Mesenchymal Stem Cells in the Umbilical Cord: Phenotypic Characterization, Secretome and Applications in Central Nervous System Regenerative Medicine

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Abstract: Mesenchymal Stem Cells (MSCs), have been defined and characterized by: 1) their ability to adhere to plastic culture flasks; 2) the positive expression of CD105, CD73, CD90 membrane antigens, and the lack of expression of others (e.g CD45 and CD34) and 3) the ability of differentiation under adequate conditions along the osteogenic, chondrogenic and adipogenic lineages. In recent years, cells with these characteristics have been isolated from the Wharton's jelly of the Umbilical Cord (UC). Similarly to bone marrow MSCs, they have shown multilineage differentiation potential and to be able to provide trophic support to neighboring cells. According to the literature, there are two main populations of cells with a mesenchymal character within the human UC: Wharton's jelly Mesenchymal Stem Cells (WJ-MSCs) and Human Umbilical Cord Perivascular Cells (HUCPVCs). In the present work our aim is to make a comprehensive review on MSC populations of the UC and how these cell populations may be used for future applications in CNS regenerative medicine. Following a brief insight on the general characteristics of MSC like cells, we will discuss the possible sources of stem cells within the WJ and the cord itself (apart UC blood), as well as their phenotypic character. As it has already been shown that these cells hold a strong trophic support to neighbouring cell populations, we will then focus on their secretome, namely which molecules have already been identified within it and their role in phenomena such as immunomodulation. The possible applications of these cell populations to CNS regenerative medicine will be addressed by critically reviewing the work that has been performed so far in this field. Finally, a brief insight will be made on what in the authors' opinion are the major challenges in the field for the future application of these cell populations in CNS regenerative medicine.

Keywords: Mesenchymal stem cells, umbilical cord, Wharton's jelly, secretome.

1. INTRODUCTION

The identification of Mesenchymal Stem Cells (MSCs) followed the pioneering work of Alexander Friedenstein and colleagues, who discovered that within the bone marrow compartment existed a population of non-hematopoietic cells with osteogenic potential [1]. Furthermore, he developed a method of isolating these cells based on their adherence to plastic surfaces, reporting that they developed into fibroblast-shaped cell cultures with high replicative and clonogenic potential [2]. Since then, human MSCs (hMSCs), or cells with a MSC-like phenotype have been identified and isolated from several adult and fetal tissues, namely bone marrow [3-5], adipose tissue [6, 7], circulating blood [4, 8], dental pulp [9-11], placenta [12, 13], amniotic fluid [14, 15], umbilical cord blood [16], subendothelial layer of umbilical cord vein [17], umbilical cord Wharton's jelly (WJ) [18, 19], umbilical cord perivascular

layer [20], pancreas [21], liver [4, 5], lung and spleen [5]. They are commonly characterized by: 1) the ability to adhere to plastic culture flasks; 2) the expression in ≥95% of the MSC population of CD105, CD73 and CD90 membrane antigens, and the lack of expression of CD45, CD34, CD14, CD11b, CD79ˍ or CD19 and HLA class II; and 3) the ability of differentiation under adequate conditions into at least osteoblasts, chondrocytes and adipocytes [22, 23].

Despite these common traits, these cells do present subtle changes in their expression profile. Such changes can be the result of tissue-specific environments; however, as already pointed by others, one should also take into account that differences in culture protocols may influence the identity of MSC populations [24, 25]. In addition to CD105, CD73 and CD90, other membrane antigens, namely CD29, CD44, CD51, CD71, CD106, CD166 and Stro-1, have been associated with a MSC-like identity [24, 26]. Besides the derivation towards the osteogenic, chondrogenic and adipogenic lineages [24, 26, 27], the derivation of other mesenchymal cell types, namely myocytes and tenocytes, from MSCs, has also been documented [26, 27]. Interestingly, several reports argued that MSCs can be derived into cell types without a mesoderm/mesenchymal origin, namely neurons or epithelial

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cells, thus disrupting the contingencies of embrionary development [26]. However, while the derivation of epithelial cells seems likely to occur, the data concerning the differentiation into neural cells is rather questionable and mined with both variations in differentiation protocols and lack of conclusive functional studies [26]. As in the case of the phenotypic profile of MSCs, the differentiation potential of each MSC population may be different. Inconsistencies in culture protocols can help explain such variation, but interestingly even when cultured under the same conditions, some MSC populations were shown to have limited capacity to derivate into certain cell types [28-31].

Due to the widespread availability of MSCs and their remarkable ability to differentiate into several mesenchymal lines, they were soon thought to be suitable candidates for the therapeutical repair of lesioned tissues. Hence, several strategies using MSCs, differentiated MSCs, genetically engineered MSCs, or MSCs combined with biomaterials were already developed for the treatment of several lesions, some of them with quite promising results [24, 27, 32]. As an example, bone marrow MSCs were shown to have beneficial effects when transplanted into patients suffering from osteogenesis imperfecta [33, 34]. However, in recent years several studies have reported that MSCs' therapeutical application may be extended beyond tissue transplantation protocols. Indeed, MSCs were found to produce and secrete multiple paracrine factors with therapeutical relevance for their anti-apoptotic, angiogenic, anti-scaring, immunomodulatory and chemoattractive activities [25, 35]. In this context, understanding the role of the various mechanisms involved in the environment of these stem cell "niches" is of the utmost importance to understand not only the concept of stem cell biology but also for the establishment of *in vitro* culture protocols meant for biomedical use. Thus, in addition to the clarification of the phenotypic identity and culture parameters of MSC populations, nowadays is becoming extremely relevant the detailed characterization of MSCs secretome, as the factors secreted by these cells may be the main effectors of their therapeutic action.

Of particular interest is the possible application of the outcomes of MSCs related research for the development of new therapies for several neurodegenerative diseases. When applied to the central nervous system (CNS), MSCs are considered as cytotrophic mediators because their secretome is rich in neurotrophic factors, which promote neurogenesis and increase neuronal survival. In fact, due to its increasing importance, this topic has been addressed in a number of studies, namely dealing with stem cells isolated from the bone marrow (BMSCs) [36-39] and adipose tissue (ASCs) [40-44]. However, in recent studies stem cells isolated from the WJ have also disclosed similar trophic properties. Therefore, the aim of the present review is to summarize published data related to the characterization of the phenotypic identity, immunologic properties and secretome of stem cells with a mesenchymal phenotype isolated from the UCs' WJ. Additionally, we also intend to review experimental data with the purpose of discussing the possible applications of these cells in future therapies dealing with the treatment of central nervous system-related injuries/diseases, namely through mechanisms mediated by their secretome.

2. UMBILICAL CORD MESENCHYMAL STEM CELLS

2.1. Phenotypic Identity

The interest in the umbilical cord as a source of MSCs developed after early studies reporting the isolation of MSC-like cells from the umbilical cord blood [16]. However, the isolation yields for MSCs reported so far have been too low to consider umbilical cord blood MSCs as a clinically viable cell population for therapeutical use [45]. In recent years, other tissues within the umbilical cord were found to be rich in MSC-like cells. The discovery of such MSC niches came to support early studies showing the presence of fibroblast-shaped, plastic-adherent cells in the UC's WJ [46]. Later on, these cells were shown to be capable of chondrogenic derivation under proper differentiation conditions [47].

The definite classification of these cells as MSCs came from the work of Wang *et al.*, who in 2004 described their phenotype and their potential to differentiate along several mesenchymal lineages [18]. Briefly, these cells presented a fibroblast shape in culture, were positive for CD29, CD44, CD51, CD105, SH2 and SH3, and negative for hematopoietic cell markers, namely CD34 and CD45 [18]. Moreover, it was shown to be possible to derive WJ-MSCs into functionally viable adipocytes and osteocytes [18]. Chondrogenic differentiation was also attained, but the expression of chondrocyte-associated markers in an undifferentiated state led the authors to hypothesize that these cells could in fact be prone to derivation along the chondrogenic lineage [18]. Further studies confirmed the phenotypic identity and differentiation potential of WJ-MSCs [19, 48-53] and provided additional data, particularly in respect to the growth pattern of human WJ-MSCs [48]. These cells presented a 6-7 day lag phase and a log phase of 6-8 days. Population doubling time (PDT) ranged from $85\pm7,2h$ in P_0 state to $11\pm1,2h$ in P_7. Morphological analysis however, showed that the isolated population could be in fact constituted by two sub-populations. The authors described cells presenting filapodia with a widened appearance and rich in pancytokeratin, designted type 1 cells, and fusiform-shaped cells with long cytoplasmic extensions but no pancytokeratin expression, which were designated type 2 cells. Moreover, and although both cell types presented chondrogenic, adipogenic and osteogenic differentiation potential, *in situ* labeling showed that type 1 cells were confined to the perivascular areas of the umbilical cord, whereas type 2 cells had a widespread distribution, except for perivascular areas. Based on these results it seems likely that type 2 cells reported by Karahuseyinoglu *et al.* [48], are in fact WJ-MSCs. In addition, type 1 cells showed tendency to diminish in number with subsequent passages and were unable to acquire a neuronal morphology, unlike type 2 cells that when exposed to a neurodifferentiation media did show such ability. Interestingly, both porcine and human WJ-MSCs cultured under neurogenic conditions were shown to acquire a neuronal-like phenotype [48, 54-56]. According to these reports, these cells acquired a neuronal shape with the formation of axon-like processes, and even expressed neuronal or neural stem cell markers including TuJ1, NSE, NFE, MAP-2, NF, NeuN, TH, and glial ones (GFAP, CD11b), both *in vivo* and *in vitro* [48, 49, 54-56]. However, expression of some of these mark-

ers is also found in basal conditions, without exposure to differentiation protocols [54, 55]. Additionally, functional evidences regarding the electrophysiological activity of MSC-derived neurons were not clearly evident [48, 49, 54-56], and therefore further studies need to be done on this topic.

In the case of type 1 cells, prior to the work of Karahuseyinoglu *et al.*, a perivascular MSC-like population had been already described by Sarugaser *et al.*, [20]. These cells were shown to be efficiently isolated from the perivascular region of the umbilical cord, and to have a colony forming unit (CFU) frequency of 1:333 [20]. Furthermore, growth profile was strikingly different from the one reported by Karahuseyinoglu *et al.* for a mixed umbilical cord MSC population mainly constituted by WJ-MSCs [48]. Human umbilical cord perivascular cells (HUCPVC) presented a 24h lag phase, a log phase of at least 96h, and PDT ranged from $59,4 \pm 42,4$h in P_0 to nearly 20h in P_6 [20]. Cells were positive for CD44, CD73, CD90 and CD105, and negative for CD34, CD45, CD106, CD123, CD235a, SSEA-4, HLA-DR, -DP, -DQ, HLA-G, Oct4, and also for HLA-ABC after freezing/thawing [20]. HUCPVCs were found to be highly prone for osteogenic differentiation, even under standard culture conditions, and unable to acquire a neuronal-like phenotype [20]. Subsequent reports showed that HUCPVC have a high expression of CD146 and that the presence of this marker is limited to the perivascular area of the umbilical cord and inexistent in WJ [31]. Furthermore, the derivation of chondrocytes and osteocytes from CD146$^+$ HUCPVCs was also accomplished [31]. Finally Sarugaser and colleagues [57] revealed that HUCPVCs, contributed to rapid connective tissue healing *in vivo* by producing bone, cartilage and fibrous stroma. Additionally, they also exhibited a high clonogenic frequency, allowing the isolation of definitive SCD parent and daughter clones from mixed gender suspensions as determined by Y-chromosome fluorescent *in situ* hybridization [57].

Taken together, these studies suggest that WJ-MSCs and HUCPVCs are in fact two different populations of MSCs isolated from the umbilical cord. Although they share some phenotypic traits and have similar mesenchymal tri-differentiation potential, WJ-MSCs present lower growth rates, lack the expression of pancytokeratin and CD146, seem to be prone for chondrogenic differentiation and are able to acquire a neuronal-like phenotype; whereas HUCPVCs are a fast-growing MSC population, present in the perivascular area of the umbilical cord, with high expression levels of pancytokeratin and CD146, having tendency to derive along the osteogenic lineage and an inability to acquire neuronal-like shape.

Interestingly, other authors proposed the existence of yet another MSC population in the umbilical cord, specifically within the endothelial/subendothelial area of the blood vessels [17]. These cells, with a fibroblast-like morphology, are deprived of hematopoietic and endothelial cell markers but are positive for ASMA and cell adhesion markers [17]. Moreover, derivation into adipocytes and chondrocytes was efficiently accomplished [17]. Further studies complemented the characterization of these cells, for they showed that endothelial/subendothelial MSC-like cells are CD14, CD31, CD34, CD45, CD51/61, CD106, CD117 and HLA-DR negative; CD13, CD29, CD44, CD49d, CD54, CD73, CD90 and HLA-ABC positive; and able to acquire a neuronal-like shape, while expressing several neuronal markers, when cultured in neurodifferentiation medium [58-61]. More recently, Ishige and colleagues have isolated MSCs from umbilical cord arteries and veins, and compared these two subpopulations [51]. No differences were found neither in respect of the phenotypic profile, nor regarding the mesenchymal tri-differentiation ability of the cells isolated from those two sources [51]. However, artery-derived MSCs presented a higher growth rate with lower CFU frequency, whereas vein-derived MSCs presented low growth rate but the highest CFU frequency [51]. Despite these differences the reported data do not suggest that artery and vein-derived MSCs are in fact two separate MSCs populations.

2.2. Immunomodulation

In recent years, and much like to what is already described for BMSCs and ASCs, umbilical cord-derived MSCs were also found to have relevant immunomodulatory activity. WJ-MSCs, for example, were found to be able to hamper both Con-A and PHA-induced proliferation of *in vitro* splenocyte and peripheral mononuclear blood cells (PMBC) cultures [50, 52, 53]. This effect was even found to be higher than the one presented by BMCS [50, 53]. Furthermore, in one-way mixed lymphocyte reactions, WJ-MSCs did not stimulate the proliferation of allogenic T cells, and in two-way mixed lymphocyte reactions WJ-MSCs decreased the level of alloreactivity [50]. Importantly, it was also reported that WJ-MSCs express the mRNA for the immunosuppressive HLA-G; lack the expression of CD40, CD80 and CD86, co-stimulatory receptors essential for immune response; and express mRNA for several cytokines, with IL-6 and VEGF being the most emphasized given their previously shown immunosupressive role [50]. Several other mRNAs for cytokines and proliferation, growth, and anti-apoptotic factors, namely IL-1, IL-8, IL-11, IL-14, BMP1, CSF3, FAM3C, GDF15, PDGFB, TNF-4, -11b and -12, were detected as well [50]. Further studies also reported that WJ-MSCs in basal culture conditions express mRNAs for HGF, TGF-β, COX-1 and COX-2 [52]. In accordance to the results of *in vitro* surveys, *in vivo* studies showed the low immunogenic profile of WJ-MSCs. When transplanted into rat brains, human WJ-MSCs were able to survive for 1 week with no signs of immune reaction against them [19]. Similar results were found after pig WJ-MSCs were transplanted into rat brains [62, 63], thus confirming that these cells can be seen as valid tools for future therapeutic applications.

The immunologic properties of HUCPVCs were also recently studied. Briefly, HUCPVCs were found to promote no proliferation when cultured with either resting or activated allogenic lymphocytes [64]. Additionally, both in one-way and two-way mixed lymphocytes reactions, HUCPVCs were shown not only to reduce alloreactivity but also to attenuate lymphocyte's activity, as seen through the reduction of CD25 expression [64]. Remarkably, the immunosuppressive activity of HUCPVCs was found to be exerted, at least in part, through soluble factors, given that reduced levels of lymphocyte proliferation in mixed lymphocyte reactions were also seen when HUCPVCs and allogenic lymphocytes

were cultured in transwell systems [64]. More recently, our group also showed that conditioned media of these cells were able to reduce the densities of microglial cells, which are important mediators of the immune reaction within the CNS [65].

2.3. Secretome

Proteomic/secretome studies performed on WJ-MSCs are still sparse, although they are of extreme importance for the identification of trophic factors relevant for biomedical application, or cytokines taking part in the immunomodulatory interaction between WJ-MSCs and the immune system. In this respect, Yoo *et al.*, already showed that WJ-MSC-derived culture supernatants present several cytokines and other secreted factors, namely IL-2, IL-6, IL-8, IL-12, IL-15, MCP-1, MIP-1β, RANTES and PDGF-AA [52]. The presence of chemoattractants such as MCP-1, MIP-1β, RANTES and PDGF-AA suggests that WJ-MSCs, and more importantly their secretory activity, can beneficially interact with the immune system by boosting the prompt mobilization of immune cells. The presence of pro-inflammatory cytokines in the conditioned medium of WJ-MSCs [52], as well as the expression of pro-inflammatory cytokine mRNAs [50] are in conflict with the immunosuppressive activity of these cells. Given that expression of mRNA for the immunosuppressive cytokine IL-10 was not found, and no IL-10 was detected in WJ-MSCs conditioned medium, a possible explanation for such results might again be the secretion of TGF-β, whose mRNA expression was detected [52]. Besides that, it is reasonable to think that WJ-MSCs might suffer a change in their secretory profile when exposed to an immunoreactive environment, much like what was already reported for ASCs [66]. Also noteworthy is the fact that no VEGF was found in the secretome of WJ-MSCs [52], suggesting that although VEGF mRNA was detected by Weiss *et al.*, the protein may not be secreted, or even synthesized. Interestingly, some reports in which WJ-MSCs were transplanted into animal models of brain stroke [67] or myocardial infarction [68] have suggested that indeed WJ-MSCs are able to secrete VEGF *in vivo*. Taking into account that no VEGF was found in *in vitro* studies, such results may indicate that on the context of a lesion, where cells are exposed to an inflammatory environment, WJ-MSCs can suffer a change in their secretory profile. Other secretome studies were able to prove the presence of HGF in WJ-MSCS culture medium, as well as appreciable levels of prostaglandin E-2 (PGE$_2$) [53]. Interestingly, in the same report it was shown that stimulation of WJ-MSCs with either IFN-γ or TNF-α, major players in immune responses, does not compromise their low immunogenic profile, nor their ability to attenuate alloreactivity in *in vitro* mixed lymphocytes reactions [53]. Remarkably, the ability of WJ-MSCs to reduce alloreactivity was even increased upon IFN-γ stimulation [53]. These results, although performed *in vitro*, suggest that the immunomodulatory/immunosuppressive activity of WJ-MSCs might not be affected when transplanted into organs or tissues where active immune response is underway. Moreover, since exposure to pro-inflammatory stimuli was able to boost the immunosuppressive character of WJ-MSCs, this report further supports the hypothesis that the immunomodulatory activity of WJ-MSCs, and thus the secretome profile underpinning

such phenotype, might change when cells are exposed to an immunologically active environment. Still, *in vivo* data need to be further provided in order to have a clearer picture on this.

The trophic action of MSCs isolated from the WJ in the perivascular regions has also been shown. For instance, our group has recently revealed how HUCPVCs secretome modulates the action of CNS cells [65]. Moreover, we have recently observed that HUCPCVs also secrete HGF, VEGF, NGF, SCF and FGF (unpublished data). In another study the comparative proteomic analysis of HUCPVCs, placenta-derived MSCs and BMSCs, revealed that HUCPVCs express higher levels of manganese superoxide dismutase (MnSOD) and plasminogen activator inhibitor-1 (PAI-1) [69]. These results suggest that HUCPVCs may have reduced migration ability, given that both these proteins are known to be involved in the migration of cells [69]. Such hypothesis was confirmed through a trans-matrigel migration assay, in which HUCPVCs presented the lowest migration ability [69]. Furthermore, they also presented higher levels of GRP-75, a protein widely implicated in cell proliferation [69], and that can help explaining the higher proliferation rate of HUCPVCs when compared with other MSCs, namely WJ-MSCs.

Regarding MSCs isolated from the endothelial/subendothelial portion of the umbilical cord blood vessels, a gene expression study revealed that these cells present several overexpressed genes when compared to umbilical cord blood MSCs. Among those overexpressed genes are *synpo2* (synaptoidin) and *nrp2*, involved in neurogenesis, and *flt1* (vascular endothelial growth factor – VEGF), which is implicated in angiogenesis [61]. In yet another gene expression comparative analysis was described the high expression of several genes coding for proteins implicated in angiogenesis, namely CXCL6 and IL-8, in endothelial/subendothelial MSCs, when compared to BMSCs [59]. More recently, Koh *et al.*, [60] performed a quite objective analysis of their secretome, targeting growth factors with known neuroprotective role. They were able to identify the presence of relevant amounts of G-CSF, VEGF, GDNF and BDNF. The secretory activity of endothelial/subendothelial MSCs was even higher than that of BMSCs.

2.4. MSCs from the Umbilical Cord and CNS Regenerative Medicine

Much like BMSCs and ASC, WJ-MSCs also present several characteristics that make them suitable alternatives for cell-based therapeutical use. To assess this hypothesis, specifically in the context of CNS lesions, several studies employed WJ-MSCs as experimental treatments. For instance, WJ-MSCs were already experimentally used for the treatment of spinal cord lesions in animal models. After complete transection of the rat spinal cord the transplantation of human WJ-MSCs into the lesion site and both rostral and caudal stumps was able to promote axonal re-growth across the injured area [70]. Remarkably, transplanted animals also presented increased density of neurofilament-positive fibers in the transected area, which was concomitant with significant functional improvements [70]. As discussed elsewhere, one of the major hurdles for the regeneration of spinal cord

injury sites, is the formation of a glial scar [71, 72]. In this respect, WJ-MSC-treated animals showed, when compared to untreated controls, a clear reduction in microglial activation and glial scar formation [70]. Interestingly, three weeks after lesion several cytokines and growth factors were found to be overexpressed in transplanted animals, namely neurotrophin-3 (NT-3) and bFGF, which are known to exert a protective and regenerative action in spinal cord lesions [70, 73, 74].

The beneficial action of WJ-MSCs was also reported in experimental models of cerebral ischemia. Rat WJ-MSCs were shown to have a protective action when transplanted three days before a cardiac arrest-induced global ischemia [75]. When unilaterally injected into the dorsal thalamic nucleus, dorsal hippocampus, corpus callosum and dorsal cortex, these cells significantly reduced the extent of neuronal damage in the hippocampus [75]. Remarkably, such effects were seen in both ipsilateral and contralateral hemispheres [75]. To further understand the impact of the treatment with rat WJ-MSCs, another study used the same experimental design and assessed the levels of astrocytic and microglial activation [76]. As hypothesized by the authors, the reduction in Vimentin (VIM)- and Nestin (NES)-positive cells in treated animals suggests a decrease in inflammatory reaction after global ischemia. These markers are usually overexpressed during astrocytosis; however, they are also present in neuronal precursors. Although no double stainings for VIM/NES and specific astrocyte markers were performed, significant positive correlations between VIM/NES staining and neuronal damaged were reported [76]. Additionally, regarding microglia response to global ischemia, the authors describe a decrease in activation, positively correlated with reduced neuronal damage [76]. Besides rat WJ-MSCs, several lines of evidence have reported that human WJ-MSCs may also be beneficial for the treatment of brain ischemia. Ding *et al.*, described that in stroke-subjected rats the transplantation of human WJ-MSCs is able to promote functional recovery of behavioral deficits, as seen through locomotor activity evaluations and neurochemical imaging techniques [49]. Furthermore, transplanted animals presented reduced lesion size and a higher extent of vascularization in ischemic areas [49]. An also important result came from the co-transplantation of WJ-MSCs with a β1-integrin antagonist. As reported, β1-integrin levels were increased in WJ-MSCs treated animals; however, the addition of such antagonist blocked both the functional recovery and the angiogenic process, showing the direct implication of β1-integrin in the recovery of ischemic tissues [49]. Still in the same study, higher expression of SDF-1, BDNF and GDNF was found in ischemic tissues following WJ-MSCs treatment, which suggests that these cells have the ability to induce an upregulation in molecular pathways implicated in neuroprotection and angiogenesis.

Similar results were also described in studies using human MSCs isolated from the endothelial/subendothelial portion of umbilical cord blood vessels [60] or a mixed population of umbilical cord-derived MSCs, certainly comprising WJ-MSCs, HUCPVCs and endothelial/subendothelial MSCs [67]. In the later, besides a significant functional recovery and reduction of the ischemic area, the authors also reported

increased levels of VEGF and bFGF near lesioned areas [67]. Importantly, immunohistochemical analysis strongly suggested the involvement of transplanted cells in the secretion of the factors [67].

Common to some studies was the presence of labeled WJ-MSCs expressing neuronal and glial markers, suggesting the *in vivo* differentiation of these cells towards those lineages [49, 60, 67]. The possibility of cell fusion cannot be excluded, but even if differentiation occurs, the incidence of such events so far reported is certainly too low to account for the positive action of the transplantation protocols. Instead, given the growing body of evidence proving that umbilical cord-derived MSCs secretome is rich in growth factors, the trophic activity of these cells seems to be much more relevant for their *in vivo* impact.

Apart from spinal cord lesions and brain ischemia, the therapeutical potential of umbilical cord-derived MSCs for the treatment of Parkinson's disease was already assessed. In a 6-OHDA-injected hemiparkinsonian rat model, the transplantation of human WJ-MSC into the striatum was able to promote functional recovery at the level of the dopaminergic system, as seen by the reduction in apomorphine-induced turning behavior [19]. Moreover, the authors also described improvements at the histological level, where increased staining for tyrosine hydroxylase was found in WJ-MSC-transplanted animals. Noteworthy is the fact that the authors reported an absence of WJ-MSCs three months post-transplantation on the injected sites, and the inexistence of neurons/glial cells differentiated from them.

Finally, data regarding the possible applications of HUCPVCs in the CNS is scarcer than that concerning WJ-MSCs use. Nevertheless, our group has recently shown that HUCPVCs conditioned media, and thus their secretome, increase glial cell viability and proliferation [65]. Furthermore, it was also observed that glial cell cultures exhibited higher numbers of GFAP positive cells (astrocytes) and O4 positive cells (oligodendrocytes) when incubated with the CM. Additionally, it was also observed that the growth factors present in the CM did not induce an increase on the microglial cells number, as mentioned earlier. For hippocampal neurons similar results were obtained, as cultures exposed to HUCPVCs CM disclosed higher numbers of MAP-2 positive cells. Moreover, it was also observed that the cell viability and proliferation in this primary hippocampal cell culture system were also higher, when compared to control cultures. Therefore, these experiences allowed us to conclude that the secretome of these cells may have a modulatory effect on CNS cells [65]. Moreover, current experiments performed in our lab have shown that the CM of HUCPVCs is able to induce an increase of GFAP, MAP-2 and nestin positive cells (without co-localization with HUCPVCs) when injected in the rat hippocampus (unpublished data). Finally we were also able to observe a similar trend to that reported by Weiss *et al.* [19] when HUCPVCs were transplanted into a unilateral 6-OHDA-lesioned PD rat model (unpublished data).

CONCLUDING REMARKS

There are now several studies strongly suggesting that MSCs isolated from different areas of the umbilical cord, other than the blood, present low immunogenic potential and

an ability to modulate the activity of immune cells to the extent of decreasing immune reaction towards allogenic cells. Additionally, there is also robust evidence on the beneficial actions of these cells in different *in vitro* and *in vivo* CNS models of survival, proliferation, differentiation and injury. On the light of these discoveries, these MSC populations might be good candidates for future clinical applications within CNS regenerative medicine. The expression of several growth factors also raises the possibility that these cells can be further used to exert protection/differentiaton under the same context. However, and contrary to what happens with BMSCs and ASCs, the proteome and secretome of these umbilical cord derived MSCs are still poorly studied and more experiments need to be conducted in order to further characterise their properties. Moreover, it would be also interesting to study how these cells integrate within the neuro-oncology field. For instance, a number of recent papers have revealed the tropism of MSCs towards malignant tumours, making these cells a potential vehicle for delivery of therapeutic genes to disseminated glioma cells. Finally *in vivo* studies should be designed in order to adequately assess if the histological improvements frequently observed, are matched by relevant functional outcomes. By doing so it will be possible to better understand the true potential for these cells to be used in CNS regenerative medicine.

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