invention has as its object an isolated population of genetically modified cells, the method for their preparation and their use in therapy.

TECHNICAL BACKGROUND

In recent years, scientific research has paid special attention to the cell therapy based on the administration to the patient of preparations containing cells, possibly
10 genetically modified, in order to obtain a therapeutic, diagnostic or preventive effect. The cells most commonly used in cell therapy are adult stem cells: multipotent undifferentiated cells characterized by high autogenerative capacity. Adult stem cells exist in almost all tissues and can easily be isolated for example from the umbilical cord, bone marrow, adipose tissue, from the muscle, from the corneal stroma and
15 from the dental pulp and subsequently expanded in vitro. A type of adult stem cell is haematopoietic that gives rise to all blood cells. Another type of adult stem cell is one that originates from the mesoderm and is called mesenchymal or stromal; mesenchymal stem cells (MSC) have the capacity to autoregenerate, to proliferate in an extended way and to differentiate into bone, adipose, cartilage and muscle in
20 vitro and in vivo. MSCs have a high expression of markers CD105, CD73, CD90 and low expression of antigens CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR and therefore exert immunosuppressive activity. In addition, as a result of particular stimuli such as tissue damage caused for example by ischemia, inflammation or tumor, MSCs migrate into the blood to reach the site where the
25 damage occurred. This is because on the surface of MSC are specific receptors of chemokines, such as CXCR4 which is the receptor for SDF-1 (stromal derived factor from cell) the secretion of this passes into the damaged part induced by HIF-1alpha (inducible factor 1 'hypoxia), c-Met that is the receptor for HGF and which is UPAR receptor for urokinase. These chemotactic factors are secreted in response to
30 hypoxia and inflammation (see Young-Wook Won et al., Biomaterials 2014; 35: 5627-5635); thus the MSC have tropism for damaged areas, inflamed and hypoxic (see Uchibori et al., Int J Hematol 2014; 99: 377-382). The intrinsic characteristics of the MSC, with almost no immunogenicity and the tropism toward damaged tissue, the sites of inflammation, and hypoxic tumor cells make these cells especially suited to

the treatment of tissue damage, degenerative diseases, inflammatory disorders, immunological, neurological, bacterial infections , in gene therapy, in the treatment of viral diseases and tumors.

Another particular feature of the MSC is that they produce exosomes and extracellular vesicles that contain various constituents of the cell of origin as for example proteins, lipids, mRNAs and microRNAs. It was shown that exosomes and the extracellular vesicles are able to convey towards target cells the RNA endogenous and exogenous ones, functioning as artificial liposomes. Once they reach the target site exosomes and extracellular vesicles empty their contents via vesicle fusion with the plasma membrane of target cells. For therapeutic success it is essential that the cells administered to the subject who needs them, reach the site in which they must act in a quantity sufficient to achieve the therapeutic effect. Significant limitation on use of cell therapy is the fact that the vast majority of MSC administered systemically in healthy subjects are found in the lung (see Lama et al., J Clin Invest 2007; 117: 989-996 quoted Wei et al., Acta Pharmacologica Sinica 2013; 34: 747-754). It was also reported that MSC administered systemically to rats after ischemic attacks are concentrated in the lungs while only 1% of the MSC injected systemically reach the area of ischemic myocardium after 4 hours of the infusion (see Barbash et al., Circulation 2003; 108: 863-868). Another limitation to the application of cell therapy is the fact that MSCs undergo apoptosis and die after a few days, for example it has been reported that over 90% of the MSC transplanted die within the first 24 hours following either tissue hypoxia or the lack of nourishment (see Tang et al., J Am Coll Cardiol 2005; 46: 1339-1350) and that only the 0,44% survive four days after transplantation in ischemic hearts (see Thomas et al., Circulation 2002; 105: 93-98 quoted in Xian-Bao Liu et al., J Cell Biochem 2009; 106: 903-911).

This is due to the fact that the ischemic environment is characterized by hypoxia and nutrient deprivation that cause apoptosis of the cells. For all these reasons the use of non-genetically modified MSC (MSC WT) in the clinic has not been widely used ,even increasing the amount injected, because excessive number of MSC injected leads to pulmonary embolism (see Furlani et al., Microvasc Res. 2009; 77: 370-376).

MSC can also be used to release pharmacologically active agents to cells, or tissues or target organs. The international patent application WO2013 / 090 523 describes , for example a method for releasing miRNA to cells, or tissues or target organs, by the

administration of MSC or exosomes that contain these miRNAs. The MSC described in this patent application are not genetically modified in order to improve survival tropism and, therefore, from the therapeutic point of view they suffer from the same limitations of the MSC WT. To promote the tropism and / or survival of MSC WT were applied several research strategies.

For example, because the expression of CXCR4 receptor is important for the tropism of the MSC and the levels of this receptor on the cell surface are low (see Wynn et al., Blood 2004; 104: 2643-2645.), genetic modification were carried out on MSC by inducing the expression of CXCR4 on their surface (see Young-Wook Won et al., Biomaterials 2014; 5627-5635). The transcription factor HIF-1 alpha (hypoxia inducible factor) is the major protein that regulates hypoxia. Also HIF-1alpha regulates the expression of several genes in determining effective angiogenesis, preventing apoptosis and induce cell tropism toward the site of ischemia (see Peterson et al., Life Sci 2011; 88: 65-73 quoted in Razban et al., BioResearch Open Access 2012; 1: 174-183). Finally, HIF-1alpha increases the production of exosomes (see King et al., BMC Cancer. 2012; 12: 421) fundamental to convey the RNA exogenous to the target cells. Hypoxic preconditioning of stem cells induces HIF-1 alpha and causes a reduction of apoptosis and improved survival and differentiation of these cells (see Kim et al., J Biol Chem 2009; 284: 33161-33168 mentioned Razban et al., BioResearch Open Access 2012; 1 (4): 174-183).

It was, however, reported that MSC expressing HIF-1alpha even at medium-high level, in hypoxic environment and with the lack of nutrients are still being damaged by hypoxia and the cells have little migration to hypoxic sites (see Palomäki et al., Stem Cells. 2013; 31: 1902-1909). Therefore, to promote survival in hypoxic condition which occur in damaged tissues, for example ischemic, inflamed, and / or tumor, some researchers have been studying how to stabilize or upregulate the transcription factor HIF-1alpha. To this end, some researchers have carried out genetic modification on MSC by the introduction of the gene HO-1 (heme oxygenase 1) (see Tang et al., J Am Coll Cardiol 2005; 46: 1339-1350). Other researchers have achieved stabilization of HIF-1alpha inhibiting the activity or expression of 'enzyme prolyl hydroxylase 2 through competitive inhibitors such DMOG (dimetilossalilglicina) (see Xian-Bao Liu et al., J Cell Biochem 2009; 106: 903 -911). Other research groups have achieved genetically modified MSC by the overexpression of HIF-1alpha (see Razban et al., BioResearch Open Access 2012; 1: 174-183 and in-Ho Park et al.,

Experimental & Molecular Medicine 2013; 45: 1-11). In the literature it has been reported that in order to obtain a migration and survival of MSCs such as to achieve the therapeutic effect, it is necessary to overexpress both HIF-1alpha and HIF-2alpha and that only by silencing the gene PHD-2 is this result possible (see Sun et al., PLoS One 2014; 9 (3): e90667). Another research group has developed genetically modified MSC through the silencing of PHD-2 transfecting cells with siRNA and obtaining a localized and transient activation of several genes favoring pro-angiogenic and the survival (see Kiave-Hyne HoWangYin, et al., Stem Cells 2014; 32: 231-243).

The ROS (reactive oxygen species) mitochondrial, produced from the complex III in a direct way following hypoxia and through the activation of romo1 upstream of the complex III in the case of lack of nourishment, have a pro-apoptotic effect. ROS are the main cause of apoptosis of MSCs, therefore suppressing their production causes increased survival of these cells. The suppression of the production of ROS can be obtained through different strategies.

A research strategy to suppress the production of mitochondrial ROS and promote survival in hypoxic condition has led to the development of MSC genetically changed with the introduction of the SIRT3 gene (see Xue-Qing Wang et al., J Cell Mol Med 2014; 1-13). Indeed the protein SIRT3 (mitochondrial sirtuin 3) is involved in cell resistance to various types of stress maintaining genomic stability and integrity of mitochondrial. Sirt3 protect different types of cell lines by hypoxia or cell death caused by taurosporin. Increased expression of SIRT3 inhibits the induction of MPT (mitochondrial permeability transition), the loss of membrane potential and the accumulation of ROS (reactive oxygen species); SIRT3 also prevents binding of HKII (hexokinase II) to the mitochondria and increases the activity of carbonic anhydrase VB then maintains the physiological pH preventing cell death by apoptosis (see Pellegrini et al., Cell Death and Diff 2012; 19: 1815-25). Another research strategy to suppress the production of mitochondrial ROS, the main cause of apoptosis of MSCs, and promote survival in hypoxic condition is represented by 'functional inhibition of gene UQCRB both by genetic and pharmacological means (see Cho Yoon Sun et al., Biochem.Biophys.Res.Comm. 2013; 433: 396-4004; Hye Jin Jung et al., J. Biol. Chem. 2010; 285: 11584-11595). In fact UQCRB represents a subunit of complex III and its blocking or silencing inhibits ROS production at the mitochondrial level without negatively affecting the mitochondrial respiration so

having no side effects . A strategy to suppress the production of cellular ROS and promote cell survival in hypoxic condition is represented by the modulation of SOD1, and SOD3 SOD2 (superoxide dismutase 1-3) increasing activity by overexpression directly or indirectly through the use of inductors transcriptional such as for example the system nrf2 / Keap1 or with the use of molecules or peptide mimetics or inhibitors of Keap1 (see Kolamunne et. al., Redox Biol. 2013; 1: 418-426). The suppression of the production of cellular ROS can be carried out also through modulation of other systems, such as for example Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (Catalase), Grx (glutathione-glutaredoxin system), PGC- 1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx). Hypoxia and ROS mitochondrial inhibit RNase Dicer and Drosha, enzymes essential in the processing of exogenous RNA. (see Q Dong et. al. J Invest Dermatol. 2011 Jun; van den Beucken T et. al. Nat Commun. 2014 Oct; Bandara V, et. al. BMC Cancer. 2014 Jul; Wiesen JL et. al. Mol Immunol . March 2009; JJ David Ho et. al. J Biol Chem. Aug 17, 2012;). Therefore, the reduction of ROS also favors the processing of exogenous RNA without which the therapeutic function of the MSC cannot take place .

SUMMARY OF THE INVENTION

The present inventors have addressed the problem of improving cell therapy by improving the tropism and the survival of the cells used, in particular adult stem cells, even more particularly of mesenchymal stem cells (MSC) adult. The present inventors have found that silencing PHD-2 gene through the formation of a knock out causes overexpression of HIF-1 α and subsequent induction of C-Met receptor, and UPAR CRCR4. Furthermore, the present inventors have found that adult stem cells in which the PHD-2 gene has been silenced, possess an improved tropism both in vitro and in vivo. To achieve this effect also contributes the fact that the silencing of the PHD-2 gene thus obtained also determines the overexpression of HIF-2 α over that of HIF-1 α .

In adult stem cells of the present invention, the silencing of the gene PHD-2 is not made via the use of pharmacological inhibitors, therefore, adult stem cells of the present invention have the advantage of being more secure than adult stem cells described in the prior art, because you do not use molecules potentially toxic to the

body intended to receive the cell therapy. Furthermore, the silencing of PHD2 gene through the formation of a knock-out system has the advantage of carrying out of permanent block of the protein of interest, contrary to what takes place by means of pharmacological inhibition and / or by means of siRNA.

- 5 Furthermore, the silencing of the gene PHD-2 determines the further advantage to increase in addition to the tropism, also the survival of adult stem cells of the present invention, as such silencing active telomerase which is a transcriptional target of HIF-1alpha.

The present inventors have found that adult stem cells in which has been silenced
10 the UQCRB gene possess an improved in vitro survival. The silencing of the UQCRB gene determines also the advantage of increasing the permanence of adult stem cells of the present invention in a differentiated state and to decrease the number of mutations, the main cause of neoplastic transformation which occurs in the absence of oxygen. Furthermore, the reduction of mitochondrial ROS due to the silencing of
15 UQCRB gene allows RNase Dicer and Drosha to carry out their action, that is to determine the ripeness of exogenous RNA after transfection occurred, without which the therapeutic effect of MSCs engineered is not expressed. Therefore this is an additional advantage for the success of the therapy in vivo by administration of adult stem cells of the present invention.

20 The improvement of the tropism toward hypoxic areas, such as for example the area of ischemic, inflamed and / or tumor, allows the adult stem cells of the present invention to reach the area affected by the disease in effective doses and improvement of the survival allows these cells stay connected for longer with the area affected by the disease. Also, because adult stem cells tend to concentrate in
25 the lung, high doses of these cells can cause occlusion of pulmonary capillaries resulting in pulmonary embolism; therefore, the improvement of the tropism and survival means that the dosage of adult stem cells of the present invention can be reduced and that therefore can be decreased the side effect of this type of cell therapy, that is, the occlusion of pulmonary capillaries, resulting in pulmonary
30 embolism. In conclusion, the silencing of PHD2 and UQCRB genes improve cell therapy performed through the administration of adult stem cells of the present invention.

The present inventors have also found that by varying the qualitative content of the exogenous RNA introduced into adult stem cells, you get a therapy targeted to the type of pathology.

In addition, the present inventors have found that by combining the dosage of endogenous RNA expressed in a particular disease in a particular subject by administration to said subject of adult stem cells comprising exogenous RNA specific to counteract said pathology, not only do you get a targeted therapy of the type of disease but also a specific therapy is obtained for the subject type. In addition, the present inventors have found that adult stem cells by inserting a suicide gene and administering to the patient, after a successful therapeutic effect, a drug activated by phosphorylation by an enzyme encoded by the suicide gene, you can eliminate these cells from the subject to which they were administered.

In this way there is provided a control on the growth and survival of the cells and avoids the possible side effects of cellular therapy, such as for example the effects due to an uncontrolled growth of the cells (tumor-like transformation).

Since the 'overexpression of HIF-1alpha following the silencing of PHD-2 induces angiogenesis, adult stem cells in which it was overexpressed HIF-1alpha are not suitable for tumor therapy. The present inventors have found that the gene silencing HIF-1alpha after that adult stem cells have reached the target tissue, inhibition of angiogenesis is obtained, and therefore these cells become also suitable for therapy of tumors.

The cells of the present invention, in particular adult stem cells, even more particularly mesenchymal stem cells (MSC) adult, genetically modified by means of transfection of specific exogenous RNA, and the silencing of PHD-2 gene and / or the suppression of the production of ROS cell by means of silencing UQCRB gene or overexpression of SIRT3 gene or modulation of one or more of the following systems through the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (Superoxide dismutase 1-3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPxs) are useful in cell therapy of conditions and / or diseases selected from, but not limited to, the following group : tissue damage, degenerative diseases, inflammatory disorders,

immunological, neurological, bacterial infections, in gene therapy and in the treatment of viral diseases. Moreover, these cells further modified genetically by means of the silencing of HIF-1alpha after the completion of migration to the target tissues, are useful in tumor therapy.

- 5 The silencing of the PHD-2 gene determines the further advantage of increasing the proliferation of adult stem cells of the present invention and the production by these cells of exosomes, fundamental to convey the exogenous RNA to the target cells. Therefore, exosomes produced by a population of adult stem cells, in particular adult mesenchymal stem cells, genetically modified by the silencing PHD-2 gene and in
10 which has been induced the expression of at least one RNA exogenous to anti-inflammatory activity, they are useful in the cell therapy of conditions and / or inflammatory diseases.

A first object of the present invention relates to an isolated population of cells characterized in that

- 15 - (I) comprises at least one exogenous RNA,
- (II) having the PHD-2 gene silenced, and / or
- (IIIa) have the UQCRB gene silenced or (IIIb) have the SIRT3 gene overexpressed or
(IIIC) have one or more of the following systems modulated by the use of inhibitors or
20 inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1-3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO
25 (myeloperoxidase), glutathione peroxidase (GPx);
optionally
- (IV) comprises at least a suicide gene, and / or
- (V) have the HIF-1alpha gene silenced.

A second object of the present invention relates to a method for producing an
30 isolated population of cells as defined above, comprising the subjecting of the isolated cells to the following stages:

- a) induce the expression of at least one exogenous RNA;
- b) silencing the PHD-2 gene, and / or
- c) decrease mitochondrial ROS and / or cytoplasmic by

c1) or c2 silencing UQCRB gene) overexpression SIRT3 gene or c3) modulation of one or more of the following systems through the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (Superoxide dismutase 1-3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx);

and optionally

- d) introducing at least one suicide gene, and / or
- e) silencing the HIF-1alpha gene .

A third object of the present invention relates to an isolated population of cells as defined above for use as a medicament.

A fourth object of the present invention relates to an isolated population of cells as defined above for use in the treatment of a disease chosen from, but not limited to, the following group: tissue damage, degenerative diseases, inflammatory disorders, immunological, neurological and bacterial infections, in gene therapy, in the treatment of viral diseases and tumors.

A fifth object of the present invention relates to exosomes produced from an isolated population of cells as defined above for use in the treatment of a disease chosen from, but not limited to, the following group: tissue damage, degenerative diseases, inflammatory disorders, immunological, neurological and bacterial infections, in gene therapy, in the treatment of viral diseases and tumors.

A sixth object of the present invention relates to exosomes produced from an isolated population of cells genetically modified by means of silencing PHD-2 gene and in which has been induced the expression of at least one RNA exogenous to anti-inflammatory activity, for use in the treatment of a condition or inflammatory disease.

A seventh object of the present invention concerns a method to prevent and / or treat a condition or disease in a subject by administration of an isolated population of cells as defined above in an amount / number in pharmaceutically effective to prevent and / or treat this condition or pathology.

An eighth object of the present invention relates to a pharmaceutical composition comprising an isolated population of cells as defined above and at least a pharmaceutically active substance.

A ninth object of the present invention concerns a method to prevent and / or treat a condition or disease in a subject by administration of exosomes produced from an isolated population of cells as defined above in an amount / number in pharmaceutically effective to prevent and / or treat the condition or disease.

- 5 A tenth object of the present invention concerns a method to prevent and / or treat a condition or inflammatory disorder in a subject by administration of exosomes produced from an isolated population of cells genetically modified by means of silencing PHD-2 gene and in which the expression of at least one RNA exogenous to anti-inflammatory activity was induced in an amount / number in pharmaceutically
10 effective to prevent and / or treat the condition or pathology.

DEFINITIONS

As used herein an RNA, miRNA, siRNA, shRNA, anti-miRNA, and / or mRNA is "exogenous" in respect to a cell if it has been artificially introduced into the cell or in the precursor cells of that cell. As used herein the term "prevent" a condition or
15 disease is to reduce the incidence of the damage associated with the condition or disease.

As used herein the term "treating" a condition or disease means slow down, stop or reverse the progression. In the preferred embodiment, treatment of a subject suffering from a condition or disease means reversing the progression, ideally to the
20 point of eliminating the condition or disease itself.

As used herein the expression "administering" refers to a population of cells means that this population has been introduced into the subject to be treated in one of the veins or arteries, by injection and / or infusion. In an alternative, as used herein this term means that this population has been introduced into the tumor of the subject to
25 be treated, by injection and / or infusion. In another alternative, as used herein this term means that this population has been introduced in the adipose tissue of the subject to be treated by injection and / or infusion, that is practicing an injection and / or infusion subcutaneously.

As used herein the expression "administering" refers to exosomes produced by the
30 cells of the present invention and isolated from their supernatant means that these exosomes have been introduced into the subject to be treated in one of the veins or arteries, by injection and / or infusion, or which they have been applied to the subject to be treated topically, for example by means of aerosol, cream or spray. Such administration can be carried out, for example, once, a plurality of times and / or on

one or more extended periods. A single administration is preferred, but repeated doses over time (for example, every day, every third day, weekly, bi-weekly, monthly, quarterly, semiannual or annual) may be necessary in some cases.

Such administration is preferably performed using a population of adult mesenchymal cells and a pharmaceutically acceptable carrier. Vehicles pharmaceutically acceptable for injection and / or infusion are well known to those skilled in the art and include, but are not limited to, 0.01 to 0.1 M and preferably 0.05 M phosphate buffer or 0.8% solution saline. Additionally, such pharmaceutically acceptable vehicles can be aqueous solutions or non-aqueous solutions, suspensions and emulsions.

Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous vehicles include water, alcohol solutions, emulsions and suspensions, including saline and buffered media. Vehicles suitable for parenteral use include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Vehicles suitable for use include intravenous fluids and nutrients fillers, electrolytes fillers such as Ringer's dextrose and those based on Ringer's dextrose and the like. Vehicles used commonly for intravenous administration are, for example, in Remington (see Remington: The Science and Practice of Pharmacy, 20th, p. 808, Lippincott Williams & Wilkins (2000)).

Vehicles pharmaceutically acceptable for topical administration are those known to those skilled in the art (see Remington cited above). They may also be present preservatives and other additives, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases, and the like.

The administration and dosage of the population of cells or exosomes produced by them subject are made in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and the method of administration, administration schedule, the 'age, sex, body weight of the patient and other factors known to the doctor.

As used herein the expression "an amount / number in pharmaceutically effective" it is therefore determined on the basis of the above considerations. This amount must be effective to achieve improvement including but not limited to, decrease the damage, or to improve or eliminate the symptoms and other indicators chosen as appropriate by the expert of the branch. As used herein the term "allogeneic cell" with

respect to an entity means that the cell or its precursor cells are from another person of the same species.

As used herein the term "autologous cell" with respect to a subject means that the cell or one of its precursor cells are from the same subject.

5 DETAILED DESCRIPTION OF THE INVENTION

As will be discussed in more detail in the experimental part, the cells of the present invention are useful for improving cell therapy of various diseases, such as for example tissue damage, degenerative diseases, immunological and inflammatory disorders, bacterial infections, in gene therapy, in the therapy of viral diseases and
10 cancers. A first object of the present invention relates to an isolated population of cells characterized in that

- (I) comprises at least one exogenous RNA,
- (II) having the silenced PHD-2 gene , and / or
- (IIIa) have the UQCRB gene silenced or (IIIb) have the SIRT3 gene overexpressed
15 or (IIIC) have one or more of the following systems modulated by the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1- 3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs
20 (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx); optionally

- (IV) comprises at least a suicide gene, and / or
- (V) have the HIF-1 α gene silenced.

25 According to a realization of the first object of the present invention said population is a population of adult stem cells, preferably mesenchymal stem cells (MSC) adult. Said population of cells can be of animal or human origin. According to another realization of the first object of the present invention at least one exogenous RNA is selected from the group comprising at least one miRNA, siRNA, shRNA, anti-miRNA,
30 and / or mRNA.

In one realization of the present invention said cells comprise at least one miRNA chosen from anti-tumor, but not limited to, the following group: let-7 ((lung, breast); miRNA-221/222 (glioblastoma); miRNA-181c (stomach); miRNA-31 (breast, stomach, ovary), miRNA-34b (colon, ovary, glioblastoma); miRNA-200c (breasts) miRNA-107

(colon, pancreas); miRNA-126 (stomach, breasts) miRNA-96 (pancreas); miRNA-196 (pancreas); miRNA-29 (non-breast), miRNA-99a, miRNA-362-3p, miRNA16, miRNA-203, miRNA-34a, miRNA-16, miRNA-15a, miRNA- 15b, miRNA-192/215, miRNA-138, miRNA-106b, miRNA-107, miRNA185, miRNA-194 and miRNA-20a. In another realization of the present invention the cells comprise at least one anti-miRNA antitumor chosen from, but not limited to, the following group: miRNA-210, 9-miRNA, miRNA-21 (lung, colon, liver, esophagus, pancreas, breast, glioblastoma, myeloma), miRNA-34, miRNA-106a ~ 363 cluster, miRNA-135b, miRNA-221 (breast, lung, liver), miRNA-181b (liver, myeloma), miRNA-200a / b (ovary) , miRNA-10b (breast, esophagus, glioblastoma), miRNA-196 (esophagus, glioblastoma, colon), miRNA-17/92 and miRNA-30-c.

In another realization of the present invention said cells comprise at least one anti-HIV miRNA chosen from, but not limited to, the following group: miRNA-149 (vpr), miRNA-29a, 29b (nef), miRNA-378 (env), miRNA-3245p (vIP) miRNA-23b (NOX4), miRNA-21 (gag), miRNA-185 (pol1), miRNA-122 (pol2), miRNA-16-1 (tat) miRNA-20a (env) and miRNA -150 (vif).

In another realization of the present invention the cells comprise at least one anti-HIV miRNA chosen from, but not limited to, the following group: miRNA-217 miRNA-34a; miRNA-117 and miRNA-182 (induced tat).

In another realization of the present invention said cells comprise at least one miRNA with anti-ischemic and / or reperfusion favoring selected from, but not limited to, the following group: miRNA-494, 499-miRNA, miRNA-125a, miRNA-214 (p22phox) (CYBA), miRNA-126 and miRNA-210.

In another realization of the present invention said cells comprise at least one anti-miRNA with anti-ischemic and / or reperfusion favoring selected from, but not limited to, the following group: miRNA-199a, 92a-miRNA, miRNA-320, miRNAs -920, miRNA-320 and miRNA-21.

In another realization of the present invention the cells comprise at least one miRNA to anti-inflammatory activity selected from, but not limited to, the following group: miRNA-214 miRNA-491-5p (NFkB), miRNA-873 (fos), miRNA- 429 (jun) and miRNA-1405p (tnf).

In another realization of the present invention said cells comprise at least one miRNA to regenerative activity, in particular following spinal cord injury, selected from, but not limited to, the following group: miRNA- 214, miRNA-491-5p (NFkB), miRNA-140-

5p (tnf) (anti-inflammatory), miRNA-125a-5p, miRNA-214 (anti p22phox), miRNA-193a-3p (anti mpo), miRNA-222 (PTEN), miRNA-320, miRNA -21, miRNA-431, miRNA-132 and miRNA-125b.

In another realization of the present invention the cells comprise at least one anti-miRNA to regenerative activity, in particular following spinal cord injury, selected from, but not limited to, the following group: miRNA-124 and miRNA-138.

In one realization of the present invention, said isolated population of cells is characterized in that

- (I) comprises at least one exogenous RNA and

10 - (II) have the PHD-2 gene silenced,
optionally

- (IV) comprises at least a suicide gene, and / or

- (V) have the HIF-1alpha gene silenced .

In another realization of the present invention, said isolated population of cells is characterized in that

- (I) comprises at least one exogenous RNA and

- (IIIa) have the UQCRB gene silenced or (IIIb) have the SIRT3 gene overexpressed or (IIIC) have one or more of the following systems modulated by the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1- 3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx);

25 Optionally

- (IV) comprises at least a suicide gene, and / or

- (V) have the HIF-1alpha gene silenced.

Preferably, the isolated population of cells of the present invention is characterized in that

30 - (I) comprises at least one exogenous RNA,

- (II) having the PHD-2 gene silenced , and

- (IIIa) have the UQCRB gene silenced or (IIIb) have the SIRT3 gene overexpressed or (IIIC) have one or more of the following systems modulated by the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1- 3), Nrf2 / keep1 (NF-

E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx);
optionally

- (IV) comprises at least a suicide gene, and / or

- (V) have the HIF-1 α gene silenced.

Even more preferably the isolated population of cells of the present invention is characterized in that

- (I) comprises at least one exogenous RNA,

- (II) have the PHD-2 gene silenced,

- (IIIa) have the gene UQCRB silenced or (IIIb) have the SIRT3 gene overexpressed or (IIIC) have one or more of the following systems modulated by the use of inhibitors

or inducers: SOD1 / SOD2 / SOD3 (superoxide dismutase 1- 3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx); is

- (V) have the gene HIF-1 α silenced,

optionally

- (IV) comprises at least one suicide gene

wherein said at least one exogenous RNA is selected from the group comprising at least one miRNA, siRNA, shRNA, anti-miRNA, and / or antitumor mRNA.

More preferably at least one exogenous RNA is selected from the group comprising at least one miRNA and / or at least one anti-miRNA antitumor.

More preferably said population comprises at least one miRNA selected from anti-tumor, but not limited to, the following group:

let-7 ((lung, breast); miRNA-221/222 (glioblastoma); miRNA-181c (stomach); miRNA-31 (breast, stomach, ovary), miRNA-34b (colon, ovary, glioblastoma); miRNA-200c (breasts) miRNA-107 (colon, pancreas); miRNA-126 (stomach, breasts) miRNA-96 (pancreas); miRNA-196 (pancreas); miRNA-29 (non-breast), miRNA-99a, miRNA-362-3p, miRNA16, miRNA-203, miRNA-34a, miRNA-16, miRNA-15a, miRNA-15b,

miRNA-192/215, miRNA-138, miRNA-106b, miRNA-107, miRNA185, miRNA- 194 and miRNA-20a

and / or

at least one anti-miRNA antitumor chosen among, but not limited to, the following group: miRNA-210, 9-miRNA, miRNA-21 (lung, colon, liver, esophagus, pancreas, breast, glioblastomas, myeloma), miRNA-21, miRNA-34, miRNA-106a ~ 363 cluster, miRNA-135b, miRNA-221 (breast, lung, liver), miRNA-181b (liver, myeloma), miRNA-200a / b (ovary), miRNA-10b (breast, esophagus, glioblastoma), miRNA-196 (esophagus, glioblastoma, colon) and miRNAs-17/92 and miRNA-30-c.

Preferably said suicide gene is selected from, and not limited to, the following group HSV-tk and caspase-9.

More preferably said suicide gene is HSV-tk.

A second object of the present invention relates to a method for producing an isolated population of cells as defined above, comprising subjecting the isolated cells to the following stages:

a) induce the expression of at least one exogenous RNA;

b) silencing the PHD-2 gene , and / or

c) decrease mitochondrial ROS and / or cytoplasmic by

c1) or c2 silencing UQCRB gene overexpression SIRT3 gene or c3) modulation of

one or more of the following systems through the use of inhibitors or inducers: SOD1 / SOD2 / SOD3 (Superoxide dismutase 1-3), Nrf2 / keep1 (NF-E2-related transcription factor 2), CAT (catalase), Grx (glutathione-glutaredoxin system), PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) FoxOS (forkhead box protein family), Trx (thioredoxin system), NOX-DUOXs (NADPH oxidase family), phox family PRXs (peroxiredoxins), MPO (myeloperoxidase), glutathione peroxidase (GPx);

and optionally

d) introducing at least one suicide gene, and / or

e) silencing the HIF-1alpha gene .

According to an realization of the second object of the present invention said population is a population of adult stem cells, preferably adult mesenchymal stem cells (MSC). Said isolated population of cells can be of animal, for example, non-human primate, mouse, rat, guinea pig or rabbit; or it can be of human origin. This

population can be isolated for example from umbilical cord, bone marrow, adipose tissue, from muscle, corneal stroma and the dental pulp.

In accordance with a realization of the second subject of the invention, step a) induce the expression of at least one exogenous RNA, can be achieved by the use of a vector, said vector can be a plasmid or a virus. Preferably, step a) is carried out by transfection using a plasmid as a vector, preferably said plasmid is selected from the group comprising PMRI-mCherry, PMRI-ZsGreen1 and pCMV-Tet3G.

More preferably, said plasmid is PMRI-mCherry or PMRI-ZsGreen1.

In accordance with another realization of the second subject of the invention, step a) can be realized with the technique of the inducible promoter, by chemical means such as calcium phosphate, DEAE-Dextran, liposomes (for example with lipofectamine); or by physical methods such as electroporation, microinjection and bombardment by particles (gene gun). Preferably, the step a) is realized by means of the technique of the inducible promoter, even more preferably by means of a system regulated by tetracycline or its analogs such as doxycycline (system TET-ON).

In accordance with a preferred realization of the second object of the invention, the step a) is carried by transfection with the plasmid PMRI-mCherry or PMRI-ZsGreen1 and the technique of the inducible promoter by means of TET-ON.

In accordance with a realization of the second subject of the invention, step b)

silencing the PHD-2 gene can be made through the formation of a knock out system or by introduction of at least one miRNA or siRNA (RNA interference) brand.

Preferably, the step b) is made by the formation of a knock-out system, even more preferably by formation of a knock out system with the use of the plasmid PHD-2 CRISPR / Cas9 KO.

Alternatively, step b) is effected by transfezione with miRNA-338-3p using the agent transcription siPORTTM NeoFXTM (Life Technologies) after cloned miRNA-338-3p using the method Pre-miRTMmiRNA Starter Kit (Life Technologies). In accordance with another realization of the second subject of the invention, the step c1) muting the UQCRB gene can be realized through introduction of at least one miRNA or siRNA (RNA interference) specific, or via the formation of a knock-out system.

Preferably, the step c1) is performed by introduction of at least one miRNA or siRNA (RNA interference) specific, even more preferably through the introduction of miRNA194. Alternatively, silencing UQCRB gene can be performed also by RNA under other inducible promoters or by the formation of a knock-out system.

In accordance with another realization of the second subject of the invention, the step c2) overexpress the SIRT3 gene can be achieved through the expression of a plasmid containing the cDNA of SIRT3 under a strong viral promoter type cytomegalovirus. In accordance with another realization of the second subject of the invention, the step c3) modulation of one or more of the systems mentioned above can be realized through the use of inhibitors or inducers selected from, but not limited to, non-enzymatic antioxidants such as glutathione, lipoic acid, resveratrol, lycopene, curcumin; mimetics, both peptidic and non-peptidic of mitochondrial complexes I, II, III, IV, V; mimetic peptide that is non-peptide, or inhibitors, both synthetic and natural, that have relationships with the aforementioned biochemical enzyme systems and / or protein, or all those systems that reduce mitochondrial ROS and / or cytoplasmic, and / or activate or inhibit molecular targets downstream of the aforementioned enzymes, transcription factors, proteins, such as for example HIF-1 alpha, HIF-2alpha, Nrf2 etc. or, in the case of transcription factors, the various transcriptional target as telomerase, for hif-1alpha etc. In addition, the phase c3) can be made through the modulation of molecular targets of direct and indirect downstream of ROS as for example, Src, PI3K, AKT, MAPK, Bcl2, AP1, NFkB, etc. that upon activation increased cell survival in response to stress (ROS, hypoxia, lack of nourishment).

In particular, system modulation SOD1 / SOD2 / SOD3, can be achieved by the use of mimetics or overexpression of transcription factors inducing nrf2 or inhibition of keep1, or by use of non-enzymatic antioxidants such as glutathione, lipoic acid, resveratrol , lycopene, curcumin. The silencing of the UQCRB gene or alternatively overexpression of SIRT3 or modulation of one or more of the systems mentioned above in order to suppress the mitochondrial ROS and / or cytoplasmic antigens is decided in relation to the various cases regimens (see Xue-Qing Wang et al., J Cell Mol Med 2014; 1-13; Kovac et. al. Biochim Biophys Acta 2014; piou: S0304-4165 (14) 00398-5).

In accordance with a further realization of the second subject of the invention, step d) introducing at least one suicide gene, it can be made with the technique of liposomes, electroporation or calcium phosphate.

It is preferably accomplished using lipofectamine.

In accordance with a further realization of the second subject of the invention, step e) silencing the HIF-1alpha gene can be effected by insertion of at least one miRNA or

siRNA (RNA interference) or through the formation of a knock out system . Is preferably accomplished by inserting at least one specific mRNA, even more preferably it is realized by means of the insertion of miRNA-199a-5p and the technique of the inducible promoter by means of TET-ON.

- 5 A third object of the present invention relates to an isolated population of cells as defined above for use as a medicament.

A fourth object of the present invention relates to an isolated population of cells as defined above for use in the treatment of a disease chosen from, but not limited to, the following group: tissue damage, degenerative diseases, inflammatory disorders,
10 immunological, neurological and bacterial infections, in gene therapy, in the treatment of viral diseases and tumors.

In accordance with a preferred realization of the fourth object of the present invention, said isolated population of cells is useful for use in the treatment of specific tissue damage selected from, but not limited to, the following group: ischemia,
15 myocardial infarction. In accordance with another preferred realization of the fourth object of the present invention, said isolated population of cells is useful for use in the treatment of specific types of cancer selected from, but not limited to, the following group: carcinoma of the bladder, breast, colon, kidney, liver, lung, including small cell lung cancer, esophagus, gall bladder, ovary, pancreas, stomach, cervix, thyroid,
20 prostate, and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage including acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's disease, non-Hodgkin lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias,
25 myelodysplastic syndrome and promyelocytic leukemia, myeloma; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioblastoma, glioma and schwannomas; other tumors, including melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderma pigmentosum,
30 keratoxanthoma, follicular thyroid cancer and Kaposi's sarcoma. A fifth object of the present invention relates to exosomes produced from an isolated population of cells as defined above for use in the treatment of a disease chosen from, but not limited to, the following group: tissue damage, degenerative diseases, inflammatory

disorders, immunological, neurological and bacterial infections, in gene therapy, in the treatment of viral diseases and tumors.

A sixth object of the present invention relates to exosomes produced from an isolated population of cells genetically modified by means of silencing PHD-2 gene and in
5 which has been induced the expression of at least one RNA exogenous to anti-inflammatory activity, for use in the treatment of a condition or inflammatory disease.

A seventh object of the present invention concerns a method to prevent and / or treat a condition or disease in a subject by administration of an isolated population of cells as defined above in an amount / number in pharmaceutically effective to prevent and
10 / or treat this condition or pathology.

In accordance with an realization of the seventh object of the present invention, said isolated population of cells may be allogeneic with respect to said subject, or may be autologous with respect to it; preferably is autologous with respect to said subject. Preferably in accordance with the seventh object of the present invention the
15 administration of said population of cells is introduced into a vein or artery of said subject by injection and / or infusion.

Alternatively, in accordance with the seventh object of the present invention the administration of said population of cells is introduced into the fatty tissue of said subject by injection and / or infusion subcutaneously.

20 In accordance with another preferred realization of the seventh object of the present invention, the condition or disease which is the tumor and the administration of said population of cells is introduced into a vein or artery of said subject by injection and / or infusion upstream of the tumor.

Conditions or neurological diseases such as for example Parkinson's disease, depression and schizophrenia that do not factor peculiar inflammation or hypoxia, cannot use the migratory capacity of adult stem cells. In accordance with another preferred realization of the seventh object of the present invention, if the condition or disease to be treated is neurological, as for example a condition selected from, but not limited to, Parkinson's disease, depression and schizophrenia, the administration
25 of said population of cells is introduced into the fatty tissue of said subject by injection and / or infusion subcutaneously. If so, then the exosomes produced by said cell population to convey at least one exogenous RNA from adipose tissue of said subject to the cells and / or target tissues. An eighth object of the present invention
30

relates to a pharmaceutical composition comprising an isolated population of cells as defined above and at least one pharmaceutically acceptable vehicle.

In accordance with an eighth realization of the present invention said pharmaceutically effective carrier is selected from aqueous solutions or non-
5 aqueous solutions, suspensions and emulsions.

A ninth object of the present invention concerns a method to prevent and / or treat a condition or disease in a subject by administration of exosomes produced from an isolated population of cells as defined above in an amount / number in pharmaceutically effective to prevent and / or treat the condition or disease.

10 A tenth object of the present invention concerns a method to prevent and / or treat a condition or inflammatory disorder in a subject by administration of exosomes produced from an isolated population of adult cells genetically modified by means of silencing PHD-2 gene and in which was induced the expression of at least one RNA exogenous to anti-inflammatory activity, in an amount / number in pharmaceutically
15 effective to prevent and / or treat the condition or pathology. Preferably In accordance with the tenth object of the present invention the administration of said exosomes in said subject is via topical route, by means of aerosol, cream or spray. More preferably In accordance with the tenth object of the present invention, said condition or disease is inflammation and pulmonary administration of said exosomes
20 is via aerosols.

In accordance with a realization of the seventh, ninth and tenth object of the present invention, said subject can be human or animal, for example, non-human primate, mouse, rat, guinea pig or rabbit; preferably said subject is human.

EXPERIMENTAL PART

25 Withdrawal and isolation of adult mesenchymal stem cells (MSC)
a) from adipose tissue.

Adult MSCs were obtained from male mice C57BL / 6 (Charles River) by biopsy.

The adipose tissue was first weighed and then washed with Dulbecco solution (DPBS) containing 200 units' / ML and 200 micrograms / ml respectively of penicillin
30 and streptomycin to remove contaminants plasma.

The fabric obtained was crushed gently for 10 minutes with scissors until you have pieces of 1 mm². The extracellular matrix was enzymatically digested by the use of collagenase type I (37 ° C, 30 min, 0.5%), which later was inactivated with an equal volume of KO-DMEM 10% fetal bovine serum (FBS).

The adipose tissue digested was centrifuged at 1500 rpm for 5 minutes to obtain a pellet.

The pellet was resuspended in KO-DMEM and filtered in a filter 100 μ M BD Pharmingen to remove debris, and a lysis buffer was added (10X cellsignal product) lisa h and erythrocytes, was put into culture plates T75 cm² a growth medium containing Mesenchymal Stem Cell Basal Medium (ATCC), Penicillin-Streptomycin-Amphotericin B Solution (ATCC) and 10% FBS and finally was placed at 37 ° in an incubator with a humidified atmosphere containing 95% CO₂ to 5% .

Non-adherent cells were removed 48 hours after the seeding, changing the medium every three days until the cells have reached 90% confluence.

MSC were obtained from the patient even with liposuction.

The cells obtained from liposuction were washed twice in sterile DPBS (Invitrogen) to remove contaminants.

After washing the tubes containing the aspirated were treated with 0.075% collagenase type I (Invitrogen) in DPBS (Dulbecco's Phosphate buffered saline) for 60 minutes at 37 ° C under slow agitation, then the collagenase was inactivated with an equal volume of KO-DMEM (Invitrogen), 10% fetal bovine serum (FBS).

The pellet obtained was centrifuged at 600 rpm for 10 minutes and resuspended in the culture medium KO-DMEM (Invitrogen) after having been washed twice in sterile DPBS (Invitrogen) and subsequently filtered through a filter 100 μ m in example with a filter BD Pharmingen to remove debris.

The remaining cells were placed in T75 cm² culture plate with culture medium containing Mesenchymal Stem Cell Basal Medium (ATCC) and 10% FBS and finally was placed at 37 ° in an incubator with a humidified atmosphere containing 95% CO₂ at 5%.

With a similar method, the MSC can also be obtained, for example, from the umbilical cord, from adult muscle, from the corneal stroma and from the dental pulp.

b) from the bone marrow

Bone marrow (MOC) had been vacuumed from femurs and tibias of male mice C57BL / 6 (Charles River) after having sacrificed the animals.

The MO was diluted with a 1: 1 solution of Mesenchymal Stem Cell Basal Medium (ATCC) and Penicillin-Streptomycin-Amphotericin B (ATCC) and centrifuged at 1800 rpm for 10 minutes. The supernatant was discarded and the pellet was washed twice

after having been resuspended in a culture medium and plated into culture plates with the medium Mesenchymal Stem Cell Basal Medium (ATCC), 10% FBS (Hyclone).

Non-adherent cells were removed 48 hours after the seeding changing the medium every three days until the cells have reached 90% confluence.

PHD-2 gene silencing

Adult MSCs isolated from adipose tissue or bone marrow of mice as described above have been genetically modified by means of silencing PHD-2 gene .

The silencing of PHD-2 gene was carried out through the formation of a knock out system using the technique of CRISPR with the use of the plasmid-PHD 2 CRISPR / Cas9 KO (Santa Cruz Biotechnology, Inc.) In accordance with manufacturer's instructions.

In short, in a plate for tissue culture in 6-well plates were seeded approximately 2.5×10^5 cells per well 24 hours before transfection.

The cells were grown to a confluence of 70-90% in a culture medium devoid of traditional antibiotics and supplemented with FBS. Healthy cells below the confluence are needed for a thorough transfection of plasmid associated with CRISPR / Cas9 KO.

300 microliters of the complex plasmid DNA / transfection reagent UltraCruz were added to the cells which were subsequently incubated for 48-72 hours under normal culture conditions. There was no need to change the culture medium.

Silencing UQCRB gene

Adult MSCs isolated from adipose tissue or bone marrow of mice as described above have been genetically modified by silencing UQCRB gene by miRNA-194 expressed constitutively using the kit Pre-miR[™] miRNA Starter Kit (Life-technologies) in accordance with the manufacturer's instructions.

Transfection of RNA therapeutic under inducible promoter.

Adult MSCs isolated from adipose tissue or bone marrow of mice as described above were genetically modified by silencing PHD-2 gene and UQCRB gene as described above, these have been modified also by means of the induction of the expression of exogenous RNA specific means of the technique the inducible promoter.

Multiplexed shRNA-miRs

When the number of exogenous RNA to transfect was greater than one, shRNA were cloned into the vector-miRs pLVX [Clontech plasmid 631,987 (mCherry) and

631,982 (ZsGreen / GFP)], which were summarized as ultrameri (IDT Technologies) and cloned into vectors pLVX; shRNA were cloned using the restriction sites EcoRI and BamHI, the miRNAs having been positioned downstream of the promoter ef-1 α .

- 5 Using the method of shRNA-miRs in multiplexed, exploiting the flanking regions of 30NT in tandem, one has the processing by the enzyme Drosha / Dgcr8 and the consequent formation of individual exogenous RNA from the primary transcript, thus making possible the expression more exogenous RNA in the same plasmid.

The exogenous RNA were transfected into cells using as a method of transfection
10 CalPhos™ Mammalian Transfection Kit (Clontech) vector cloning them in Mir-X™ Inducible miRNA System TET-ON (Clontech) and inducing them with doxycycline according to manufacturer's instructions.

In short, the sequence of exogenous RNA was cloned using as vector the plasmid PMRI-mCherry (or PMRI-ZsGreen1) using as a method of transfection CalPhos™
15 Mammalian Transfection Kit (Clontech); to select clones that had stably transfected plasmid in their genome was made a selection with G418 (400 mg / ml.), replaced with fresh medium containing G418 every 4 days; after 2 weeks the first resistant clones appeared. Adding to the plate doxycycline (0.01-1.0 mg / ml) MSCs that express the exogenous RNA desired are obtained.

- 20 The exogenous mRNA were transfected into cells using transfection method xfect Transfection (Clontech) cloning them in carrier pCMV-Tet3G and inducing them with doxycycline according to manufacturer's instructions.

In short, the cDNA of interest was cloned into pCMV-Tet3G using xfect Transfection Reagent and later tranfected clones were selected with G418 or (400 micrograms /
25 ml.)

The selection of the transfected cells can be done using a vector with other selection markers other then G418 (geneticin 418) such as thymidine kinase (tk), other aminoglycoside phosphotransferase (neo), hygromycin B phosphotransferase, asparagine synthetase, xanthine-guanine phosphoribosyl transferase; Finally, the
30 selection of transfected cells can be effected by means of vectors encoding for green fluorescent protein (GFP).

In one realization of the present invention was induced the expression of miRNAs anticancer selected from the following group: let-7 ((lung, breast); miRNA-221/222 (glioblastoma); miRNA-181c (stomach); miRNA-31 (breast, stomach, ovary), miRNA-

34b (colon, ovary, glioblastoma); miRNA-200c (breasts) miRNA-107 (colon, pancreas); miRNA-126 (stomach, breasts) miRNA-96 (pancreas); miRNA-196 (pancreas); miRNA-29 (non-breast), miRNA-99a, miRNA-362-3p, miRNA16, miRNA-203, miRNA-34a, miRNA-16, miRNA-15a, miRNA-15b, miRNA- 192/215, miRNA-
5 138, miRNA-106b, miRNA-107, miRNA185, miRNA-194 and miRNA-20a.

In another realization of the present invention was induced the expression of anti-miRNA anticancer selected from the following group: miRNA-210, 9-miRNA, miRNA-21 (lung, colon, liver, esophagus, pancreas, breast, glioblastoma, myeloma), miRNA-21, miRNA-34, miRNA-106a ~ 363 cluster, miRNA-135b, miRNA-221 (breast, lung,
10 liver), miRNA-181b (liver, myeloma), miRNA-200a / b (ovary) , miRNA-10b (breast, esophagus, glioblastoma), miRNA-196 (esophagus, glioblastoma, colon), miRNAs-17/92 and miRNA-30-c.

Adult MSCs genetically modified by means of the silencing of the genes PHD-2 and UQCRB and in which was induced the expression of at least one miRNA and / or of
15 an anti-miRNA antitumor selected from the (those) group (s) mentioned above (the), hereafter defined MSC1.

In another realization of the present invention it has been induced the expression of HIV miRNA chosen from the following group: miRNA-149 (vpr), miRNA-29a, 29b (nef), miRNA-378 (env), miRNA-3245p (VIP) miRNA-23b (NOX4), miRNA-21 (gag) ,
20 miRNA-185 (pol1), miRNA-122 (pol2), miRNA-16-1 (tat) miRNA-20a (env) and miRNA-150 (vif).

In another realization of the present invention was induced the expression of anti-HIV miRNA selected from the following group: miRNA-217 miRNA-34a; miRNA-117 and miRNA-182 (induced tat).

25 Adult MSCs genetically modified by means of the silencing of the genes PHD-2 and UQCRB and in which was induced the expression of at least one miRNA and / or of an anti-miRNA chosen from among the anti-HIV (those) group (s) cited above (i), hereafter defined MSC2.

In another realization of the present invention was induced the expression of
30 miRNAs with anti-ischemic and reperfusion favoring chosen from the following group: miRNA-494, miRNA-499, miRNA-125a, miRNA-214 (p22phox) (CYBA) , miRNA-126 and miRNA-210.

In another realization of the present invention was induced the expression of anti-miRNA with anti-ischemic and / or prevention of reperfusion injury selected from the

following group: miRNA-199a, 92a-miRNA, miRNA-320, miRNA- 920, miRNA-320 and miRNA-21.

Adult MSCs genetically modified by means of the silencing of the genes PHD-2 and UQCRB and in which was induced the expression of at least one miRNA and / or an anti miRNAs with anti-ischemic and / or reperfusion favoring selected from (i) Group (i) above (i), from here on are defined MSC3.

In another realization of the present invention was induced the expression of miRNAs to anti-inflammatory activity selected from the following group: miRNA-214 miRNA-491-5p (NFkB), miRNA-873 (fos), miRNA-429 (jun) and miRNA-1405p (tnf).

Adult MSCs genetically modified by means of the silencing of the genes PHD-2 and UQCRB and in which was induced the expression of at least one miRNA antiinflammatory selected from the above-mentioned group, from here on are defined MSC4.

In another realization of the present invention the expression of miRNAs has been induced to regenerative activity after spinal damage selected from the following group: miRNA- 214, miRNA-491-5p (NFkB), miRNA-140-5p (tnf) (anti-inflammatory), miRNA-125a-5p, miRNA-214 (anti p22phox), miRNA-193a-3p (anti mpo), miRNA-222 (PTEN), miRNA-320, miRNA-21, miRNA-431, miRNA -132 and miRNA-125b.

In another realization of the present invention has been induced the expression of anti-miRNA has been induced to regenerative activity after spinal damage selected from the following group: miRNA-124 and miRNA-138.

Adult MSCs genetically modified by means of the silencing of the genes PHD-2 and UQCRB and in which was induced the expression of at least one miRNA and / or an anti-miRNA to regenerative activity selected from (i) group (s) cited above (i), hereafter defined MSC5.

Insertion of the suicide gene

MSC1-MSC5 prepared as described above were further modified genetically by the insertion of the suicide gene HSV-tk (Herpes Simplex Virus-thymidine kinase gene- Addgene) made by the method Lipofectamine 2000 (Invitrogen.) according to manufacturer's instructions.

In short ,one day before transfection in a 24 well plate were inseminated 0.5-2x10⁵ cells in 500 microliters of growth medium without antibiotic so that at the time of transfection the cells had reached a confluence of 90-95%. Just before preparing the

complex HSV-tk / Lipofectamine 2000 they were inseminated with $4-8 \times 10^5$ cells in 500 microliters of growth medium without antibiotic.

The complexes were prepared by mixing HSV-tk with lipofectamine 2000, and 100 microliters of these complexes were added to each well containing the cells and the means of growth the cells were maintained in medium containing G418 replacing it every 2 days with fresh medium, for a period of 30 days to select the clones that stably received the plasmid containing the gene HSV-tk.

After the successful therapeutic effect, the suicide gene was activated by administering to the patient ganciclovir (GCV), which, becoming phosphorylated by the thymidine kinase (TK) encoded by the suicide gene, blocks the synthesis of DNA and causes apoptosis of said adult stem cells so eliminating them by the subject that had been administered.

Silencing of gene HIF-1alpha

MSC1 prepared as described above were further genetically modified by insertion of miRNA-199a-5p cloned into TET-ON 3G (Clontech) with the gene downstream of a red fluorochrome to verify the successful expression in target tissues.

After the successful migration of the tumor cells to the zones, to achieve the silencing of gene HIF-1alpha, TET-ON was activated by administering to the patient a tetracycline derivative

Having silenced the gene HIF-1alpha the presence of the silenced protein by WB was verified.

Isolation of exosomes

The exosomes were isolated from the supernatant of adult MSCs genetically modified by means of gene silencing PHD-2 and in which had been induced the expression of at least one miRNA to anti-inflammatory activity selected from the group mentioned above.

The isolation of exosomes was performed using the kit EXOQICK (System Biosciences) according to manufacturer's instructions.

Example 1 - Tropism

The increase of the protein HIF-1 alpha and its target following the silencing of the gene PHD-2 was determined in vitro using WB (western blot), and antibody GFP (B-2): sc-9996. In the MSC of the present invention was determined an increase of HIF-1alpha protein by about 67%, of c-Met by approximately 70%, of UPAR of about

76% and CXCR4 by about 78% compared to the values respective MSC in WT (non-GM).

In vivo it has been verified the tropism towards the hypoxic zones using nude mice NU / NU-foxn1nu (Charles River) in which, by methods known in the art, had been induced areas of hypoxia.

A same amount of MSC and MSC WT was administered to different groups of mice through the carotid artery contralateral or ipsilateral respect to the area hypoxic induced.

The mice were sacrificed and samples of hypoxic tissue, for example ischemic were taken . Following the green fluorochrome (GFP) into the tissues through the technique of immunofluorescence. it was found that 24 hours after the administration, the presence of MSCs in hypoxic tissues of mice treated with the MSC of the present invention was increased by about 85% compared to the presence of MSC in hypoxic tissues of WT control mice treated with MSC WT.

From these data it is clear that the MSC in which the PHD-2 gene has been silenced possess an improved tropism both in vitro and in vivo.

Example 2 - Survival

The presence of the protein UQCRB following the silencing of the gene UQCRB was verified in vitro.

This protein was found to be silenced in the MSC of the present invention for 90% compared to WT MSC (not genetically modified).

The survival of MSCs in the present invention was also tested in vitro of after hypoxia and serum deprivation and an increase in survival of about 85% was found compared to the control represented by MSC WT (non-GM).

From these data it is clear that the MSC in which the gene was silenced UQCRB have improved survival in vitro.

Example 3 - Pharmacological activity

- Antitumor activity of MSC1

MSC1 were grown in culture with A549 tumor cells (ATCC CCL-185, lung), mda-MB-468 (ATCC HTB-132, breast) or HCT116 (ATC CCL-247, colon); for comparison were used respectively WT MSC and MSC transfected with vector alone, grown in culture with A549 tumor cells (ATCC CCL-185, lung), mda-MB-468 (ATCC HTB-132, breast) or HCT116 (ATC CCL-247, colon).

At 12, 24 and 48 hours from the start of the coculture, of MSC with the tumor cells of all three cultures the target proteins was determined by immunofluorescent WB or RT-PCR the expression of corresponding to (ai) antitumor miRNA (s) express (i) in MSC1.

- 5 After 24 hours in the cancer cells grown in culture with the MSC1 an average decrease of target proteins of about 1 '85% compared to controls was measured. In addition cancer cells grown in culture with the MSC1 were found to possess an increase of the antiproliferative effect of about 70% compared to the controls, an increase of the anti-invasive of approximately 58% compared to controls, and an
10 increase of the effect anti-migration by approximately 73% compared to controls.

The MSC1 were tested also in vivo.

- Lung ,breast or colon cancer were induced in immunodeficient nude mice nu / nu NCR (Charles River) respectively injecting cancer cells A549 (ATCC CCL-185, lung), mda-MB-468 (ATCC HTB-132, breast) or HCT116 (ATC CCL-247, colon) according
15 to known techniques.

To a group of such mice was injected MSC1 within the tumor. As a control groups of such mice that were injected respectively WT MSC and MSC transfected with the vector alone were used.

- Mice treated as described above were sacrificed at 15, 30 or 45 days and the
20 respective tumors were measured and weighed.

At 30 days after inoculation, in the mice treated with the MSC1 there was a regression of the tumor mass by approximately 80% compared to controls.

In addition there was a decrease of metastases by about 78% compared to controls.

- From these data it is clear that the MSC1 possess antitumor activity in vitro and in
25 vivo.

- Antiviral activity of MSC2.

MSC2 were grown in culture with CD4 + lymphocytes infected with HIV; for comparison were used respectively WT MSC and MSC transfected with vector alone, grown in CD4 + lymphocytes in culture infected with HIV.

- 30 In order to check the blocking of viral genes following administration of MSC2, these were measured using APO-BrdU TUNEL assay or Western blot blocking of apoptosis and expression of the viral proteins.

CD4 + lymphocytes infected with the HIV virus and grown in culture with the MSC2 a block apoptosis by approximately '89% and a decrease in the expression of viral proteins of about ' 85% compared to controls war observed .

These data show that the MSC2 possess antiviral activity in vitro.

5 - Migration, survival and anti-ischemic activity of MSC3

The MSC, taken from bone marrow of male mice C57BL / 6 (Charles River) and genetically modified to give MSC3, was injected into the bloodstream of mice females C57BL / 6 (Charles River).

10 In one experiment, the successful migration of MSC3 in cardiac tissues of female mice, as described above coming from male mice, was tested 7 days after the treatment, highlighting by PCR in real time, the presence of the male sry gene in cardiac tissue of female mice. Alternatively the technique of research of green fluorochrome (GFP) can be used ,by cloning genes into plasmids downstream with the fluorochrome (GFP), and analyzed by immunofluorescence sections of the target
15 tissue.

In another experiment, infarction was induced in female mice C57BL / 6 (Charles River) by blocking the flow of the left descending coronary artery; a group of these mice were treated with MSC3. As a control groups of such mice that were injected respectively WT MSC and MSC transfected with the vector alone were used.

20 Also in this case an increase in the migration and survival of around 80% of the MSC3 compared to controls was observed .

After sacrifice the density of the capillary vessels in the mice, was measured by immunohistochemistry on tissue sections of 5µm with anti-factor VIII (santa cruz) the presence of immature capillaries in the ischemic heart tissue.

25 2 weeks after treatment with MSC3 an increase of angiogenesis by approximately 45% and the tubulogenesis by about 50% compared to controls was found .

These data show that the MSC3 in vivo possess the therapeutic activity desired in addition to possessing an increase of migration and survival.

- Anti-inflammatory activity of MSC4.

30 In order to experimentally induce lung inflammation, female mice were made to inhale endotoxin lipopolysaccharide LPS Pseudomonas aeruginosa (Sigma-Aldrich, catalog number L9143) Balb / C (19-22 g) (Charles River).

After inalation of LPS the presence of inflammatory cells such as neutrophils, macrophages and lymphocytes and inflammatory mediators such as TNFa, IL-1b,

MPO, MMP-9 and fibrinogen in BAL (bronchoalveolar lavage) was measured in these mice .

In all groups of such mice a maximum peak of increase in neutrophils in 24 hours, of macrophages and lymphocytes in 48 hours in 96 hours after inhalation was recorded.

To a group of such mice MSC4 was injected, to another control group only the vehicle. was injected

In the mice treated with the MSC4 a decrease of neutrophils to about 70%, of macrophages by approximately 75% and lymphocytes of about 80% occurred compared to the mice treated with the vehicle only.

Moreover, in the mice treated with the MSC4 after 48 hours from inhalation of LPS a decrease of TNFa by about 98%, fibrinogen of about 97%, of MMP-9 to about 95%, of MPO about 91% and IL-1b by about 61% was verified compared to the mice treated with vehicle only.

These data show that the MSC4 possess anti-inflammatory activity in vivo.

- Activities of regenerative MSC5

MSC 5 were grown in culture with the cell line PC12 (ATCC CRL-1721); for comparison WT MSC and MSC transfected with vector alone, grown in culture on the same cell line were used respectively.

In order to check the growth of neurites and the resistance to inflammation and ROS, following administration of MSC5, axonal growth of PC12 cells and their survival to stress, both of oxidative and inflammatory type were measured.

In PC12 cells grown in culture with MSC5 an increase of about 40% of the axonal growth and an increase of about 65% of survival stress was found compare to the controls.

Furthermore MSC5 were tested in vivo.

A damage spinal cord at T8 was induced experimentally by methods known in mice.

To a group of such mice MSC5 was injected . As a control groups of such mice were injected respectively with WT MSC and MSC transfected with the vector alone.

The mice were filmed for four minutes and their ability to move was evaluated by the system Basso, Beattie and Bresnahan (BBB).

The mice treated with the MSC5 showed an increase in walking capacity by about 57% compared to controls.

These data show that the MSC5 possess regenerative activity in vitro and in vivo.

In total from the above data it is shown that the MSC of the present invention exceed the current limits of cell therapy.

In fact, the MSC of the present invention, in addition to possessing the desired therapeutic activity, possess an increased capacity for migration and survival.

CLAIMS

1. An isolated population of of adult stem cells characterized by the following:

- (I) comprises at least one exogenous RNA,
- (II) has the PHD-2 gene silenced inducibly
- 5 - (III) has the suppression of mitochondrial and/or cellular ROS production

2 The isolated population of of adult stem cells in accordance with claim 1, in which the population of of cells is a population of of adult mesenchymal stem cells (MSCs).

3. The isolated population of of adult stem cells in accordance with claim 1 or 2, wherein at least one exogenous RNA comprising at least one miRNA, siRNA, shRNA, anti-miRNA, and / or mRNA is selected from the group.

4. The isolated population of of adult stem cells in accordance with any of claims 1 to 3, wherein said exogenous RNA is:

- At least one anticancer miRNA chosen from: let-7 miRNA-221/222, miRNA-181c, miRNA-31, miRNA-34b, miRNA-200c, miRNA-107, miRNA-126, miRNA-96, miRNA-196, miRNA-29, miRNA-99a, miRNA-362-3p, miRNA16, miRNA-203, miRNA-34a, miRNA-16, miRNA-15a, miRNA-15b, miRNA-192/215, miRNA-138, miRNA-106b, miRNA-107, miRNA185, miRNA-194 and miRNA-20a, and / or at least one anti-cancer miRNA chosen from: miRNA-210, 9-miRNA, miRNA-21, miRNA-34, miRNA-106a ~ 363 clustered miRNAs -135b, miRNA-221, miRNA-181b, miRNA-200a / b, miRNA-10b, miRNA-196, miRNA-17/92 and miRNA-30-c; and / or
- At least one anti-HIV miRNA chosen from: miRNA-149, miRNA-29a, 29b, miRNA-378, miRNA-3245p, miRNA-23b, miRNA-21, miRNA-185, miRNA-122, miRNA-16-1, 20a-miRNA and miRNA-150, and / or at least one anti-HIV anti-miRNA selected from: miRNA-217 miRNA-34a; miRNA-117 and miRNA-182; and / or
- At least one anti-ischemic and / or reperfusion miRNA with favoring selected from miRNA-494, miRNA-499, miRNA-125a, miRNA-214, miRNA-126 and miRNA-210, and / or at least one anti-miRNA to anti-ischemic and / or reperfusion favoring selected from miRNA-199a, miRNA-92a, miRNA-320, miRNA-920, miRNA-320 and miRNA-21; and / or
- At least one anti-inflammatory miRNA chosen from: miRNA-214, miRNA-491-5p, miRNA-873, miRNA-429 and miRNA-1405p; and / or
- At least one miRNA with regenerative activity, preferably after spinal cord damage, chosen from: miRNA- 214, miRNA-491-5p, miRNA-140-5p, miRNA-125a-5p, miRNA-

214, miRNA-193a-3p, miRNA-222, 320-miRNA, miRNA-21, 431-miRNA, miRNA-132 and miRNA-125, and / or at least one anti-miRNA with regenerative activity, preferably after spinal damage, selected from miRNA-124 and miRNA -138.

5 The isolated population of of adult stem cells in accordance with any one of claims 1-4, wherein the inducible silencing of the gene PHD2 is obtained by knock-out of the gene.

6. The isolated population of of adult stem cells in accordance with any one of claims 1-5, in which the suppression of the production of mitochondrial ROS and / or cell is obtained by:

10 - silencing UQCRB gene silenced; and / or
- Overexpression of SIRT3 gene; and / or
- Modulation of one or more of the following systems, preferably through the use of inhibitors and / or inducers, said systems being selected from: SOD1 / SOD2 / SOD3, Nrf2 / keep1, CAT, Grx, PGC-1 α , FoxOS, Trx, NOX-DUOXs, phox family PRXs, MPO
15 and glutathione peroxidase.

7. The isolated population of of adult stem cells in accordance with any one of claims 1-6 characterized in that it further comprises at least one suicide gene.

8. The isolated population of of adult stem cells in accordance with claim 7, wherein said suicide gene is selected from HSV-tk, and / or caspase-9.

20 9. The isolated population of of adult stem cells in accordance with any one of claims 1-8 further characterized by the fact of having the gene HIF-1alpha silenced.

10. The isolated population of of adult stem cells in accordance with any one of claims 1-9, wherein said cells are isolated from umbilical cord, bone marrow, adipose tissue, muscle, corneal stroma, or dental pulp.

25 11. The isolated population of of adult stem cells in accordance with any one of claims 1-9, wherein said cells are of human or animal origin, preferably mouse, rat, guinea pig or rabbit.

12. A method for obtaining an isolated population of of adult stem cells in accordance with any one of claims 1-11, comprising the steps of:

30 (I) isolating a population of adult stem cells;
(li) inducing in said population of adult stem cells the expression of at least one exogenous RNA;
(lii) inducibly silencing the gene PHD-2, and
(Iv) suppressing the production of mitochondrial ROS and / or cytoplasmic.

13. The method in accordance with claim 12 further comprising an induction phase of at least one suicide gene and / or a phase of silencing the gene HIF-1alpha.

14. Method in accordance with claim 12 or 13, wherein the adult stem cells are of animal or human origin, preferably isolated from umbilical cord, bone marrow, adipose tissue, muscle, corneal stroma, or dental pulp, preferably are mesenchymal stem cells (MSC) adult.

15. Method in accordance with any one of claims 12-14, wherein the step (ii) is performed:

- By use of a vector, preferably a plasmid, preferably PMRI-mCherry, PMRI-ZsGreen1 or pCMV-Tet3G, or a virus;

- By use of an inducible promoter, preferably by chemical means, preferably calcium phosphate, DEAE-Dextran, liposomes, or by physical methods, preferably electroporation, microinjection or by particle bombardment;

- By transfection, preferably using the plasmid PMRI-mCherry or PMRI-ZsGreen1, and the technique of the inducible promoter by means of system TET-ON.

- Through the formation of a knock-out or by introduction of at least one particular miRNA or siRNA (RNA interference) .

16. A method in accordance with any one of claims 13-15, wherein the suicide gene is introduced into the cell by means of the technique of liposomes, electroporation, or calcium phosphate, preferably with lipofectamine.

17. Method in accordance with any one of claims 13-15, wherein the silencing of gene HIF-1alpha is carried out by insertion of at least one miRNA or siRNA or through the formation of a knock-out system.

18. A pharmaceutical composition comprising an isolated population of adult stem cells in accordance with any one of claims 1-11 and at least one pharmaceutically active substance.

19. Exosomes produced from the isolated population of adult stem cells in accordance with any one of claims 1-11.

20. Population of isolated adult stem cell in accordance with any one of claims 1-11, or pharmaceutical composition in accordance with claim 18, or exosomes in accordance with claim 19 for use as medication .

21. Population of isolated adult stem cell in accordance with any one of claims 1-11, or pharmaceutical composition in accordance with claim 18, or exosomes in

accordance with claim 19 for use in the treatment of a tissue injury, a degenerative disease, an inflammatory disorder, immunological, neurological, bacterial infection .

22. Population of isolated adult stem cell in accordance with any one of claims 1-11 for use, or pharmaceutical composition in accordance with claim 18, or exosomes in
5 accordance with claim 19 in gene therapy, preferably of viral diseases and tumors.

23. isolated population of adult stem cells, or pharmaceutical composition, or exosomes in accordance with claim 21 wherein the tissue damage is ischemia or myocardial infarction.

24. Population of isolated adult stem cell, or pharmaceutical composition, or
10 exosomes in accordance with claim 22 in which the tumor is selected from: cancer of the bladder, breast, colon, kidney, liver, lung, small cell lung cancer, esophagus, gall bladder, ovary, pancreas, stomach, cervix, thyroid, prostate and skin, squamous cell carcinoma, hematopoietic tumors of lymphoid lineage preferably acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma,
15 Hodgkin's disease, lymphoma Non-Hodgkin's lymphoma, hairy cell lymphoma and Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, preferably acute and chronic myelogenous leukemias, myelodysplastic syndrome and promyelocytic leukemia, myeloma; tumors of mesenchymal origin, preferably fibrosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, preferably
20 astrocytoma, neuroblastoma, glioblastoma, glioma and schwannomas; melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderma pigmentosum, keratoxanthoma, follicular thyroid cancer and Kaposi's sarcoma.

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2016/050185

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113
ADD.

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)

C12N

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 practicable, search terms used)

EP0-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.



See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2016/050185

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INTERNATIONAL SEARCH REPORT

International application No

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

International application No

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