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Review

Characteristics, applications and prospects of mesenchymal stem cells in cell therapy☆

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ABSTRACT

Recent advances in the field of cell therapy and regenerative medicine describe mesenchymal stem cells (MSCs) as potential biological products due to their ability to self-renew and differentiate. MSCs are multipotent adult cells with immunomodulatory and regenerative properties, and, given their therapeutic potential, they are being widely studied in order to evaluate their viability, safety and efficacy. In this review, we describe the main characteristics and cellular sources of MSCs, in addition to providing an overview of their properties and current clinical applications, as well offering updated information on the regulatory aspects that define them as somatic cell therapy products. Cell therapy based on MSCs is offered nowadays as a pharmacological alternative, although there are still challenges to be addressed in this regard.

Características, aplicaciones y perspectivas de las células madre mesenquimales en terapia celular

Palabras clave:
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RESUMEN

Los recientes avances en el área del tratamiento celular y la medicina regenerativa describen las células madre mesenquimales (*MSC*) como potenciales activos biológicos debido a su capacidad de autorrenovación y diferenciación. Las *MSC* son células multipotentes, con propiedades inmunomoduladoras y regenerativas, y debido a su potencial terapéutico están siendo ampliamente estudiadas con el objetivo de evaluar su viabilidad, seguridad y eficacia. En esta revisión, se describen las principales características y fuentes de obtención de las *MSC*, se da una visión global sobre sus propiedades y aplicaciones clínicas actuales, así como una actualización de los aspectos regulatorios que las definen como medicamentos de tratamiento celular somático. El tratamiento celular basado en *MSC* se presenta a día de hoy como una gran alternativa farmacológica aunque todavía quedan retos por abordar.

Introduction

Cell therapy is a new therapeutic approach, based on the use of cells as therapeutic agents.[1] Thus, in regenerative medicine, the study and correct determination of the type of cell to be applied to a specific treatment is essential for success. For that reason, their safety and ability to repair, replace or restore the biological function of damaged tissues and organs needs to be defined.[1]

Studies carried out to date suggest that stem cells are suitable for use in regenerative medicine. The main feature of stem

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cells is that they are unspecialized cells with the ability to self-renew for long periods of time as well as differentiate themselves (plasticity) into specialized cells with specific functions. Stem cells are classified according to their differentiation ability: totipotent, pluripotent, multipotent and unipotent, with pluripotent and multipotent stem cells being the most studied for clinical application. Pluripotent stem cells can be differentiated from any cell of the body. They are divided into embryonic and induced; whereas multipotent adult somatic or tissue-specific stem cells[2] are those whose potential for specialization is restricted to one or more cell lineages.

Stem cells currently used in cell therapy are adult stem cells. *Mesenchymal stem cells* are among the most extensively studied (*MSC*). Despite having less proliferative potential and lower plasticity compared with embryonic stem cells and induced stem cells, they are easier to obtain from tissues, their manipulation does not create ethical problems,[3] have high *in vitro* expansion capacity plus a low potential for the formation of teratomas.[4,5] All this coupled with their ability to produce cytokines and growth factors, migrate to the region where tissue damage has occurred and exert immunomodulatory actions in that site means that the study and development of *MSC* as biological assets can help provide new therapeutic alternatives with high potential in regenerative medicine and cell therapy[6] for diseases that, so far, have no effective conventional treatments such as cancer, diabetes, chronic critical limb ischaemia, myocardial infarction, Parkinson's, *etc.*

Definition and characteristics of mesenchymal stem cells

MSC also known as stromal stem cells were first described in 1968 by Friedenstein et al. as colony forming units, fibroblastoid in appearance, which adhered to plastic in culture and had the ability to regenerate bone tissue *ex vivo*.[7] In 2006, the minimum characteristics required for a cell to be considered *MSC* were redefined in the *International Society of Cellular Therapy* (*ISCT*) Congress, these *being* as follows: (1) MSC must be capable of adhering to plastic in culture, (2) express the surface antigens CD73, CD90 and CD105 in the absence of other hematopoietic antigens of type CD34, CD45 and typical B lymphocytes, monocytes and macrophages markers, (3) be multipotent and have a high plasticity to differentiate *in vitro* under standard culture conditions into osteoblasts, adipocytes and chondrocytes.[8,9]

Other characteristics, in addition to those proposed by the *ISCT*, which can help to define and classify a cell as *MSC* is having a mesodermal origin, sharing some fibroblast characteristics, being able to self-renew, for which, during cell division, only one of the two resulting cells will start cell differentiation programs, exhibiting a relatively low immunogenicity and having the ability to differentiate under certain conditions into different lineage cells, "differentiation plasticity".[10,11]

Sources and cultures of mesenchymal stem cells

MSC can be obtained from bone marrow, adipose tissue, cord blood, dental pulp, smooth, skeletal and cardiac muscle, liver, spleen, testes, menstrual blood, pancreas, periosteum, synovial membrane, dermis, pericytes, trabecular bone, lung, placenta, peripheral blood, periodontal ligament and amniotic fluid aspirates.[12–16] Of these, the most important are bone marrow, adipose tissue and cord blood[17] (Fig. 1). Once *MSC* are isolated by plastic adherence and are cultured *in vitro*, may, under certain conditions, differentiate into mesodermal lineages such as osteocytes (bone cells), chondrocytes (cartilage cells), adipocytes (fat cells), myoblasts (precursors of muscle cells) and cardiomyocytes (heart cells),[8,18–20] or they can even differentiate into endodermal (hepatocytes, pancreatic cells) and ectodermal (keratinocytes, astrocytes and neurons)[21–23] lineage cells. Even though *MSC* have a broad differentiation capacity, they are considered multipotent and not pluripotent cells because their tetraploid complementation could not be proven. This consists on a test whereby, after injecting *MSC* in a mouse blastocyst, chimeric mice are expected to be obtained, in which, after the development of the embryo, the injected cells lead to cells belonging to the embryonal layers (endoderm, mesoderm and ectoderm).

MSC are easy to isolate, expand *in vitro* and handle throughout the cell culture process to which they must be subjected in order to obtain the number of cells needed to define the cell dose for patient administration. They can also be cryopreserved without experiencing phenotypic alterations, losing their proliferative capacity or their differentiation ability after the thawing process.[24] However, it has been described that when these undifferentiated cells are subjected to culture division processes for more than 7 passages, their cytogenetic characteristics and telomerase activity begin to be affected.[25] This leads to culture ageing and the emergence of chromosomal alterations[26] while causing the loss of cell multipotentiality and replicative senescence.[25,27] All this results in an accumulation of genetic and epigenetic alterations in these cells[28] inducing the transformation of *MSC* into immortalized cells with ability to form tumors.[26] In fact, some studies defend that cellular senescence acts as a tumour suppressor mechanism, able to stop cell growth, thereby reducing the risk of transformation of *MSC* into cancer cells,[29,30] while other studies have shown that the performance and differentiation potential may vary depending on age, donor condition, tissue collection conditions as well as media and conditions employed during cell culture.[31]

Therefore, when designing the study and development of a therapeutic treatment based on the administration of *MSC*, the characteristics and availability of donor tissue (autologous and allogeneic), the number of cell doublings during *in vitro* expansion and specificity and differentiation capacity of these cells should be taken into account.[32]

Characterization of mesenchymal stem cells

Because no *MSC* can be identified by a unique specific marker, these cell types must be defined based on a combination of phenotypic markers and functional properties. Thus, following the guidelines set by *ISCT*, one of the minimum criteria required today for a cell to be considered a *MSC* is the expression of certain surface antigens: CD73, CD90, CD105, CD166 and varying levels of stromal markers STRO-1, CD29, CD44, CD71, CD271 and GD2 ganglioside[14]; and the absence of hematopoietic antigens or other antigens typical of other cell populations present in the same tissues as the *MSC* such as CD45, CD11b, CD34, CD14, CD19, CD79a and major histocompatibility complex class II.[8,9,14,33] In addition, some authors have reported that *MSC* can express ligands essential for interaction with T cells, such as VCAM-1, ICAM-2, LFA-3[14,33] but they never express costimulatory molecules like CD80, CD86, CD40, CD40L and Fas ligand[14,34] (Table 1).

Depending on the origin of *MSC*, expression of surface markers varies; for example, adipose tissue *MSC* express higher surface antigens levels for: CD34, CD49d, CD54; bone marrow *MSC* express higher levels of CD106 surface antigen and *MSC* obtained from cord blood rarely express CD90 surface antigen.[17]

Similarly, although the current guidelines established by the *ISCT* still apply when defining a cell as *MSC*, one must resort to other available cell identification tools to distinguish *MSC* from other cell types, like in the case of fibroblasts. *MSC* have a fibroblastic