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Review

Wharton's jelly derived mesenchymal stromal cells: Biological properties, induction of neuronal phenotype and current applications in neurodegeneration research

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ABSTRACT

Multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSC), can be isolated from bone marrow or other tissues, including fat, muscle and umbilical cord. It has been shown that MSC behave *in vitro* as stem cells: they self-renew and are able to differentiate into mature cells typical of several mesenchymal tissues. Moreover, the differentiation toward non-mesenchymal cell lineages (*e.g.* neurons) has been reported as well. The clinical relevance of these cells is mainly related to their ability to spontaneously migrate to the site of inflammation/damage, to their safety profile thanks to their low immunogenicity and to their immunomodulation capacities. To date, MSCs isolated from the post-natal bone marrow have represented the most extensively studied population of adult MSCs, in view of their possible use in various therapeutical applications. However, the bone marrow-derived MSCs exhibit a series of limitations, mainly related to their problematic isolation, culturing and use. In recent years, umbilical cord (UC) matrix (*i.e.* Wharton's jelly, WJ) stromal cells have therefore emerged as a more suitable alternative source of MSCs, thanks to their primitive nature and the easy isolation without relevant ethical concerns. This review seeks to provide an overview of the main biological properties of WJ-derived MSCs. Moreover, the potential application of these cells for the treatment of some known dysfunctions in the central and peripheral nervous system will also be discussed.

Introduction

Mesenchymal stromal cells (MSC) from bone marrow have initially gained much attention by hematologists involved in hematopoietic stem cell transplantation. However, it soon became clear that MSCs have biological properties making them suitable for use in regenerative medicine and immunomodulation. Moreover, MSCs obtained from other sources could offer theoretical advantages over bone marrow derived ones. In particular, MSC derived from the Wharton's jelly of the umbilical cord (WJMSCs) exhibit unique features (*e.g.* primitive nature, multilineage potential immunomodulatory ability, ease of isolation, extensive proliferation) that may make them more valuable therapeutic tools for the treatment of various diseases or tissue damage. This review will first attempt to provide a brief summary of the main biological properties of WJMSCs, and then discuss their efficacy to promote anatomical and functional recovery upon transplantation in rodent models of central and peripheral nerve dysfunction.

Isolation, characterization and growth of mesenchymal stromal cells

Multipotent mesenchymal stromal cells (MSC), also known as mesenchymal stem cells (MSC) were first isolated from animal bone marrow by Friedenstein et al. (1970) and were initially described

Abbreviations: WJ, Wharton's jelly; UC, umbilical cord; MSC, mesenchymal stromal (stem) cells; WJMSC, Wharton's jelly mesenchymal stromal cells; HLA, human leukocyte antigen; HSC, hematopoietic stem cells; GvHD, graft *versus* host disease; NK, natural killer; TNF, tumor necrosis factor; IFN, interferon; bFGF, basic fibroblast growth factor; BHA, butylated hidroxy-anisole; DMSO, di-methyl-sulf-oxide; RA, retinoid acid; NGF, nerve growth factor; FCS, fetal calf serum; NCM, neuronal conditioned medium; NIM, neuronal induction medium; BDNF, brain derived neurotrophic factor; EGF, epidermal growth factor; NFM, neurofilament M; TH, tyrosine hydroxylase; GFAP, glial fibrillary acidic protein; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; NSC, neural stem cell; PNS, peripheral nervous system; BMSC, bone marrow MSC; ASC, adipose-derived stem cell; GDNF, glial cell line-derived neurotrophic factor; PLCL, poly DL-lactide-epsilon-caprolactone.

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Table 1
Factors used to obtain differentiation of MSCs from various sources into relevant cell types.

Differentiation into	Culture medium factors	References
Osteocytes	Ascorbic acid, β glycerol phosphate and dexamethasone	Pittenger et al. (1999)
Adipocytes	Dexamethasone, insulin, and isobutyl-methyl-zantine	Pittenger et al. (1999)
Chondrocytes	Transforming Growth Factor beta (TGF-β)	Baksh et al. (2007)
Cardiomyocytes	5-Azacytidine	Xu et al. (2004)
Endothelial cells	2% Fetal calf serum and vascular endothelial growth factor	Oswald et al. (2004)
Neurons	DMSO (di-methyl-sulf-oxide), butylated hydroxy-anisole (BHA), β-mercaptoethanol, forskolin, KCl, valproic acid and hydrocortisone	Woodbury et al. (2000) and Ishii et al. (1993)

as precursors of fibroblasts or stromal cells. MSCs represent a rare population (0.001–0.01% of nucleated cells) of adult human bone marrow cells, but they can also be identified in muscle, periosteum, adipose and other connective tissues (Castro-Malaspina et al., 1980; Pittenger et al., 1999).

Adult MSC can be readily isolated, exploiting their marked adhesive properties, and extensively expanded. These cells are heterogeneous, showing at least two subpopulations of cells in culture: small, spindle-shaped, rapidly self-renewing MSC and larger, slowly renewing MSC (Colter et al., 2001).

There is no specific marker for MSC, rather, the validation of MSC identity is based on a combination of phenotypic and functional characteristics. MSC express several surface proteins, including CD29, CD44, CD73, CD90, CD105, but low levels of HLA class I and undetectable hematopoietic markers such as CD14, CD34 and CD45, endothelial markers (CD31), HLA class II and costimulatory molecules (CD80, CD86) (Bobis et al., 2006; Conget and Minguell, 1999; Djouad et al., 2005; Fibbe and Noort, 2003; Gronthos et al., 2003; Pittenger et al., 1999).

The physiological function of MSCs in the bone marrow is to contribute to the formation of the hematopoietic stem cell (HSC) niche. Here, MSC preserve the HSC pool by maintaining HSC in a quiescent state (anti-proliferative activity) until, after appropriate stimulation, they differentiate and are released in the sinusoidal vascular system (Uccelli et al., 2008).

MSCs as a product for cell therapies: from immunomodulation to regenerative medicine

MSCs gained much attention about a decade ago, owing to their ability to differentiate into several cell types and to their immunosuppressive potential (Uccelli et al., 2006). Since then, it has been consistently shown that MSC can differentiate into different cell types of mesenchymal origin (adipocytes, chondrocytes and osteocytes) and can even trans-differentiate toward non-mesenchymal cell lineages, such as neurons, cardiomyocytes, endothelial cells (Baksh et al., 2007; Lu et al., 2006; Oswald et al., 2004; Pittenger et al., 1999; Woodbury et al., 2000; Xu et al., 2004) (Table 1).

The easy obtainment of large amounts of MSC from healthy donors, together with the differentiation ability of these cells have raised great expectations about their potential use in regenerative medicine (Taddio et al., 2012). In addition, it has become apparent

Box 1: Characteristics making MSC ideal candidates for cell therapies.

- Easily expanded from adult and fetal tissues
- Multilineage capabilities
- Immune privileged cells
- Immunomodulatory and anti-proliferative action
- Release of trophic factors
- Homing to damaged sites

that MSC may exert a beneficial trophic effect on injured tissues, even without necessarily replacing dying cells (Crigler et al., 2006; Shen et al., 2013). Thus, some characteristics of MSCs make these cells even more promising for the development of cell therapies (Box 1).

First, MSCs are poorly immunogenic (*i.e.* they escape detection by cells from the immune system thanks to the low expression of HLA molecules) and, depending on the preparation and on cell delivery route, can survive in recipients and exert their action for weeks (Kurtz, 2008).

Secondly, MSCs have been proven able of spontaneous migration towards the site of damage/inflammation when infused intravenously (Chamberlain et al., 2007). Even if most infused cells can be withheld in lungs, selective homing may allow concentrating the action of these cells just where it is needed, limiting possible undesired effects.

Third, it is increasingly clear that MSC have potent immunomodulatory properties. Indeed, it has been shown that these cells can inhibit proliferation of activated peripheral blood mononuclear cells both *in vitro* and *in vivo* (Uccelli et al., 2006). The immunomodulatory effect of MSC has been exploited in subjects who developed intractable graft *versus* host disease (GvHD) after haplo-identical hematopoietic stem cell transplantation (Dhir et al., 2014; Le Blanc and Ringden, 2007; Tyndall et al., 2007). Although there is no randomized clinical trial conclusively proving the advantage of MSC based therapies, this kind of treatment is currently used in many centers and several studies are ongoing to improve efficacy (Martin et al., 2014). In fact, based on preliminary clinical experiences and *in vitro* studies, there are several factors related both to the preparation of MSC and to the activation of immune system that can affect the outcome of MSC therapy. For example, activated natural killer (NK) cells are able to kill MSC (Spaggiari et al., 2006; Uccelli et al., 2008), while M1 macrophages, by producing IL-1ß, IL-6, TNF-α and IFN-γ, can inhibit the MSC growth *in vitro* (Freytes et al., 2013). On the other hand, the immunosuppressive potency of MSCs could be increased *in vitro* by the exposure to exogenous molecules (licensing), such as interferon-gamma (IFN-γ). For example, MSC that have been previously exposed, *in vitro*, to IFN-γ are resistant to NK-mediated lysis, probably because of an upregulation of HLA-I on their surface (Spaggiari et al., 2006; Uccelli et al., 2008).

In particular, we have previously shown that MSC are not able to suppress proliferation of pre-stimulated lymphocytes, while the IFN-γ treatment increases this activity (Valencic et al., 2010). In these experiments, the pre-stimulation of lymphocytes was meant to mimic what happens *in vivo*, in conditions such as GvHD, where the therapeutic use of MSC is proposed for patients with already active immune responses.

Overall, the combination of direct or indirect regenerative properties and immunomodulatory action can represent the added value of this therapy in conditions in which tissue damage is worsened by inflammatory or autoimmune mechanisms. For example, there is consistent evidence that hypoxic ischemic damage in newborns is greatly enhanced by secondary activation of an immune response to necrotic tissue, which can expand the effects

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