Potential use of adipose tissue stem cells in the control of aging

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Abstract

Cell therapy with adult stem cells is a new battle front for the control of aging. Before being used for this purpose, we need to answer several basic questions about the biochemistry and physiology of these cells. This paper presents some aspects and preliminary results obtained in our laboratory using stem cells from adipose tissue.

Introduction

Various strategies are used to control the effects of aging on the normal functions of an organism. Antioxidants, diet, exercise and even activating enzymes of phase II detoxification appear to delay the decay function to a greater or lesser extent. However, none of these strategies prevent biological aging. Hence, to control the effects of aging it will be necessary to emphasize the need to explore the mechanisms of tissue repair and regeneration as a complementary methodology to other prevention strategies. In this sense, Regenerative Medicine may represent a fascinating alternative to control the aging process.

One of the aspects of Regenerative Medicine is based on the regenerative capacity of adult stem cells are in most tissues and contribute to this homeostasis and repair. After tissue damage several intracellular and intercellular pathways are activated in a coordinated attempt to restore the tissue integrity. With aging occurs general decline in the regeneration potential. Besides this loss of regenerative capacity is produced by alterations of the stem cells with aging, it is thought that stem cells transplanted into damaged or aged tissues may have therapeutic and restorative capacity. In fact, stem cells represent a huge promise in the therapy of many aging-related degenerative disorders aging some diseases such diabetes, heart disease, stroke, Parkinson, etc [1-4]

Stem cells

Stem cells have the ability to self-renew through symmetric divisions and the potential of differentiating into several different cell types depending on their degree of multipotentiality through asymmetric division [1-3,5-8]. It is now known that most of the tissues have a very specific population of adult stem cells that allow their regular renewal or regeneration [6,8]. Thus, it has been described the existence of these SC in tissues such as muscle, skin, liver, pancreas, brain [9], intestines, fatty tissue [10,11].

In higher animals, stem cells have been classified into two groups: embryonic stem cells and organ-specific stem cells or stem cells from adult tissue. The latter are able to originate cells of a particular organ in the adult. Our work focuses on adult stem cells. The activity of these stem cells varies greatly from one organ to another: those of the bone marrow that form blood cells are very active and are continually dividing, while those which are, for example, in the small intestine are more inactive. Some adult stem cells are capable of differentiating into more than one cell type as mesenchymal stem cells.

Adipose tissue mesenchymal stem cells (ADSC)

Within the adult stem cell group are the mesenchymal stem cells (MSC). The MSC belong to the mesenchyma, which by a differentiation process will lead to the blood vessels, smooth muscle, mesothelium, lymphatic system and connective tissue itself. These cells can be obtained from different organs including fetal liver, umbilical cord, and bone marrow [12,13]. Another important source is adipose tissue, which contains progenitor cells called adipose tissue stem cells (ADSC) [2,10,14,15].

Advantage of ADSC

The easy access to the subcutaneous fat by liposuction, allows ADSC to be obtained under local anesthesia and with minimal discomfort to the patient. In addition, its high abundance with not ethical problems associated with its use, make adipose tissue an important source of MSC [16-18]. Another advantage is that the proportion of MSC is 500 times higher [19,20] in adipose tissue than in the bone marrow, so that a large number of cells can be obtained without a large number of passes, decreasing the risk of chromosomal abnormalities induced senescence in cultures [21]. An additional advantage is its potential to differentiate into bone, cartilage, tendons, skeletal muscle, fat, endothelial tissue and macrophages when grown under specific conditions of each lineage [11,13,22]. Surprisingly, the ADSC not only have the potential to differentiate into cells of mesodermal origin and organs, but also they have the ability to differentiate into neurons, endocrine cells of the pancreas, hepatocytes, endothelial cells and cardiomyocytes [10].

ADSCs can repair and regenerate the tissues by several mechanisms: First, the ADSC transplanted into a damaged or diseased tissue can secrete cytokines and growth factors that stimulate the recovery in a paracrine manner. The ADSC could modulate the host stem cell niche by stimulating the recruitment of endogenous stem cells to a particular site and promote their differentiation into the required lineage. Also, ADSC can provide antioxidants so that toxic substances released into

the local environment are eliminated, thereby promoting the recovery of the surviving cells. Another mechanism is thought their in vitro differentiation into the desired line, prior to their autologous transplantation [2]. They can also act as immune modulators [23].

Today it is possible to isolate and culture adult stem cells for therapeutic use. Once transplanted, these cells can be used to rejuvenate damaged tissue by aging or other causes. However, no one knows for sure the consequences of the stem cells treatments because there are many basic questions about the biology of these CM that must be answered before its therapeutic use. Obviously, these cells have to survive and perform their function in unfavourable conditions as the damaged tissue microenvironment, where many factors needed for stem cells are lacking. Under these adverse conditions, the survival of the stem cells will depend on his "molecular health" and its responsiveness when implanted in the tissue. Therefore, stem cells must have a robust repair mechanisms and resistance in order to participate in tissue regeneration. It is likely that this molecular health and stress response depends on many biochemical and physiological aspects related to donor age, lifestyle, etc. As these cells are continuously receiving "on-off" signals to divide, it could be that old cells are less sensitive to these signals. Consequently, the therapeutic applications of stem cells in adult tissue repair requires a better understanding of the biology of these cells, of the environment of the damaged tissue or both.

The work we are carrying out in our laboratory try to answer several of these basic questions about ADSC.

First of all, we focused on the isolation methods. These methods are based on magnetic cell sorting (Miltenyi Biotech), where magnetic microparticles linked to antibodies recognize surface antigen in the stem cells. The separation is performed on a column inserted in an extremely powerful magnet to retain the labeled cells. This methodology allows us to build a cell bank of patients of different ages that meet the criteria of "Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy."

The first criteria is that cells must be adherent to the plastic. The photographs of Figure 1 show that the human ADSC are adherent to the culture flask without addition of any substrate.

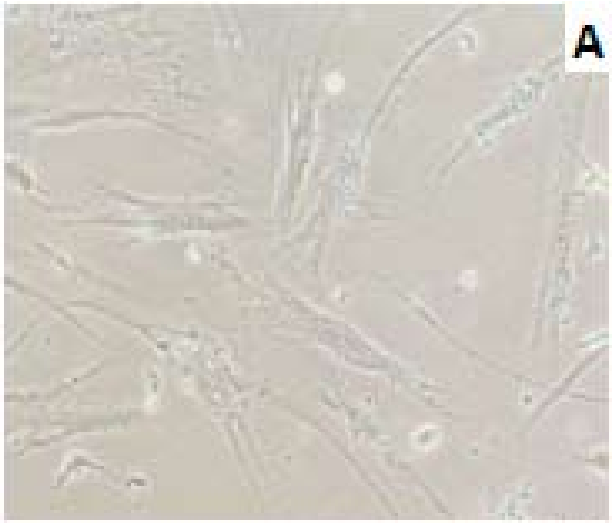
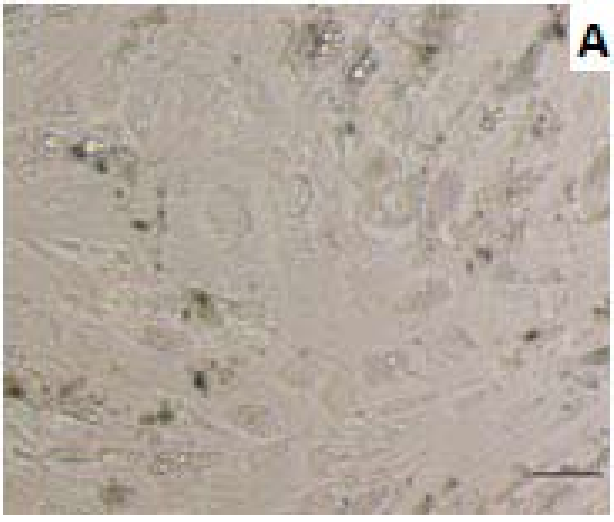
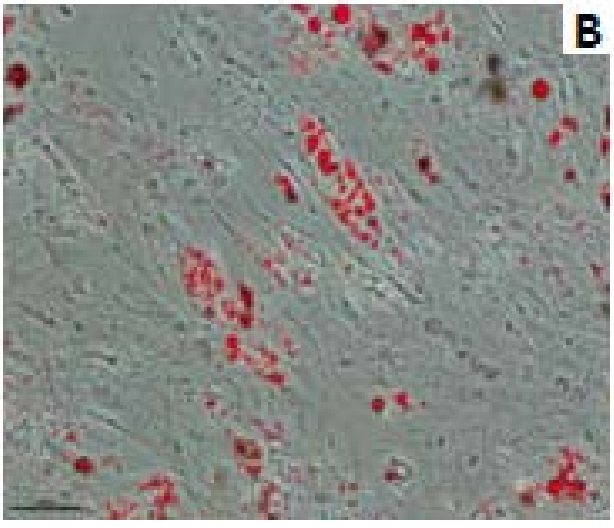


Figure 1. Human ADSC (42 yrs) in culture flask. 10x magnification.

A prerequisite for considering the cultured ADSC as MSC, is to demonstrate that they have the potential to differentiate into at least two different cell types [11,13,22,24]. For this, cells were cultured in specific differentiating media. Figure 2 shows photographs taken before and after

differentiation into adipocytes, where it can be observed fusiform cells containing lipid vacuoles. The red staining of these vacuoles (Fig. 2 and B) shows the differentiation into adipocytes ADSCs. Furthermore, the presence of black-purple shown by the presence of alkaline phosphatase in osteoblasts generated by ADSC differentiation (Fig. 2C)





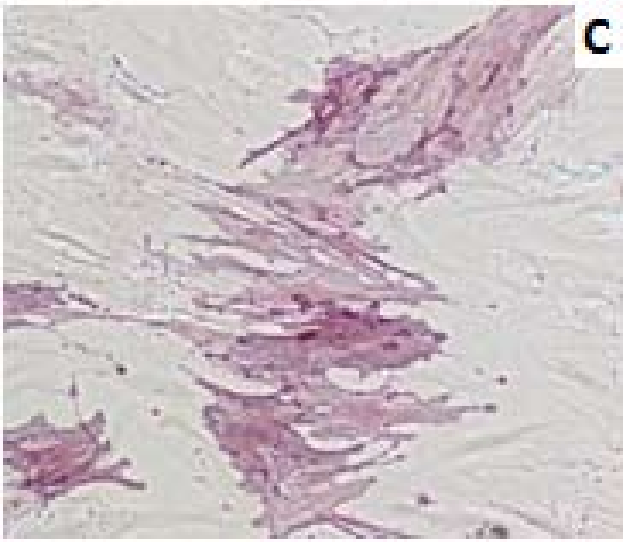
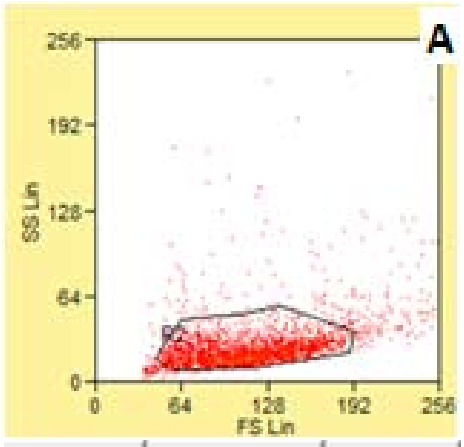


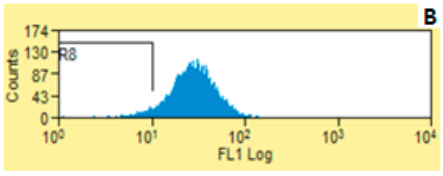
Figure 2. Differentiation of human ADSC into adipocytes and osteoblasts. A) Picture of human adipocytes obtained from ADSC (patient of 37 years) prior to staining, 10X magnification. B) Photograph after fixation and staining process. C) Photograph of osteoblasts after fixing and staining 20X magnification.

In addition to the ability of adherence to plastic and differentiation, the results provided by flow cytometry serve to confirm the nature of the cells obtained. Figure 3 show the results obtained from a cell population of human ADSC. These graphs represents side scatter (Side Scatter, SS), which gives information about the existence of different cell populations. The forward scatter (forward scatter, FS), gives an insight on cell size. As can be seen, the sample contains a unique cell population, reflected in the graph as a single cloud.

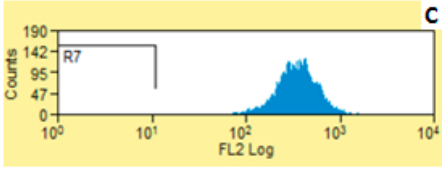
To verify that the obtained cells meet the minimum standards required by the committee of mesenchymal stem cells and tissues of the international cell therapy, cells must have the surface markers CD73, CD90 and CD105. On the contrary, they should not contain CD14, CD34, CD45, CD19, CD79α and human leukocyte antigen (HLA)-DR.



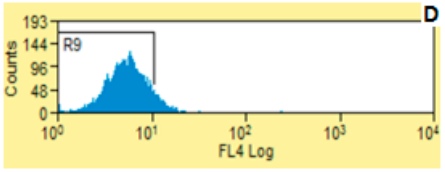
Region	Count	% Hist
Total	4139	100.00
R2	3690	89.15



Region	Count	% Hist
Total	3690	100.00
R8	202	5.47



Region	Count	% Hist
Total	3690	100.00
R7	13	0.35



Region	Count	% Hist
Total	3690	100.00
R9	3323	90.05

Figure 3. Study of the degree of cellular homogeneity and phenotyping of human ADSC. Data were obtained from the flow cytometer FC-500 (Beckman-Coulter). The results of positive events to the fluorophore are in %.

The histograms represent the number of events counted on each exposed channel. In our case, the channel FL1, FL2 and FL4 are set to excite the fluorophores FITC, PE, PerCP, respectively. Considering the antibodies used, (CD14-PerCP, CD20-PerCP, CD34-PerCP, CD45-PerCP, CD105-FITC and CD90-PE), FL1 channel gives us information on the number of cells expressing CD90 marker (Fig. 3B); FL2 channel gives information about the proportion of CD105-expressing cells (Fig. 5C) and FL4 channel indicates the percentage of positive cells is any of the following markers: CD14, CD20, CD34 and CD45 (Fig. 3D). The first histogram (Fig. 3B) indicates that 94.53% of the sample is positive for the CD90 marker. The second histogram (Fig. 3C) shows that almost 100% of the sample expressed the marker CD105. Only 9.5% of the cells have one of the following markers: CD14, CD20, CD34, CD45.

Finally, we have used a method for labeling and tracking that allow us to monitor stem cells once they are injected in vivo. This method is based in transfecting the ADSC with the luciferase gene by using lentivirus. After intraperitoneal injection of luciferin, cells carrying the luciferase gen can be observed by using an in vivo imaging system. Figure 4A shows a culture of transfected ADSC in a 25 cm 2 flask prior to administration of 3 luciferin used as a control of bioluminescence. Figure 4B shows the same culture after the administration of D-luciferin.

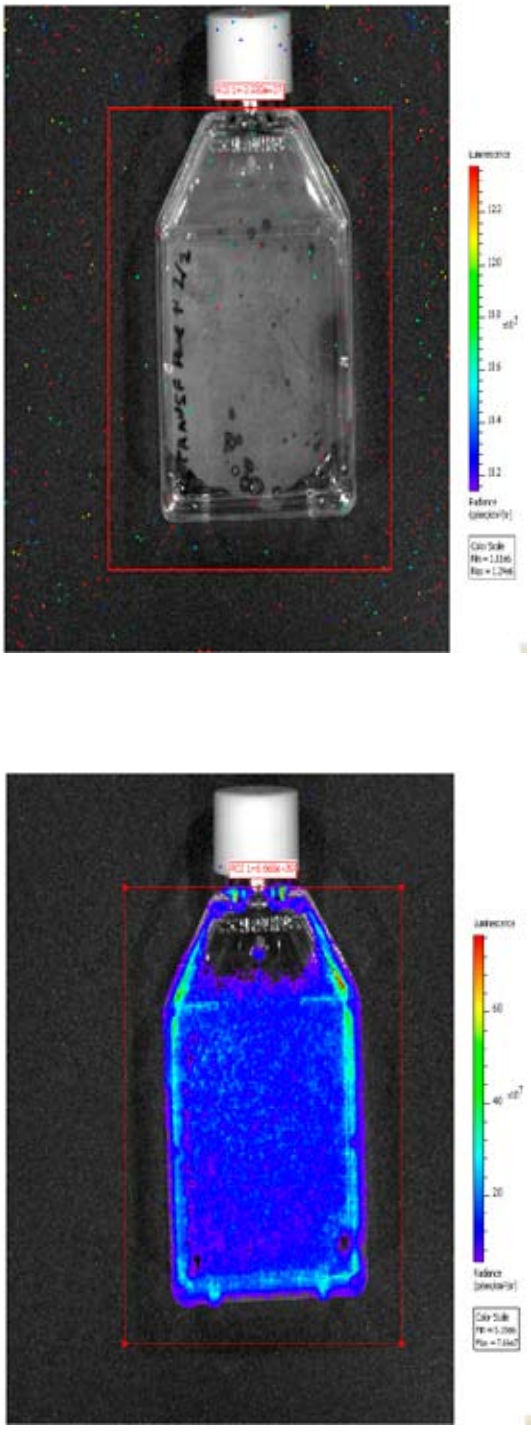


Figure 4. ADSC cells transfected with lentivirus containing the luciferase gene. A) vial containing the cells prior to the addition of luciferin. B) Same bottle treated with luciferin.

Conclusion

In summary, we can say that cell therapy open a new battle front in the control and treatment of aging related diseases. However, there are many basic questions about the biology of these stem cells that should be answered to predict the therapeutic potential of these cells, some of which are going to be known after the completion of the the present study because. The main objective of this work is to identify the factors affecting the "robustness" of the biochemistry and physiology of ADSC.

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