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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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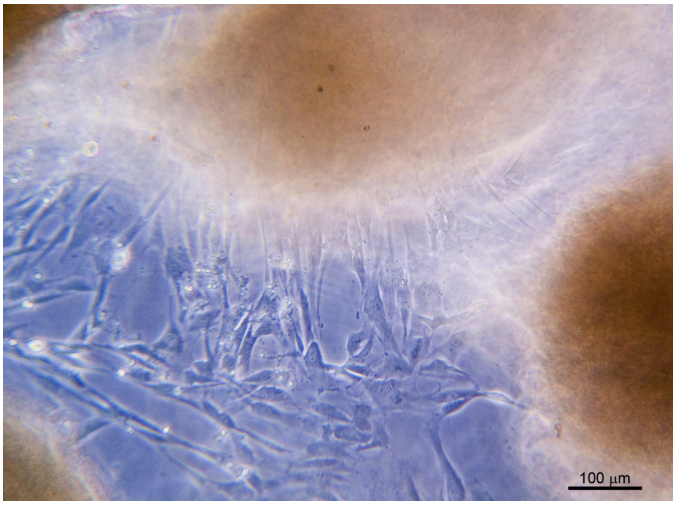

Fig. 1. Spontaneous migration of mesenchymal stromal cells from fragments of Wharton's jelly to the plastic well. The Wharton's jelly, after it has been diced in small fragments, is transferred on a plastic support (six-well plates) and incubated at 5% CO_2, 37 °C. After 10 days of undisturbed incubation the cells begin to migrate from the tissue to the surface well. Scale bar = 100 µm.

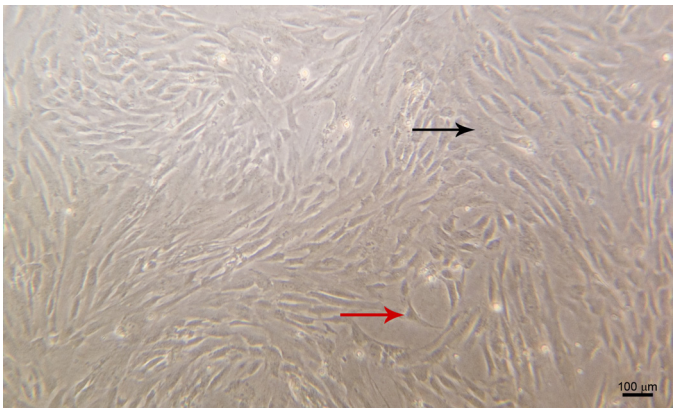

Fig. 2. Mesenchymal stromal cells at confluence, cultured in Dulbecco's modified eagle medium plus fetal bovine serum. They are heterogeneous showing at least two subpopulations: the first one displays a fibroblast-like morphology, is spindle-shaped and rapidly self-renewing (lower arrow, red); the second one exhibits a rhomboid-like morphology, is larger and slowly renewing (upper arrow, black). Scale bar = 100 µm.

of the initial lesion (Barone et al., 1997; Emerich et al., 2002; Ladeby et al., 2005; Rothwell et al., 1997). Immune mechanisms with activation of macrophages and microglia have been shown to play a fundamental role also in neurodegenerative disorders (Streit et al., 1999). Thus the ability of MSCs to control immune activation and to provide trophic signals for tissue repair can be the basis of MSC based therapies in several conditions (van Velthoven et al., 2010).

Wharton's jelly mesenchymal stromal cells: easy and young

Wharton's jelly mesenchymal stromal cells (WJMSC) can easily be obtained in large numbers and could represent a valuable alternative to BM-derived cells also thanks to their younger origin (Batsali et al., 2013; McElreavey et al., 1991; Wang et al., 2004). Many protocols have been proposed to isolate cells from Wharton's jelly, depending on the possibility to remove umbilical arteries and vein, and on an enzymatic or mechanical dissection approach. The enzymatic treatment with collagenase, trypsin, or hyaluronidase disrupts the cellular matrix and then the isolated cells will be washed and cultured (Wang et al., 2004). More simply, the tissue can be mechanically dissociated into fragments (very small or a couple of centimeters depending on different protocols) and transferred in culture plates until the cells will migrate to the plastic bottom (La Rocca et al., 2009; Mitchell et al., 2003) (Fig. 1). Isolated cells or the fragments require medium such as low-glucose Dulbecco's modified Eagle medium containing fetal bovine serum or other supplement (platelet rich plasma) (Mitchell et al., 2003; Wang et al., 2004; Wen et al., 2014) (Fig. 2).

Similar to the bone marrow cells, umbilical cord, MSCs grow in adhesion on plastic supports where they typically exhibit a rhomboid-like and a fibroblastoid-like morphology. These two cell types differ in the amount of vimentin (mesenchymal marker) and pancytokeratin (ectodermal marker) (Colter et al., 2001).

Several studies have shown that WJMSCs can be differentiated into osteocytes, adipocytes, endothelial cells, chondrocytes and neural lineages (Chen et al., 2009, Karahuseyinoglu et al., 2007; Lu et al., 2006; Saben et al., 2014; Wang et al., 2004; Wu et al., 2007; Zuk et al., 2001). The immunomodulatory properties of WJMSCs were shown to be similar to bone marrow-derived MSCs (Weiss et al., 2006). Thanks to their strong proliferation potential and the large quantity in which they can be obtained, WJMSCs have recently emerged as a promising source of cells to be employed in the treatment of several neurological disorders (Zhou et al., 2012).

Neuron-like differentiation of WJMSCs

Under adequate stimulation, WJMSCs can differentiate into neuron-like cells *in vitro*, exhibiting both morphological and biochemical properties of neural cells and expressing typical neuronal proteins such as Nestin and Beta III Tubulin (Ishii et al., 1993; Woodbury et al., 2000) (Fig. 3). Likewise, WJMSCs can also differentiate into astrocytes and neurons *in vivo* after being exposed to peculiar culture media enriched with different factors and implanted into the mouse brain (Zhang et al., 2006).

Mitchell et al. (2003) were the first who observed neuronal differentiation of WJMSCs following a multi-step neuronal induction process. They used the protocol of Woodbury et al. (2000) for neuronal differentiation of the bone marrow MSCs. This protocol entails the use of a medium with low concentration of serum containing basic fibroblast growth factor (bFGF), butylated hidroxyanisole (BHA), forskolin, KCl, valproic acid, hydrocortisone and di-methyl-sulf-oxide (DMSO, which increase cAMP levels). DMSO, in particular, proved to be capable of inducing the differentiation of several cell types towards a neuronal phenotype. Thus, differentiated neurons were detected or, more precisely, they were cells with neuronal morphology, expressing typical neuronal proteins (Woodbury et al., 2000).

To achieve neurons from WJSCs, different protocols have been adopted. Mitchell et al. (2003) found that WJMSCs can be induced to form neurons and glial cells after having been exposed to bFGF and low-serum media plus DMSO. It has been hypothesized that the DMSO may act *via* the regulation of protein kinase C pathway (Cheung et al., 2006), the down-regulation of c-myc gene, involved in cell growth and proliferation (Jiang et al., 2006), or both these mechanism combined.

Human WJMSCs differentiation may also occurs using retinoic acid (RA), nerve growth factor (NGF) and fetal calf serum (FCS), but the number of yielded neuronal cells may vary widely using different culture conditions.

Retinoic acid, a vitamin A derivative, is essential for the maintenance of normal cell growth and development and it promotes neuronal differentiation in various tissues, particularly in the nervous system, of both developing and adult animals, where it is present (Scintu et al., 2006).

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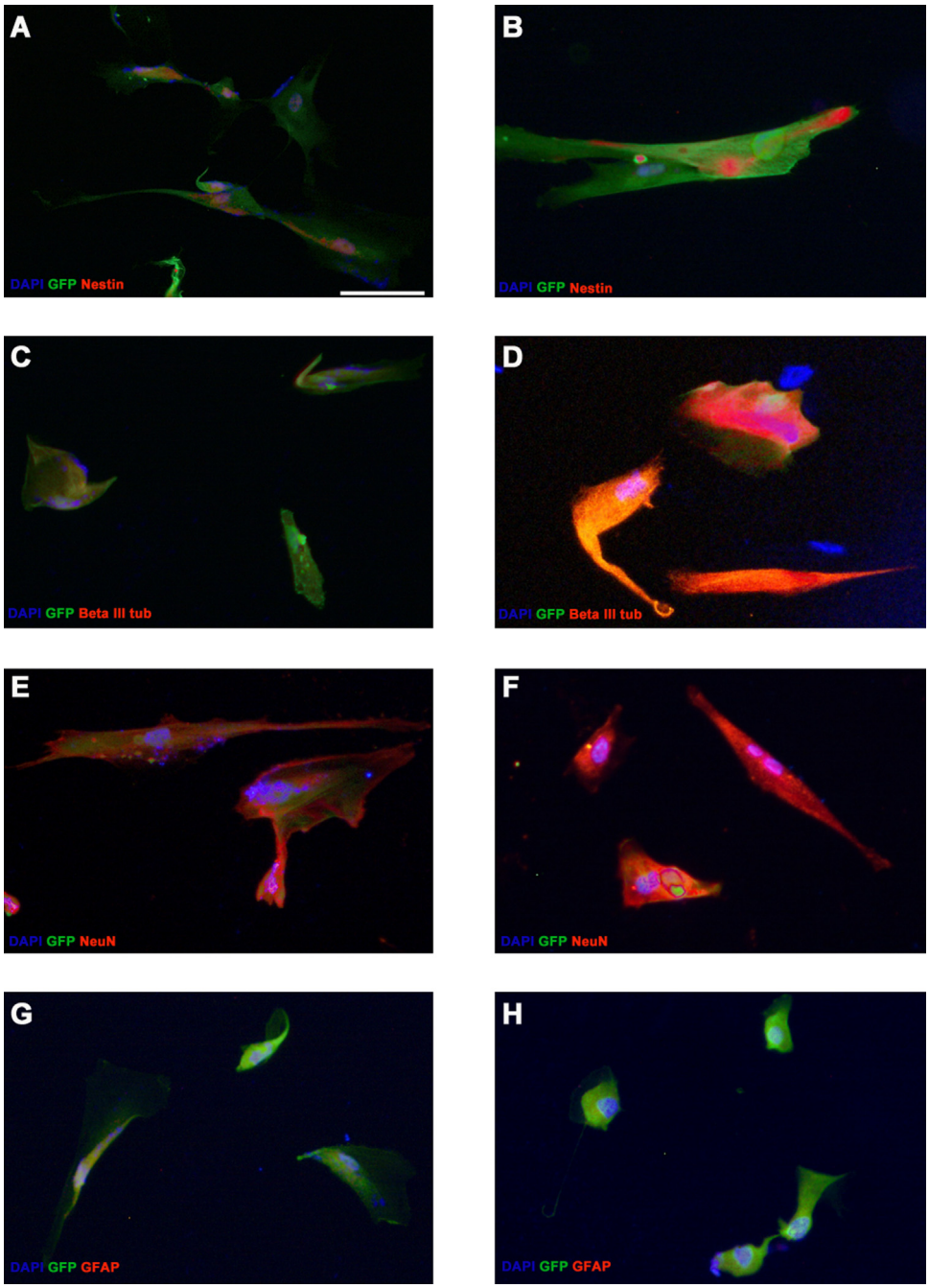

Fig. 3. Unpublished observations: immunocytochemical expression of neuronal markers in cultured Wharton's jelly mesenchymal stromal cells predifferentiated (A,C,E,G) and differentiated (B,D,F,H). Human Wharton's jelly mesenchymal stromal cells appeared respectively positive and very positive to all neuronal specification markers such as Nestin (A,B), β III tubulin (C,D), NeuN (E,F). A limited number of Wharton's jelly mesenchymal stromal cells belonging to both treatment groups appeared weakly positive for the glial marker Glial Fibrillary Acid Protein (G,H), classically considered as a mature astrocytes marker, but also expressed by neural progenitors with not yet defined phenotype. These findings suggest that predifferentiation and differentiation protocols employed in this study have probably directed cells toward a neuronal-like rather than a glial-like specification. Scale bar = 100 μm (A).

The limit of such approaches is given by the toxicity of the differentiating agents, which at high concentrations can induce widespread mortality in cell culture.

A method that does not make use of toxic molecules for the neuronal differentiation of WJMSCs requires them to be exposed to a neuronal conditioned medium (NCM), namely a medium obtained from cultures of neural stem cells or immature neurons, which is enriched in factors released by the cells themselves (Fu et al., 2004, 2006). Other strategies include treatment with a neuronal induction medium (NIM) consisting mainly of brain-derived neurotrophic factor (BDNF) and low-serum media (Zhang et al., 2012) or with a cytokine mix that include NGF, epidermal growth factor (EGF), bFGF and BDNF together with forskolin. Even if these combinations may appear somewhat milder, they proved to be equally effective in inducing differentiation compared to the previous more toxic methods (Li et al., 2012).

Another possibility to induce differentiation of WJMSCs towards a specific neuronal phenotype may require co-culturing them with

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some other types of cells. For example, Zhou et al. (2012) found that, when co-cultured with injured neural cells ($A\beta_{1-40}$ injured PC12 cells) in a Transwell® co-culture system, WJMSCs expressed neuronal markers at higher level than when other culture conditions were used.

Taken together, these findings suggest that human umbilical cord blood (Sanchez-Ramos, 2002) and Wharton's jelly (Karahuseyinoglu et al., 2007) contain cells that can be induced to neural antigenic and morphological phenotypes.

Neuron like phenotype in NSC-conditioned WJMSC

The marked differentiation capacity of mesenchymal stem cells has led the scientists to believe that they could be a good source of neural-like cells. As said before; different neural induction protocols have been developed; each giving rise to neural and/or glial-like cells. The differentiation level achieved after a neural induction has been addressed by analyzing the wide range of neural markers expressed by the differentiated WJMSCs both *in vitro* and *in vivo*. Several studies have therefore characterized the cells at various phases of differentiation or have compared various differentiation protocols.

Mitchell et al. (2003) showed that cells from Wharton's jelly are a rich source of primary cells that are readily expanded in culture and can be induced to form neurons and glia. In their study, they observed that, under the conditions imposed by the Woodbury's protocol, WJMSCs underwent profound changes in morphology, with the development of multiple dendrite-like, and single axon-like processes extending from the cell body, as well as granular structures reminiscent of Nissl substance in most cases (Guan et al., 2014; Mitchell et al., 2003). These changes in morphology were paralleled by a robust variation of the cell markers expressed. When tested for the presence of c-kit (a surface protein normally expressed in bone marrow stromal cells and hematopoietic stem cells), WJMSCs exhibited high expression levels which, however, were greatly diminished after their induction into neural cells. By contrast, steady increases in the expression of NFM, a neuron-specific intermediate filament, and of TuJ1, a class III neuron-specific β-tubulin, both markers for neural differentiation, were detected. To determine whether WJMSCs could become fully differentiated into a specific neuronal phenotype, the expression of the biosynthetic enzyme tyrosine hydroxylase (TH), a marker for catecholaminergic neurons, was investigated, together with that of GFAP and CNPase, markers for astrocytes and oligodendrocytes, respectively. TH was seen expressed in neurosphere-like colonies in fully induced cells, whereas GFAP-positive cells had a stellate morphology and lacked long process. By contrast, expression of CNPase was nearly identical in untreated and fully induced Wharton's jelly cells.

Hermann et al. (2004) went on and addressed the differentiation properties of the adult human bone marrow stromal cells. They characterized undifferentiated hMSCS, neuroprogenitor-like cells and clonogenic neural stem cells (NSCs) after differentiation, focusing their attention on mesodermal and neural markers and also the mRNA expression level of different proteins associated with the various stages of differentiation. In all cases they found a decreased expression of mesodermal markers and an increase of neural markers during the differentiation. The neuroprogenitor-like cells showed a strong increase in Nestin expression and a dosage of mRNA levels on neural-like cells reminiscent of mature neural cell type (GFAP, MBP, TUBB4/III, SNCA an TH) (Guan et al., 2014; Hermann et al., 2004).

Kogler et al. (2004) used cells derived from human cord blood to analyze their capacity to migrate, integrate and differentiate into neural-like cells *in vivo* following implantation into the hippocampus of the intact adult rat. They identified cells widely distributed throughout the brain, indicating a high migratory activity *in vivo*. Immunohistochemistry revealed the highly differentiated morphology of implanted cells.

Potential of MSC in the treatment of peripheral nerve injury

The ultimate goal of research in the field of stem cells is to develop a feasible source of cells for transplantation in patients, the so-called *cell therapy*. This is a mere concept more than an actual therapeutic intervention; several steps need to be fulfilled before its realization, from practical and ethical point of view (Hyun et al., 2008).

Cell therapy follows different goals, based on the chosen target. In animal models of neurodegenerative diseases, the researchers have looked for two aspects when dealing with transplantation: the *cell replacement*, that is, the ability of the implanted cells to survive develop and integrate into the host tissue environment, so as to acquire the anatomical and functional properties of the cells lost as a result of the pathology and the *functional recovery* promoted by the transplanted cells. However, since many neurodegenerative diseases are progressive in nature, a third possible aspect researchers have to deal with is the ability of transplanted cells to promote *protection* of the host's tissue, in order to slow or reverse its degeneration.

In recent years, there has been much interest in the regeneration of the peripheral nervous system (PNS). Traumatic lesions of the peripheral nerves are now rather frequent and could be devastating. The strategies used for repairing these lesions have improved in last decades but they still show many limitations, and often result in an incomplete anatomical and functional restitution of the injured nerve. Thus, there is presently much interest towards the possibility to associate novel surgical procedures to the use of stem cells in order to improve nerve regeneration.

The nerve injury, with or without tissue loss, consists in the interruption of the nerve, with a consequent impossibility for the two parts to communicate, which in turn leads to a loss in functionality.

Repair may be achieved through spontaneous regeneration of severed axons, reconnection of the separate endings and reinnervation of the target tissues, although this is a rather remote event, resulting in very poor or no recovery.

In the absence of spontaneous reinnervation, as it happens, for example, when the injury creates a gap, surgical intervention with nerve repair becomes necessary. The aim of nerve repair is to help fibers regenerate with a minimal loss of tissue, so as to achieve a good recovery of sensory-motor functions. Nowadays, there are several surgical techniques that include the *direct repair* (Matsuyama et al., 2000), *neurotization* (Karol, 2003) and *nerve grafting* (Mafi et al., 2012). Most of these reconstruction strategies, however, have met with only modest success (Matsuyama et al., 2000; Rodriguez et al., 2012). Therefore, recent advances in nanotechnology and tissue engineering have attracted much interest to possibly provide feasible solutions for a broad range of applications in regenerative medicine and to help designing more effective strategies for anatomical and functional repair (Subramanian et al., 2009).

One of the most promising strategies that show good results is *tubulization*. The use of nerve guide has demonstrated strong potential and capacity to circumvent the many problems inherent to the old techniques (Luis et al., 2007). However, even *tubulization* has a series of limitations related, for example, to the size of the gap to be covered, the considerable diameter

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of the scaffold and its slow reabsorbtion rate and, last but not least, the relatively modest anatomical and functional recovery observed.

For these reasons, there has recently been an increasing interest towards the possibility to enrich the nerve guides with cells (Daly et al., 2012).

The goal of the cell therapy in a PNS lesion is not cell replacement *per se* but, rather, the possibility to sustain the regeneration process. Thus, several studies have considered the use of different cell types, such as autologous Schwann cells, associated with the *tubulization* as a good method to improve the nerve regeneration (Udina et al., 2004). Unfortunately, however, these cells have a number of disadvantages associated with their use: their dissection is much invasive and painful, and their growth and expansion in culture is difficult and time-consuming (di Summa et al., 2010).

Recently, an alternative approach has considered the use of stem cells. These cells can create a good environment, by secreting factors that nourish, protect and stimulate both neuronal and non-neuronal cells involved in the regeneration of the severed nerve. Taken together, these features could help accelerating the regenerative process and may result in a better functional recovery (Ding et al., 2010; Kaewkhaw et al., 2011; Oliveira et al., 2010).

Owing to their peculiar functional characteristics (*i.e.* the marked capacity for differentiation, immunomodulation and growth support), MSCs are nowadays considered as one of the most suitable candidates for cell therapy in different diseases.

So far, either bone marrow-derived mesenchymal stem cells (BMMSCs) or adipose-derived stem cells (ASCs) have been tested preclinically. Both ASCs (Kingham et al., 2014) and BMSCs (Ishikawa et al., 2009) possess the ability to secrete multiple neurotrophic factors, including GDNF, NGF, NT-3 and BDNF (Kaewkhaw et al., 2011; Radtke et al., 2009). These cells have therefore been used in a number of studies in both differentiated and undifferentiated states in order to investigate their ability to promote peripheral nerve regeneration (Dezawa et al., 2001; Zarbakhsh et al., 2013).

However, there are several drawbacks that appear to greatly limit the suitability of these donor structures as a source of MSC: first, the procedure of cell isolation is invasive and painful; second MSCs normally occur at relatively low frequency in aspirates, thus many passages are needed to obtain sufficient numbers of cells to be used therapeutically, increasing the risk for cell contamination; third, MSC properties greatly vary with the donor's age. In this respect, WJMSC may represent a much better choice, as they are much easily attainable, exhibit excellent proliferative and differentiation potential, pose no ethical concern, and are of invariant, consistent, young age. However, only recently has interest emerged towards WJMSCs and their promising therapeutic potential (Aguilera et al., 2014; Batsali et al., 2013; Hsieh et al., 2013).

In our lab, we have recently investigated the promotion of the anatomical and functional regeneration of rat sciatic nerve after the formation of a 5 mm gap and the subsequent tubulization with a PLCL (poly DL-lactide-epsilon-caprolactone, Neurolac®, courtesy of Polyganics, Groeningen, The Netherlands) copolyestere tube. The guide was filled with hWJMSCs previously subjected to a predifferentiation treatment using media derived from NSCs culture.

Using a Walking track analysis test administered weekly over 20 weeks post-surgery, we have found significantly better functional recovery in the lesioned animals treated with hWJMSC-loaded PLCL guides, as compared to animals implanted with PLCL guide-only. Assessment of nerve retrograde transport capacity with fluorescent retrograde tracers, and post-mortem morphological analysis of the tubulized nerves confirmed the functional findings, as the nerve treated with hWJMSC-loaded PLCL guides appeared better organized and structured (Fig. 4).

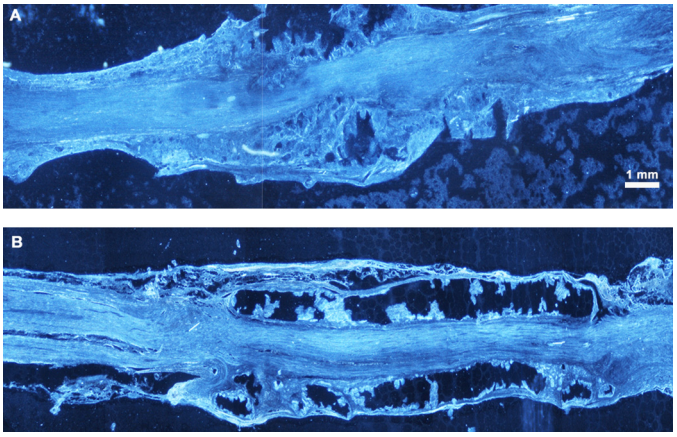

Fig. 4. Unpublished observations: photomicrographs showing the morphology of the regenerated rat sciatic nerve at about 20 weeks following tubulization, with the poly DL-lactide-epsilon-caprolactone guide containing no cells (A) or filled with human Wharton's jelly mesenchymal stromal cells (B). Note the virtually complete nerve reconstruction in (B), which also resulted in a better functional recovery than that achieved by the animal in (A) where nerve regrowth and organization appeared somewhat limited.

Potential of MSC in treatment of neurodegenerative diseases after transplantation

In recent year, several protocols have been developed for the transplantation of MSCs, which can be induced to transdifferentiate into cells of the nervous tissue *in vitro* (Sanchez-Ramos et al., 2000). Transplantation of MSCs, induced to express markers of neuronal differentiation, has shown a functional recovery in models of spinal dysfunction (Cho et al., 2009). Similar results were also obtained previously with undifferentiated MSCs derived from bone marrow (Cizkova et al., 2006) or from vessels of umbilical cord (Saporta et al., 2003), while none of these cell types exhibited a significant neuronal differentiation after transplantation. These results suggest that MSCs may release bioactive factors (Neuhuber et al., 2005) that influence regeneration, turnover and hematopoiesis. This influence was referred to as trophic because their bioactive factor secretion mediates the functional tissue outcome (Caplan and Dennis, 2006). When human mesenchymal stem cells engineered to secrete GDNF (glial cell line-derived neurotrophic factor) were transplanted into the muscles of rats with an ALS-like disease, motor function improved and disease progression was delayed (Suzuki et al., 2008). Compared with direct gene transfer, an advantage of cell-based gene delivery is that production of the trophic factor continues even if the disease process destroys the endogenous cells. Such mechanism has been proposed to underlie the ability of bone marrow-derived mesenchymal stem cells to prevent Purkinje cell death in a neurodegenerative mouse model of cerebellar ataxia (Jones et al., 2010). In this study, the observed increase in surviving Purkinje cells seemed to be the result of at least two processes, possibly responsible for this neuroprotection: the release of neurotrophic factors and, to a much lesser degree, cell fusion, without excluding the possibility of mechanical cell–cell trophic interactions (Jones et al., 2010).

Nowadays, clinical trials using MSCs are being carried out for a variety of important diseases such as stroke, multiple sclerosis, leukaemia and ALS. Clinical outcomes are variable, and generally show small improvements, but to date only few studies have been conducted over a long period of observation. There may be a direct

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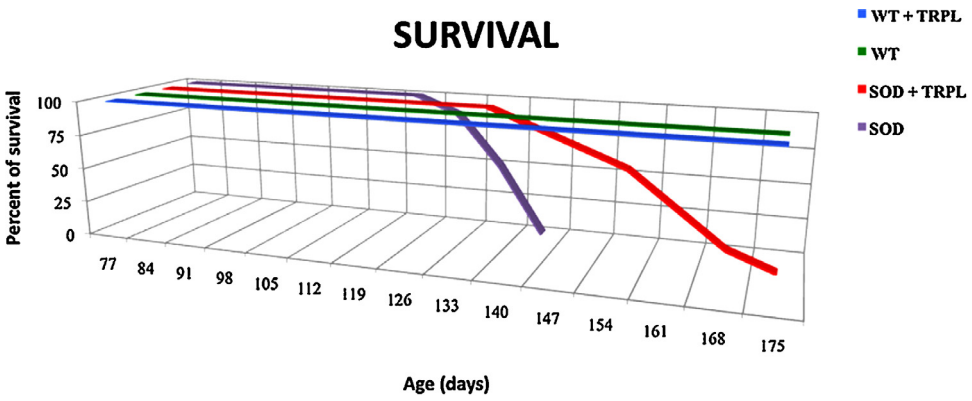

Fig. 5. Unpublished observations: Schematic diagram showing the effects upon survival of the implantation of human Wharton's jelly mesenchymal stromal cells into the lateral ventricle of mice exhibiting an amyotrophic lateral sclerosis-like phenotype. The average life span of SOD1G93A mice implanted with human Wharton's jelly mesenchymal stromal cells (158 ± 13 days) was significantly longer than that exhibited by non-implanted SOD1G93A mice (133 ± 7 days). In the best cell-treated cases, survival reached 171 days.

effect of the infused cells, but long-term clinical MSC engraftement is not yet routinely investigated (Otto and Wright, 2011).

Transplantation of MSC in experimental amyotrophic lateral sclerosis

Human MSC and mouse Bone Marrow cells delay disease onset and increase survival following delivery into the presymptomatic SOD1G93A mouse, an animal model of amyotrophic lateral sclerosis (ALS) (Corti et al., 2004; Ende et al., 2000).

Injected MSCs, however, scantly home to the central nervous system and engraft poorly. This provides support to the hypothesis that the restoring effects of transplanted cells in not due to cell replacement *per se* but, rather, it is associated with the production and release of circulating protective factors that may act both ad the CNS and PNS levels. In fact it has been shown that implanted MSCs release a series of cytokines and chemokines with anti-inflammatory properties that could be responsible of the functional improvement of mouse models of motor neuron degenerative disorder (Bigini et al., 2011; Uccelli et al., 2012).

Along a similar line of evidence, we have recently observed that implantation of hWJMSCs in the lateral ventricle of SOD1G93A mice significantly delayed the onset of the severe functional impairments typically exhibited by these animals and extended their lifespan by about 30–40%, as opposed to non-transplanted mice (Fig. 5).

Again, and in keeping with previous findings (Bigini et al., 2011), the appearance of transplanted cells, never showing morphological features of mature neurons, nor expressing any neuronal markers either, strongly suggest that, as outlined above, they may act *via* the production and release of locally acting factors with protective and/or anti-inflammatory properties, rather than by replacement of degenerating neurons.

If so, it will be necessary to identify the substances produced and released by these cells upon their maintenance in culture, as well as upon their implantation in a diseased host. The possible characterization of these factors, cytokines and chemokines with trophic, disease-modifying, and anti-inflammatory properties could corroborate the hypothesis of neuroprotective immunomodulation as a promising strategy to decelerate the progression of neurodegenerative disease in some cases or improve the regeneration process in others.

Conclusion

The unique biological features exhibited by WJMSCs, associated to their easy isolation and handling, with no ethical or legal concerns, have raised expectations concerning their possible use as disease-modifying agents in cell-based therapies. Certainly, much preclinical work is yet to be done to fully unravel the restorative properties of WJMSCs when implanted in rodent models of nervous system damage or degeneration. Thus, the promising data obtained in early and ongoing studies, some of which reported and discussed in the present review, may represent an important theoretical framework, possibly leading to the eventual clinical application of WJMSCs for the treatment of various dysfunctions in the central and peripheral nervous system.

Conflict of interest

The authors declare that there are no conflict of interests.

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