Review Article

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Mesenchymal Stem Cells X Lateral Amyotrophic Sclerosis - Las

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ABSTRACT

The mesenchymal stem cells can be expanded ex vivo and are capable to differentiate in several lineages, represent a rare subpopulation of the bone marrow cells (< 0, 01% of the mononuclear cells of bone marrow) with mitotic expansion capacity in vitro. As a result of the facility in dividing and proliferate, we concluded that the mesenchymal stem cells would be responsible for the maintenance and renovation of adult mesenchymal tissues. This cell type presents as one of its virtues a considerable immunomodulatory activity, avoiding any adverse effects. We believe that, overcoming some challenges in its isolation, preparation and infusion mode, due to these characteristics, the MSC represent promising tools for cell therapy, mostly the ones associated to bone marrow transplantation. The amyotrophic lateral sclerosis (ALS) still is a disease of unknown cause nowadays, despite knowledge of many of its aspects have advanced since Charcot's epoch. Many theories were proposed for its pathogen; however, none of them has showed unifying until now. These would have to explain the different degrees of incidence in several geographic areas, the distinct forms of clinic presentation, time of evolution, as well as the vulnerability and resistance of determined neuronal populations. It is possible that we are in front of many anatomical-clinical entities of multiple etiologies, which are presented in a similar form. This work showed that MSC cultures can be obtained starting from samples of bone marrow of healthy donors through standard of processing protocols, cultivation and characterization in vitro, and even of autologous donors.

Keywords

Mesenchymal Stem Cells, Amyotrophic Lateral Sclerosis.

Mesenchymal Stem Cells

In bone marrow, the maintenance of Hematopoietic Stem Cell (HSC) and the regulation of its auto renew and in vivo differentiation depends on the specific microenvironment to which it is submitted, historically known as "hematopoietic stem cell niche" [1]. The stem cell, by definition, are undifferentiated cells, capable of auto renew by means of asymmetrical division [2]. The main characteristics of stem cells, making them extremely interesting, are their capacity of auto renew, that is, they are capable of multiply, maintaining their undifferentiated state, providing an active reposition of its population in a constant mode in tissues; and, its potential capacity of differentiate in many cell types of the same tissue and, apparently, of different tissues [3]. The stem cells are divided in two big groups that refers to its place of origin: can be embryonic stem cells (ESC), when they're derivatives from the intern cell mass of embryonic blastocyst; and adult stem cells (ASC) which are the ones obtained in umbilical cord blood, from bone marrow and peripheral blood; additionally, we believe that there are stem cells for the tissues or specific organs all over the adult organism [4].

In humans, the embryonic stem cells are originated from embryos of eggs fertilized in vitro and donated for purpose of research with the consent of donors. They are not derivatives of the fertilized eggs present in a woman's uterus. The embryos which the human troncoembryonic-cells are derivatives have typically four or five days (blastocyst), and have three structures: the trophoblast, which is the layer of cells that surround the blastocyst; the blastocoels, which is the hollow cavity inside the blastocyst, and the cell's inner mass, which is a group of approximately 30 cells in a extremity of the blastocoels. The first ASCs studied and well characterized were the hematopoietic cells originated from bone marrow. This cells are capable of differentiate in myeloid constituents and blood lymphoid and long ago have been successfully used in transplants for patients with medullar failure or cancer [5]. With time, it

was made the isolation of another type of adult stem cell, also constituent of bone marrow, although with different properties of the hematopoietic: the mesenchymal stem cells (MSC) or stromal stem cells. They receive this denomination because they're derivatives from the intermediate embryonic leaflet, the mesoderm, which is responsible for constructing the osseous, cartilaginous and adipose tissues [6]. The mesenchymal stem cells represent a rare subpopulation of stem cells from bone marrow (< 0, 01% of mononuclear cells of bone marrow) that can be mitotically expanded in culture medium. Due to the facility in dividing and proliferating, we concluded that the mesenchymal stem cells would be the cells responsible for maintenance and renovation of adult mesenchymal tissues included the cardiac muscle [7]. Thus, until now, these cells represent the most promising source for regeneration and repair of several cell tissues [8]. Mesenchymal stem cells, the MSC are considered multipotent cells, not hematopoietic with properties of auto renew and capacity of differentiation in mesenchymal tissues and, maybe, not mesenchymal [9]. The first report of MSC was performed by the Russian researcher Friedenstein and his collaborators, in the decade of 1970 [10].

He described the isolation, in bone marrow, of clonogenic cells adhering to the substrate, in form of spike, in monolayer cultures, which were defined as Colony Forming Unit Fibroblast (CFU-Fs). The adherent cells were heterogeneous and stayed inactive for two to four days, and then started to quickly proliferate. After many passages in culture, the adherent cells became more homogeneously fibroblastoids in appearance. These cells are now denominated as mesenchymal stem cells, because of the ability to differentiate in mesenchymal cells, or stromal cells from bone marrow, because they seem to originate from the complex of support structures found in the medulla. The initial observations of Friedenstein served as basis for further studies, as the ones by Owen, in Great Britain, showing that the stromal cells in bone marrow are the common precursors of the mesenchymal tissues. As a result of the capacity of auto renew and differentiation, the stromal cells of the bone marrow were considered stem cells by Kaplan and denominated mesenchymal stem cells [11]. The MSC, especially the ones originated of the human bone marrow, differ significantly from the nomenclature used. Initially were called CFU-F (Colony Forming Unit Fibroblast), due its capacity of adhering to the plastic of cultivation bottles and form colonies of cells similar to fibroblasts [12].

Also, it was denominated as stem cells or mesenchymal progenitors, because it differentiate in a great variety of nonhematopoietic cells [13], and stromal cells of bone marrow, because it seems to originate from the support structures of bone marrow and could be used as feeder layer for the growing of HSC in culture [10]. The MSC became a therapeutic attention focus because of its immunomodulatory potential [14], although the mechanisms of immunosuppressant on the inflammatory response and on mechanisms of transplant rejection are not fully elucidated [15]. Through direct contact of the MSC to a tissue (allogeneic or autologous) or through the paracrima interaction with interferon-gamma (INF-), produced by immune cells of organism, the MSC unleash the liberation of several soluble factors, that will act on the cells of the immune system (lymphocytes and antigen-presenting dendritic cells – APC) [16]. Among these factors, are the prostaglandins (PGE2), the interleukins (IL-4, IL- 6, IL-10), the transforming growth factor beta (TGFß), the heptode growth factor (HGF) and the Indoleamine 2,3 -dioxygenase enzyme (IDO) [17].

Several further studies reported the multipower of these cells, in other words, the capacity of differentiate in cells derived from embryonic mesoderm: the osteocytes, chrondroblasts and adipocytes [18]. In the human being, the marrow bone is the most known source of CTM, but also was already isolated from other organs and tissues, such as skeletal muscle tissue and dermis [19], adipose tissue [20], synovial membrane [21], endothelium of the umbilical vein [22], and of the saphenous vein [23], kidney [24], umbilical cord and placental blood [25], marrow bone [26], articular cartilage [27], periodontal ligament [28] and lung [29]. The mesenchymal stem cells (MSC) are considered a lineage of somatic stem cells and are present in perivascular regions of all adult tissues, in small quantities, included the marrow bone (MB), the adipose tissue, the periosteum, the muscular tissue and the parenchymal organs [30]. The MB constitutes one of the main donor sites of these cells, as well as of hematopoietic and endothelial stem cells [31]. The MSC are characterized as a multipower population of cells, capable of differentiation and produce any cellular type necessary in a process of reparation, such as osteoblasts, chondroblasts, hepatocytes, neurons, epithelial cells, renal, cardiac, among others [32]. Such characteristics of plasticity suggest that this cellular type is the responsible for the turnover and for the maintenance of all tissues of organism [33]. They became the focus of innumerable researches all over the world for providing promising clinical perspectives for cell therapy. The mesenchymal stem cells, due to its easy isolation and cultivation, potential of differentiation and produce of growth factors and cytokines, have been becoming the ideal candidates for the regenerative medicine protocols [34]. As a proof, some studies using MSCs in clinical rehearsals shows that billions of MSCs isolated or bounded to biomaterials are necessary. For that, the production of MSCs need observation and adherence to good manufactures practices, to ensure the liberation of this "cellular medicine" in a safe, reproducible and efficient mode [35]. Therefore, there are many areas in medicine using stem cells, between then:

Cardiology

The coronary disease and cardiac insufficiency are responsible for the greatest index of morbidity in occidental society, with a high rate of mortality [36]. There are many diseases which can affect the good functioning of cardiac muscle: coronary diseases [37], myocardial infarction [38], Chagas disease [39], and other myocardiopathies [40]. Several cell lines like embryonic stem cells, nuclear cells of marrow bone, myoblasts, endothelial and mesenchymal progenitors and cardiac stem cells were used with varied effectiveness and safety in pre-clinical and clinical rehearsals, in the treatment of acute myocardial infarction and

chronic heart failure [41], showing an improvement of the cardiac function after the cellular therapy [42]. However, some experiments show an inability in forming cardiomyocytes and establishing electric stimulation conducting joints, which can lead to reentrant ventricular tachycardia [43].

Orthopedics

In orthopedics, the main therapeutic goal is the osteoporosis. The stem cells would be used to repopulate the bone with new and more functional cells [44]. In joint injuries with loss or deformation of cartilage discs, studies show an improvement of joints by the infusion of chondrocytes, or by reconstruction in vitro using scaffolds [45]. Besides the evidences that the MSCs can Trans differentiate in multiple cellular types, in vivo, the real contribution of MSCs to the tissue reparation is not clear. The lack of consistent in vivo Trans differentiation can be a result of the limited number of mesenchymal precursor cells derived from non-mesodermal embryonic lines, as it was recently indicated by the quick decrease in the number of MSCs of neuroepithelial origin in the adult bone marrow. Maybe, in the postnatal life, the relative importance of the MSCs from other embryonic origins decrease by the increase of the importance of the mesodermal MSCs. Therefore, besides that it has been proposed that the MSCs could be used for the regeneration in any tissue, the evidences show that the use of these cells in reconstruction exclusively through differentiation mechanisms would serve only to bone repair. Like in orthopedics, the stem cells would be used in rheumatology to help the organism to develop a new cartilage in diseases such as systemic lupus erythematous or rheumatoid arthritis, for the reestablishment of normal functions of the affected tissues [46].

Urology and Nephrology

In urology and nephrology, the cell therapy would be used in treatment of acute renal insufficiency with loss or dysfunction of renal tubule epithelial cells [47], whose proposal would be the repopulation of the area affected by the remaining cells, which presents a high proliferative capacity.

Dermatology

In dermatology, the stem cells have been evaluated in epithelial replacement of burn areas, in healing of chronic wounds and in treatment of vitiligo, and others [48].

Oncology

One of the main targets of researches, already in development with stem cells, we find in oncology and, mostly, onco-hematology [49]. The usage of stem cells, in these cases, would be related to the reconstruction of tissues. Among the many researches protocols registered in FDA, the majority is related to hematological cancers, probably because most of the studies with stem cells were, until recently, performed with cells originated from marrow bone; therefore, adult stem cells believed to have a limited ability to differentiate into other cell types. Of the research protocols registered in the FDA and participating in the database provided by the NIH bank, 76,1% of them are related to cancer, and of these, 70% are hematological. Of the protocols which define the number of individuals participating of the study, it was possible to verify that most of them participate of researches involving the study of stem cells in cancer (85,5% of individuals). The clinical applications of CD34 + stem / progenitor cells consist of either allogeneic transplantation (hematopoietic malignity, immunodeficiency, bone marrow aplasia, metabolism diseases, hemoglobinopathies) or autologous (acute leukemia, myelomas, lymphomas, breast and ovarian cancer, germinative cell tumor, autoimmune diseases) [50].

Neurology

In neurology there are many proposals. One of them is the reparation of damage in spinal cord through the replacement of neural cells, reversing cases of paralysis. In case of implantation of stem cells, with differentiation in motor neurons, it would be possible to obtain the reversion of the paralysis case51 . Another neural disease is the stroke, that, depending on its dimension or brain area affected and the kind of lesion, hemorrhagic or ischemic, can also lead to an incapacitating paralysis, generally a hemiplegia. The stem cells would be infused in the injured area in the brain, leading to a repopulation of the necrotic tissue and possibly, an improvement in motor incapacitation [52]. In amyotrophic lateral sclerosis, for the generation of a new neural tissue, along the spinal cord [53]. In Parkinson's disease, a neurodegenerative disease caused by the progressive degeneration and loss of dopamine producing neurons, leading to the arising of characteristic tremor the disease, rigidity and abnormal decrease of mobility [54], the cellular therapy would be indicated for reposition of these dopamine producing neurons, which would lead to an improvement of the general disease situation, turning it less incapacitating55 . In other neurodegenerative disease, the Alzheimer's disease, the stem cells can become a part of healing by the reposition of brain cells [56].

Endocrinology

In endocrinology, the main target of studies is diabetes. In some types of diabetes it would not be indicated the using of stem cells, as resulting of obesity. However, when the diabetes occurs due to the lack of insulin, the cellular therapy, through the provision of new insulin producing cells, could be the solution [57].

Amyotrophic Lateral Sclerosis - ALS

The Amyotrophic Lateral Sclerosis (ALS) was described in 1874 by Charcot [58]. It is a disease characterized for the progressive degeneration of motor neurons [59]. The term amyotrophic is related to muscular atrophy, weakness and fasciculation, which are indicatives of impairment of the lower motor neuron. The clinical findings of hyperreflexia, Hoffman sign, Babinski sign and clusters are related to the impairment of the higher motor neuron [58]. The ALS, also known as Lou Gehrig's disease, is a neurologic disease that causes progressive paralysis in practically all skeletal muscles, compromising the limbs' motility, the speech, swallowing and even the breathing, it is fatal. The patients frequently live from three to five years after the beginning of symptoms. There isn't, in general, any compromising of consciousness or intelligence. The symptoms are: weakness or muscular cramps in the limbs; spontaneous muscular contractions (fasciculation) in body; limbering; problems

in speaking, difficulties to swallow. Amyotrophic Lateral Sclerosis (ALS) is one of the main neurodegenerative diseases, next to Parkinson's and Alzheimer's diseases. Its incidence in populations varies from 0,6 - 2,6 by 100.000 inhabitants [60]. The age is the most important predictor factor for its occurrence, being more prevalent in patients with ages between 55 to 75 years old [61].

It is a progressive disturb that involves the degeneration of motor system in many levels: bulbar, cervical, thoracic and lumbar [62]. The clinic situation of ALS reflects the loss of neurons of motor system – from the cortex to anterior horn of the spinal cord. Physical signs of this disturb involves findings in superior and inferior motor neurons. Sensitive dysfunction is incompatible with the diagnosis of LAS, unless it is part of an underlying disturbance. The physical findings are related to the different topographies of motor nucleus degeneration: bulbar, cervical or lumbar [63]. The Amyotrophic Lateral Sclerosis (ALS) is a rare disease, but still affects an estimated 12,000 patients in Brazil. Both those who receive this diagnosis and their relatives have difficulties in access trustable information about the disease – the material in Portuguese language is especially scarce. The same obstacle repeats, in many times, for the health professionals that only eventually receive patients with ALS [64].

The amyotrophic lateral sclerosis is a motor neuronopathy characterized by the involvement of cell body of lower motor neurons (present in the brainstem and in the anterior horn of the medulla), accompanied or not by of the involvement of superior motor neuron (present in the motor area). Symptoms and characteristic signs manifest accordingly to the topographic site of involvement, allowing a better clinical and etiologic characterization. Superior Motor Neuron Dysfunction (SMN) – weakness, live tendon reflexes, presence of abnormal reflexes. Inferior Motor Neuron Dysfunction (IMN) – weakness, fasciculation (small involuntary located muscular contractions), atrophy (loss of muscular tissue), atony (loss of muscular tonus). Dysfunction of motor neurons of the brainstem – dysphagia (difficulty in swallowing), dysarthria (speaking disturb). The ALS is characterized by progressive paralysis, marked by signs of SMN compromising (hiperreflexia, over activity or reflex response, clonus – involuntary muscular contractions and Babinski sign - reflex that is measured in the plant and toes), and of IMN (atrophy, fasciculation).

The case initiates with easy weakness and, not infrequently, cramps generated by effort. Following, weakness and fasciculation are associated in the limbs or in the bulbar musculature (distony, dysarthria, dysphagia). Overtime, apart from the beginning, the weakness becomes universal. The signs of compromising of SMN are characteristics and are present in almost every case. Extrinsic ocular motor and sphincters are more resistant to aggression and are relatively preserved, even in the final stages of disease. In most of cases, the cognitive functions of patient still completely intact, besides the devastating effect that occurs in the body. The final stage of the disease is dramatic: the patients stay in bed, without movements, with breathing maintained by artificial respirator, feeding through enteral catheter and with communication injured, sometimes only achieved using eye movement. Although ALS is considered a disease of rare incidence, with about two to three cases per 100,000 people per year and prevalence of six to eight per 100,000, the illness represents a great personal and socioeconomic impact for the individual and society. Assuming that a complete family is constituted by three generations, that every individual has two kids and the period of every generation is of twenty years, we calculate that one of every two hundred subjects has a familiar member affected by ALS. Excepting a small focus in Occidental Pacific, in Guam Island, the greatest of Marianas' Islands, in which the population of Chamorro has a prevalence of fifty to a hundred times greater, the frequency is similar all over the world. Apparently, the attendance has increased uniformly and doesn't seem to be related to the better ability of doctors in recognizing the disease. In a more general form, in the study of ALS we verify that the male gender is more compromised than the female gender – in a proportion of three to two – and the white are more affected than black, with mean age of onset of symptoms of 55 to 60 years (a little more precocious in men).

About 4 to 6% of the affected cases are people with less than forty years. The sporadic form (non-genetic) is the most common form of this disease, accounting approximately 90% of all cases. The ALS still represents a great enigma for researches, and its cause remains not fully understood. We believe that its etiology has many factors, caused by exogenous or genetic factors, having glutamate excitotoxicity a direct relation with the degeneration of motor neurons. The ALS is characterized by progressive paralysis, marked by signs of SMN compromising (clonus and Babinski sign) and of INS (atrophy and fasciculation). It is the most common form of motor neurons diseases and, for that, frequently, the term ALS is indistinctly used for other forms of MND. The predominant involvement is the musculature of the limbs (mostly superior limbs), followed by bulbar compromising, usually asymmetric. In many times, preceding or following the installation of symptoms, the patients complain about cramps. Weakness, atrophy and fasciculation in limbs are the more prominent clinical signs. Later, the vocal and respiratory functions are affected. The cranial nerves, which control the vision and ocular movements, and the lower sacral spinal cord follow-up, which control the sphincters, are not usually affected. The diagnosis of ALS requires the demonstration of signs of involvement of the superior motor neuron (weakness and pyramidal release signals), along signs of compromising of the inferior motor neuron (atrophy, fasciculation). The diagnosis is based on clinical aspects, having the electromyography as fundamental exam for the characterization of diagnosis. Other subsidiary exams are performed to rule out other clinical diagnoses that may mimic ALS. Until now, there is any exam that is a definitive marker of ALS. Several exams were presented with a potential good diagnosis marker or for disease follow-up, allowing distinguish predominant compromising of SMN or INM.

The clinical diagnosis of ALS is probably correct in more than 95% of the cases. This can be aided by the electromyography for research of impairment of the inferior motor neuron and nuclear magnetic resonance with spectroscopy and magnetic stimulation

of the cortex that investigates the involvement of the upper motor neuron [58]. The ALS diagnosis is evident in patients with long evolution of the disease and generalized signs and symptoms. The precocious diagnosis of disease, when the patient has only focal symptoms in one or two regions (bulbar, superior limbs, body or inferior limbs), can be difficult and depends of the presence of signs in other affected regions and of several serial investigations [65]. The average time of the beginning of symptoms until the confirmation of diagnosis is approximately 10-13 months [66]. The diagnosis of ALS is done based in the presence of signs of compromising of superior and inferior motor neurons concomitants in different regions. The criteria of El Escorial classify the diagnosis in several subtypes [67].

Definitive ALS

Signs of superior motor neurons (SMN) and inferior (IMN) in three regions (bulbar, cervical, thoracic or lumbosacral).

Probable ALS

Signs of SMN and IMN in two regions (bulbar, cervical, thoracic or lumbosacral) with any sign of rostral SMN to IMN signs.

Probable ALS with laboratorial support

Signs of SMN and IMN in a region or signs of SMN in one or more regions associated to evidence of acute degeneration to electroencephalography in two or more segments.

Possible ALS

Signs of SMN and IMN in only one region.

Suspicious ALS

Signs of SMN in one or more regions (bulbar, cervical, thoracic or lumbosacral). Signs of IMN in one of more regions (bulbar, cervical, thoracic or lumbosacral).

In all modalities, must be an evidence of progression of the disease and absence of sensitive signs. The cause of Amyotrophic Lateral Sclerosis is not completely clarified. The epidemiological work and, mostly, the experiments with animal models, have been allowing to conclude that the disease is related to the presence of some genetic factor and its clinical expression would be related to the exposure of this individual, genetically marked, to any factor, or factors, that would act as a trigger for the unleashing the process of motor neuron degeneration (epigenetic factor).

Stem Cells

The use of stem cells as an attempt at reparative or regenerative therapy has been described as one of the most promising therapies. The isolating of the stem cells from embryonic cells or from fetal tissue, expanded in cultures, allows its differentiation in neurons and in glial cells. However, until now, the therapy with simple administration of stem cells in the locals where are cellular degeneration has not been successfully. The neurons of great projection, such as the ones involved in ALS and Parkinson's disease requires not only a regional repopulation, but also an installation of the synapse network. For the survival of these new administered cells and for the formation of the network is necessary the regional presence of innumerous neurotropic substances, highlighting GDNF (neurotropic factor derived from Glia), IGF (growth factor insulin-like), VEGF (growth factor of vascular endothelium). It is not enough; therefore, only putting the cells, we have to learn how to regionally liberate these neurotropic substances.

Casuistry

Female patient, J.F.S., 29 years old, came for the first time in the Gerobasso clinic with the diagnosis of Lou Gehrig Disease (confirmed by the neurologist who accompanied her, after electroneuromyography MMII), concomitant injury of lower motor neuron with preserved sensitive part.

Presented the face of Cuchung for prolonged use of corticoid (METICORTEN 20MG, morning and supper) and TEGRETOL 200MG (morning and supper) + OMEPRAZOL 20MG (morning and supper) + DOMPERIDONA 160MG (morning and supper).

In the physical exam, she presented normotensive with cardiac frequency 147 BPM (taquisfigmia) resting. Heart auscultation without alterations or bloating. Clean lungs with presence of vesicular murmur without adventitious sounds. Flabby abdomen to palpation, without Blumberg sign. We observed muscle hypotonia of arms and legs; patient doesn't walk (handicap), impossibility of executing fine tweeze movements.

There is no possibility of swallowing solids and presents difficulties to swallow liquid and pasty foods. Prejudiced speech.

To the laboratorial exams, presents:
Hypothyroidism, Hypoproteinemia
Total CPK higher than 1200
PTH = 84
Vit D3 =10,2

To the image exams: Bone Densitometry compatible with osteopenia and Magnetic Resonance of Hip compatible with femoral head necrosis with diagnosis hypothesis of prolonged use of corticoid (clinical studies on).

1º Implant of MSC with collect of stromal material from bone marrow of the sternum. In total, it was performed six (06) implants with cellular therapy; MSC/Growth factors like described below.
In the present, the patient presents complete normality in all aspects covered.

Method for the Implant of Stromal Cells

We prepared 44 ml of PRP - Platelets Rich Plasma, collected from superior vein of the own patient. We collected 36 ml of stromal material from bone marrow of the own patient, which was mechanically activated, chemically with growth factors, and also activated with low frequency laser. The growth factors were obtained with the using of PRP – Platelets Rich Plasma of type O negative blood, homologous – heterologous from classified donor,

through ultra-centrifugation in tubes with retention filter. It was added specific cellular inducer factors to the blend with the three components above. The product obtained was applied in patient by peripheral intravenous route, intrathecal and pedidoral.

Producing of Growth Factors, Interleukins and Cytokines

The CTMs produce a large number of cytokines, among them we can quote the LIF (leukemia inhibitory factor), the SCF (stem cell factor), SDF-1 (stromal derived factor), the oncostatina M (OSM), the BMP-4 (bone morphogenetic protein-4), the ligate Flt-3 of tyrosine kinase (Flt-3 lig) and the TGFβ.

It also produces a great variety of interleukins (IL), such as: IL-1, IL-6, IL-7, IL- 8, IL-11, IL-12, IL-14 e IL-15. When cultivated in presence of IL-1α, the MSCs produce cytokines that acts in the hematopoietic progenitors more mature, such as TPO (thrombopoietin), the GM-CSF (granulocyte macrophage colony-stimulating factor), the G-CSF (granulocyte colony stimulating factor), the M-CSF (macrophage colony stimulating factor) [68,69].

Besides, the MSCs express receptors (R) for several cytokines and growth factors, such as: IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIF-R, SCF-R, G-CSF-R, INFγ-R (interferon γ receptor), TNF1-R e TNF2-R (tumor necrosis factor receptor), TGFβ2-R, TGFβ3-R, bFGF-R(basic fibroblast growth factor receptor), PDGF-R (platelet-derived growth factor receptor) and the EGF-R(epidermal growth factor receptor) [70].

This data suggests that the MSCs contribute to the formation and functioning of the stromal microenvironment, which produces regulatory inductor signs not only for the MSCs, but also for the development of the hematopoietic progenitors and other stromal cells present in the bone marrow [71].

Stromal Vascular Factor

The stromal cells of the bone marrow were primarily described as bone progenitor cells present in its stromal fraction. Subsequent studies demonstrate that these cells possessed the capacity of differentiate in mesodermal cellular lineages, including chondrocytes, osteoblasts, adipocytes and myoblasts. Based in this capacity of differentiation in several lineages, Kaplan, in 1991, introduced the term mesenchymal stem cell [13]. The stromal tissue of the bone marrow (BM) of adult mammals was traditionally examined, basically, on its well documented role in supporting the hematopoiesis, in other words, a tissue which the main function is provide a microenvironment inside the BM that supports the proliferation, the differentiation and maturation of hematopoietic stem cells in each one of the eight distinct lineages that compose the hematopoietic system. In contrast, the possibility of the stroma from BM to be based in a model of stem cell and contains multipower stromal cells, with capacity to originate each one of the lineages of differentiated cells found in medullar stroma, in bone, and cartilage, has been receiving little attention.

However, with fast progress in this area, it is clear now that the progenitor cells/trunk of skeletal and stromal tissue of BM, unequivocally exists in multiple mammal species, and that the BM must be seen as an organ that covers at least two distinct populations of adult stem cells, which many progenies coexist in an interdependent functionally manner [72]. We also believe that there is the presence of a layer of stromal cells that would sustained to hematopoiesis In the aorta-gonad-mesonephros region in murine, that the fetal human blood contains, during the first weeks, great quantity of HSCs and of stromal cells and occurs an encounter of fetal cells in circulation and in maternal tissues, even decades after the pregnancy, that seems to participate in the tissue repair. In combination, this observations support the hypothesis of the existence of a multi or pluripotent SC that exists since the early gestational age and can persist for the entire adult life [73]. Of peripheral blood, still it's an open question [74], reported the presence of "stromal" cells in mobilized peripheral blood. The studies of Zvaifler [75], demonstrate that the MSC circulates in peripheral blood, but it is extremely rare. Another study by Kassis [76], with the help of fibrin microspheres, isolated the MSC of mobilized peripheral blood, collected by apheresis, of normal donors [76].

Pericitic Cells

The pericitic (peri = around; cito = cell) were described more than 100 years ago as perivascular cells that involve the microvassels (arterioles, venules and capillaries) [77]. It were also called Rouget cells, or referred as mural cells or, because of its contractile fibers, as smooth muscle cells (SMC) vascular [78].

The first analysis by transmission electronic microscopy revealed the ultrastrutural characteristics of pericitic cells. In general, pericitic has a stellar format, cellular body with prominent nucleus and limited perinuclear cytoplasm, from which several cytoplasmic processes extend, involving the wall of the abluminal endothelium [79]. These cells are adhered in micro vessel basement membrane, which is formed both for pericitic and endothelial cells (EC) [80].

The pericitic not only serve as a framework, as historically thought; it can communicate with the EC by direct physical contact and paracrine signaling pathways. Connections of type "gap" provide direct connections between the cytoplasm of pericitic and EC, allowing the exchange of ions and small molecules. The adhering plaques anchor the pericitic to ECs, while the contacts "peg-and-socket" allows the cells to penetrate through the discontinuities of the basement membrane of vessels, touching each other [81]. These junction complexes support the transmission of contractile mechanical forces from the pericitic to the endothelium and contain N-cadherin, molecules of cellular adherence, adherent junctions based in β-catenin, and molecules of extracellular matrix (ECM) such as fibronectin [82].

The pericitic exhibit long cytoplasmic processes, which not only can contact numerous EC, and integrate signs along the vessels length, but also can extend for more than one capillar in vasculature [83]. Interestingly, contacts cell to cell seems necessary for the activation of the transformation growth factor β1 (TGF- β1), that

induces the differentiation of pericitic in vitro [84], sustaining the notion that the direct cellular contact is a crucial communication tool for the formation and maintenance of vessels. Pericitic also exhibit a number of characteristics consistent with activity of muscular cell, such as expressing smooth muscle contractile actin, besides the involvement in vessels maturation [85].

The challenges in molecular defining the pericitic have not being easy due the fact that a specific marker for this cellular type has not yet been found. There is, however, some molecular markers that are present in the pericitic, although not exclusively, and are commonly used in its detection [86].

The ganglioside membrane 3G5 is expressed in the surface of several cellular types, including cells of pancreatic islets, follicular thyroid cells, renal glomerular cells and pericytes [87], demonstrated that the monoclonal antibody 3G5 is a marker of micro vascular pericitic in vitro and in vivo. The expression 6 of this marker also was reported in pericitic cultivated in retina and heart [88]. Because of its expression mostly in cells that has membrane processes, this ganglioside can be involved in regulation and maintenance of cellular form [89].

The CD146 is an identified member in the gene of immunoglobulin superfamily and was previously called by different names, due its identification in different tissues, by several groups of independent researches. The synonyms of CD146 include MUC18, the antigen of A32, MCAM, MEL-CAM, and S-Endo-1. Currently, it is used as a marker of mesenchymal progenitors and pericitic [90].

The pericitic has been associated mostly to processes of stabilization and hemodynamic of blood vessels. Its functions are, however, much more diverse. It can, under angiogenic stimulus, guide tubes of budding, Eliciting endothelial survival functions, and even exhibiting macrophagic activities [91].

Conclusion

The MSCs have a potential of multi lineage differentiation and can be directed to grow and differentiate in specific cellular lineages in certain conditions of microenvironment. These characteristics make these cells have great potential in various therapeutic applications, such as participate in the tissue regeneration, correct hereditary disturbs, curbing chronic inflammation, releasing biological agents. Thus, the administration of MSCs is promising as a new strategy for treating a wide range of diseases. However, the mechanisms that direct the MSCs for a determined lineage are, in great part, unknown. This situation makes the clinical applications and stem cells therapeutics still uncertain, unless that critical knowledge can be obtained, relative to several aspects, such as the signs that control survival, proliferation and differentiation, which will expand the potential therapeutic applications of these cells. In short, the promise of regenerative and curative medicine based in stem cells and, particularly, in MSCs, depends critically on the identification of mechanisms and of molecules that control and mediate the differentiation of a determined specific lineage, the establishment of these cells in determined tissue of interest, and the signaling cascades that control cell survival and proliferation. The cellular therapy with MSC is a promising therapeutic alternative, because the differentiation of these cells in mesodermal and non-mesodermal tissues broads the possibilities of clinical usage. Besides, the recent discoveries about the immunological behavior of MSC increased the therapeutic perspectives with the use of these cells by the possibility of allogeneic treatment. However, this possibility must be considered with enthusiasm and caution, since the immunomodulatory molecular mechanisms used by MSC still remain not fully understood, and the biology of MSC is still a science in formation.

The comparison between the proteomic profile and in vitro inhibition of lymphocyte proliferation in MSC derivate from different donors showed the standardization of majority protein expression, even after only one passage in culture. This similarity encourages the use of MSC in therapeutic protocols in a form independent of the compatibility of its HLA with the receptor.

This work also showed that even not very sensitive proteomic methodologies, such as two-dimensional electrophoresis associated with in-tandem mass spectrometry, are important for the study of stem cells, and capable of providing clues for the elucidation of mechanisms by which the MSC exercise their biological effect.

The Amyotrophic Lateral Sclerosis (ALS) is a disease characterized by progressive regeneration of the motor neurons. Presently, the participation of the immune response has been a target of researches that indicate the autoimmune component on the development of ALS.

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