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Umbilical Cord Mesenchymal Stem Cells: The New Gold Standard for Mesenchymal Stem Cell-Based Therapies?

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Due to their self-renewal capacity, multilineage differentiation potential, paracrine effects, and immunosuppressive properties, mesenchymal stromal cells (MSCs) are an attractive and promising tool for regenerative medicine. MSCs can be isolated from various tissues but despite their common immunophenotypic characteristics and functional properties, source-dependent differences in MSCs properties have recently emerged and lead to different clinical applications. Considered for a long time as a medical waste, umbilical cord appears these days as a promising source of MSCs. Several reports have shown that umbilical cord-derived MSCs are more primitive, proliferative, and immunosuppressive than their adult counterparts. In this review, we aim at synthesizing the differences between umbilical cord MSCs and MSCs from other sources (bone marrow, adipose tissue, periodontal ligament, dental pulp,…) with regard to their proliferation capacity, proteic and transcriptomic profiles, and their secretome involved in their regenerative, homing, and immunomodulatory capacities. Although umbilical cord MSCs are until now not particularly used as an MSC source in clinical practice, accumulating evidence shows that they may have a therapeutic advantage to treat several diseases, especially autoimmune and neurodegenerative diseases.

Introduction

Mesenchymal stromal cells (MSCs) are attractive cells due to their capacity of long-term *ex vivo* proliferation, multilineage differentiation potential, and immunomodulatory properties. These cells were first identified and isolated from the bone marrow (BM) and have emerged as powerful tools in tissue engineering and regeneration.[1] Although adult BM is the most common and best-characterized source of MSCs, Wharton's jelly (WJ) of the umbilical cord provides a novel source of MSCs with higher accessibility and fewer ethical constraints than BM holding great promise as an alternative. WJ in an extra-embryonic tissue that is easily obtained after birth, and it has initially been described by Thomas Wharton in 1656.[2] While the isolation of MSCs from BM requires an invasive procedure for the donor, MSCs can be noninvasively isolated from WJ.[3] These WJ-MSCs are believed to be more primitive than MSCs derived from more mature tissue sources and to have intermediate properties between embryonic and adult stem cells.[4] Moreover, WJ-MSCs are available in potentially large quantities, have a fast proliferation rate, a great expansion capability, do not induce teratomas, and harbor strong immunomodulatory capacities.[5,6]

In this review, we will focus on the similarities and differences between WJ-MSCs and MSCs from other sources with regard to their proliferation, their surface markers, and their transcriptome profiles. The controversy between their paracrine effects and trans-differentiation potential will be discussed. In addition, we will particularly highlight their roles as (a) immunomodulators and the mechanisms involved in their immunosuppressive properties, as (b) antitumor agents, (c) migratory curative cells with (d) a special emphasis on their clinical and therapeutic applications in autoimmune and neurodegenerative diseases.

Main Features of WJ-MSCs

Isolation methods

Isolation of MSCs from WJ requires complex processing. Many isolation and expansion protocols have been demonstrated for a fast and efficient *ex vivo* generation of large quantities of cells. Currently, ''enzymatic digestion'' and ''tissue explant'' are the two types of methods used for the isolation of WJ-MSCs.[7]

Those based on enzymatic digestion have mainly used collagenase alone or in combination with other enzymes

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(e.g., trypsine, hayluronidase) and were performed with or without the dissection of the umbilical cord into small pieces and with or without removing the blood vessels.[8] Recently, Han *et al.* have suggested that using 0.2% collagenase II at 37°C for a digestion of 16–20 h is an effective and simple enzyme digestion method.[7] Other groups have found that enzymatic digestion can alter cell population and function and, thus, have developed explant approaches without using any enzyme and taking advantage of the ability of MSCs to migrate from the tissue to adhere on the plastic.[8–10] Hua *et al.* have, very recently, compared three explant and three enzymatic methods with regard to time of primary culture, cell number, cell morphology, immune phenotype, and differentiation potential of WJ-MSCs. They have shown that the 10 mm-size tissue explant method was the optimal protocol for the isolation of MSCs.[11]

Morphology and proliferation capacity

WJ-MSCs cultured *in vitro* shared a similar fibro-blastoid shaped morphology to BM, amniotic fluid (AF), or teeth and periodontal ligament (PDL)-MSC.[12,13]

The proliferation capacity of cells is important regarding their application potential in cell therapy and tissue engineering. WJ-MSCs proliferation capacity seems to be different from other sources MSCs. Indeed, for instance, Yu *et al.* have shown that over a period of 7 days after seeding, WJ-MSCs grew much faster than PDL-MSC and had a cell doubling time of 22.23 h against 27.51 h for PDL-MSC.[14] Compared with BM-MSCs, WJ-MSCs grow much faster for the early passages and have a cell doubling time (24 h) almost twice shorter than BM-MSCs (40 h) over the 1st passage. These observations were confirmed by Abu Kasim *et al.* showing that WJ-MSCs and dental pulp-mesenchymal stem cells (DP-MSCs) were highly proliferative as compared with BM and adipose tissue (AT)-MSCs.[15] Furthermore, WJ-MSCs have a greater ability to form colony-forming unit-fibroblasts colonies *in vitro* than BM-MSCs, and their formation's frequency depends on seeding cell density.[4,16]

Other studies focusing on DP-MSCs showed that cells from both sources (WJ and teeth) initially grew slowly but their proliferation rates were increased after the first subculture.[17] However, WJ-MSCs growth is influenced by the number of culture passages *in vitro, as* amplifying these cells until passage 10 will result in a slower cell growth compared with the same cell culture at passage 5.[18]

A very recent study has evaluated the proliferation kinetics and phenotypic characteristics of MSCs derived from WJ and AT during prolonged *in vitro* expansion and found that WJ-MSCs were isolated with a high efficiency and bore a substantially increased proliferation capacity; whereas AT-MSCs exhibited a reduced proliferation potential showing typical signs of senescence at an early stage.[19]

Marker expression at protein level

A large number of studies have analyzed the surface markers of WJ-MSCs and compared their expression profiles with other sources of MSCs such as BM, teeth, or AF.

Table 1. Phenotypic Profile of WJ-MSCs Compared with MSCs from Other Sources

WJ-MSCs markers	*Compared with []*	*References*
Positive: CD29, CD105, HLA-ABC, Oct-4, Gata-4, Cx43, α-actin, cTnt Negative: CD34, HLA-DR	[AF-MSCs] Similar marker expression except Oct-4: ~25% for WJ-MSC vs. ~51% for AF MSC	18
Positive: CD44, CD13, CD56, CD61, CD73, CD105, CD90, CD166, CD29, HLA-ABC, CD59 Negative: HLA-DR	[dental pulp of milk and adult wisdom teeth-derived MSCs] Similar marker expression	17
Positive: CD73, CD105, CD90, Negative: CD34, HLA-DR, CD45, CD19, CD11b	[PDL-MSCs] Similar marker expression	14
Positive: CD68 Negative: CD34, CD45, CD163	[promyelocytic cell line (HL-60): known to express CD68] Similar level expression	20
Positive: CD13, CD29, CD44, CD105, CD106, CD73, CD166, HLA-ABC, CD90 Negative: CD14, CD34, CD38, CD45 CD31, HLA-DR	[Bone marrow MSCs] Similar marker expression except: • CD106: WJ < < < BM • HLA-ABC: WJ < < BM	13,16,21
Positive: CD105, CD146, CD73, CD90 Negative: CD14, CD34, CD31, CD45, CD3	[human MSCs from: tibial plateau (TP), trabecular bone, iliac crest (IC), BM, and WJ umbilical cord] Similar level expression for all markers except CD46 (twice more expressed for IC than for WJ and TP)	22
Positive: CD44, CD73, CD105, CD90, CD106, CD29, vimentin, laminin, Oct-4, Nanog, Negative: CD34, CD14, CD45, CD31, vWF	[Adult and fetal bone marrow (aBM-MSCs and fBM-MSCs) and adipose tissue-derived MSC (AT-MSCs)] Similar marker expression except Oct-4 and Nanog expressed only by BM-MSCs and WJ-MSCs	23

AF, amniotic fluid; AT, adipose tissue; BM, bone marrow; IC, iliac crest; MSCs, mesenchymal stromal cells; PDL, periodontal ligament; TP, tibial plateau; WJ, Wharton's jelly.

The following table summarizes the phenotypic profiles of these MSCs mentioned in the literature (Table 1).

WJ-MSCs, such as MSCs from other sources, positively express the classical mesenchymal surface markers. However, Table 1 highlights the differences in the expression levels of other markers:

- Unlike BM-MSCs, WJ-MSCs weakly expressed endoglin (SH2, CD105) and CD49e at passage 8.
- WJ-MSCs and AT-MSCs expressed CD106 at much lower levels than BM-MSCs.
- In comparison with BM-MSCs, HLA-ABC is very weakly expressed by WJ-MSCs, suggesting that these cells could be good candidates for allogeneic cell therapy.

Transcriptomic profile

Emerging data have compared the transcriptomic profile of WJ-MSCs with MSCs from other sources. The following table gives an overview of the main comparisons (Table 2).

Some studies showing a high expression of embryonic genes such as *LIFR, ESG1, SOX2, TERT, NANOG, POUF1, OCT4, LIN28, DNMT3B,* and GABRB3 by WJ-MSCs suggest that WJ could be a more primitive source of MSCs.[4,6,24–26] Furthermore, as shown in Table 2, WJ-MSCs express genes encoding for proteins that are associated with morphogenesis: *SHH*, *neuregulin-1* and 4, *SNA2*, and *WNT4*.[27]

WJ-MSCs, compared with MSCs from other tissues, differentially express genes involved in bone development. Transcription factors involved in osteoblast differentiation such as *RUNX2* were found to be expressed at comparative levels in BM-MSCs, skin-MSCs, AT-MSCs, and WJ-MSCs. However, Table 2 shows that skin-MSCs are characterized by a significantly increased expression of genes (*BMP4, BMP2*) that are associated with bone and cartilage development in comparison to the other MSCs.

WJ-MSCs reveal an important expression of genes involved in liver and cardiovascular development. The transcriptomic profile of WJ-MSCs and AF-MSCs reveals the basal expression of several mature myocardial genes: *GATA-4*, *c-TnT*, and *Cx43*, which could be associated to the potential of differentiation into myocardial cells. Interestingly, a high expression of genes encoding for GATA-binding protein 6 (*GATA6*), heart and neural crest derivatives expressed 1 (*HAND1*), inflammatory cytokine-induced intercellular adhesion molecule 1 (*ICAM1*), and vascular cell adhesion molecule 1 (*VCAM1*) was detected in WJ-MSCs (Table 2). WJ-MSCs were also shown to express genes involved in cardiovascular system development, including angiogenesis, cardiogenesis, endothelial cell (EC) development, and vasculogenesis (Table 2). In addition, other genes involved in cardiovascular development, including endoglin (*ENG*), *GJA1*, *VCAM1*, and *GATA6*, were significantly increased in BM-MSCs.[28]

The trancriptomic profile also reveals that WJ-MSCs have a significantly increased expression of genes (*AFP, DKK1, DPP4*, and *DSG2*) which are associated with liver development compared with BM-MSCs, AT-MSCs, and skin-MSCs.

WJ-MSCs express genes involved in neural development. WJ-MSCs and DP-MSCs revealed a high expression of the neuro-ectoderm lineage markers.[15] De Kock *et al.* have studied the whole gene expression profiles of four human mesoderm-derived stem cell populations: AT-MSCs, BM-MSCs, skin-MSCs, and WJ-MSCs. They have shown differences in gene expression between distinct stem cell types. Skin-MSCs predominantly expressed genes involved in neurogenesis (NES), skin, and bone (RUNX2, BMP4).[28]

Such a transcriptomic profile reveals a closer proximity between WJ-MSCs and BM-MSCs than between other combinations. Considering the genomic profile of WJ-MSCs, WJ may be considered a reliable source of MSCs useful not only in cardiovascular regenerative medicine[30,31] but also in neurodegenerative diseases. The latter will be discussed in the last part of the review.

Regenerative role of MSCs: differentiation potential versus secretome

A summary of the various comparisons between sources of MSCs that have been already described in the literature is shown in Figure 1.

WJ-MSCs differentiate into adipocytes slower than BM-MSCs.[4] Bai *et al.* have shown that AF-MSCs and WJ-MSCs could differentiate into myocardial-like cells with an important expression of myocardial genes such as GATA-4, c-TnT, α-actin, and Cx43 after myocardial induction.[18]

More recently, Chen *et al.* have worked on *in vitro* differentiation analysis of MSCs isolated from DP and WJ. They have shown that MSCs isolated from both sources exhibited the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes. However, they have noted some differences in their differentiation potentials. DP-MSCs and WJ-MSCs had a similar potential for osteogenic differentiation, but the chondrogenic and adipogenic differentiation potentials of WJ-MSCs were more important than those of DP-MSCs.[17] Meanwhile, according to Zhang *et al.*, fetal human BM-MSCs have the highest potential of *in vitro* monolayer osteogenic differentiation, come after human WJ-MSCs, human adult BM-MSCs, and then AT-MSCs.[23] Baksh *et al.* have found similar results as the previous study when comparing the *in vitro* differentiation potentials of WJ-MSCs and BM-MSCs.[44]

Jo *et al.* have studied the *in vivo* osteogenic differentiation, in a rat model, of human MSCs isolated from different sources. No differences were detectable in osteogenesis between adult AT-MSCs, BM-MSCs, and WJ-MSCs.[45] Controversial results have been described by Zhang *et al.* In fact, after a subcutaneous implantation of MSCs scaffolds in mice, better results were obtained with scaffolds elaborated with human fetal and adult BM-MSCs than those constructed with WJ-MSCs and AT-MSCs.[23] Differences in the results between the mentioned studies are probably due to the different experimental conditions. This explains that MSCs of various tissue origins have specific characteristics of differentiation or require different conditions for osteoinduction.

Table 2. Transcriptomic Profile of WJ-MSCs Compared with MSCs from Other Sources

Gene	*Gene identification/function*	*WJ-MSCs*	*BM-MSCs*	*PDL-MSCs*	*AF-MSCs*	*AT-MSCs*	*Skin-derived MSCs*	*References*
Genes related to bone development and neurogenesis								
BMP4	Induce endochondral osteogenesis	++	+	ND	ND	+	+++	28
TGFBR1	Bone development	++	+	ND	ND	ND	ND	27
OPN	Osteogenic marker	++	++	+++	ND	ND	ND	14,27
STAT1	Bone development	+	+	ND	ND	ND	ND	22
BSP	Osteogenic marker	−	ND	++	ND	ND	ND	14
OSX	Osteogenic marker	−	ND	−	ND	ND	ND	14
CXCR4	Mesoderm marker	+	+	ND	ND	ND	ND	27
BMP2	Bone development	+	++	ND	ND	+	+++	28
RUNX2	Osteogenic marker	++	+++	ND	ND	++	++	22
CDH2	Neural gene	++	+	ND	ND	+	−	27
NES	Neural development	++	++	ND	ND	−	+++	28
Gene related to liver and cardiovascular systems								
GATA-4	Mature myocardial gene	+	+	ND	+	+	++	18,28,29
c-TnT	Mature myocardial gene	+	ND	ND	+	ND	ND	18
VEGF	Cardiovascular development	+	+	ND	ND	ND	ND	27
Cx43	Mature myocardial genes	+	ND	ND	+	ND	ND	18,20
VCAM1	Cardiovascular development	++	+++	ND	ND	−	−	28
GJA1	Cardiovascular development	++	+	ND	ND	+	++	28
AFP	Liver development	+	−	ND	ND	++	+	28
DSG2	Liver development	++	−	ND	ND	+	−	28
ENG	Cardiovascular development	++	+++	ND	ND	−	−	28
HAND1	Cardiovascular development	++	+	ND	ND	+	++	28
GATA6	Cardiovascular development	++	−	ND	ND	+	−	28
DPP4	Liver development	+	−	ND	ND	++	−	28
DKK1	Liver development	+	−	ND	ND	++	−	28
Gene related to stemness								
ACTG2	Stemness-related genes	+	++	ND	ND	ND	ND	27
TERT	Stemness-related genes	++	+/−	ND	ND	ND	ND	4,27
ESG1	Stemness-related genes	++	++	ND	ND	ND	ND	27
Oct3/4	Stemness-related genes	+	++	ND	ND	ND	ND	27
ABCG2	Stemness-related genes	+++	++	ND	ND	ND	ND	27
LIFR	Stemness-related genes	+++	+++	ND	ND	ND	ND	27
SOX2	Stemness-related genes	+	ND	+	ND	ND	ND	14,27
Nanog	Stemness-related genes	+	ND	++	ND	ND	ND	14
Oct-4	Stemness-related genes	++	++++	++	ND	ND	ND	14
THY1	MSCs marker	++	+	ND	ND	+	−	28
Adipogenic and chondrogenic genes								
PPARγ	Adipogenic marker	+++	+++	+++	ND	ND	ND	13,14
FABP4	Adipogenic marker	+	++	ND	ND	ND	ND	13
LPL	Adipogenic marker	−	++	+	ND	ND	ND	13,14
LEPR	Adipogenic marker	−	+	ND	ND	ND	ND	13

(*continued*)

Table 2. Continued

Gene	*Gene identification/function*	*WJ-MSCs*	*BM-MSCs*	*PDL-MSCs*	*AF-MSCs*	*AT-MSCs*	*Skin-derived MSCs*	*References*
CD36	Adipogenic marker	−	ND	+	ND	ND	ND	14
CEBPA	Adipogenic differentiation	+	ND	++	ND	ND	ND	14
COL2	Chondrogenic marker	+	+	+	ND	+	+	14
SOX9	Chondrogenic marker	+		+	ND	ND	ND	14
Genes implicated in morphogenesis, adhesion, cell structure, and other mesodermal markers								
ACTA	Maintains the cytoskeleton	+	ND	ND	+	ND	ND	18
ACTB	Cell motility, structure, and integrity	+++	+	ND	ND	ND	ND	27
CDH5	Controls the cohesion and organization of the intercellular junctions in endothelial cells	++	+	ND	ND	+	+	28
ITGB1	Extracellular adhesion molecule	++	++	ND	ND	ND	ND	18
STAG1	Encodes for component cohesion	+	+	ND	ND	+++	++	28
WT1	Kidneys and gonads development	++	−/+	ND	ND	−/+	−/+	28
WNT4	Associated with morphogenesis	+	+	ND	ND	ND	ND	18
SNA2	Associated with morphogenesis	++	++	ND	ND	ND	ND	18
SHH	Associated with morphogenesis	−/+	+	ND	ND	ND	ND	18
Neuregulin 4	Associated with morphogenesis	++	++	ND	ND	ND	ND	18
COL1A1	Mesodermal marker	+++	+++	ND	ND	−	−	28
CXCR4	Mesoderm marker	+	+	ND	ND	ND	ND	18
ICAM1	Mesodermal lineage specification	+++	+	ND	ND	+	+	28
CD44	Mesoderm marker	++	++	ND	ND	ND	ND	18
PECAM1	Cell adhesion marker	++	+/−	ND	ND	+/−	+	28
CD9	Implication in differentiation, adhesion, and signal transduction	+	−	ND	ND	++	++	28
Collagen X	Mesoderm marker	−	+++	ND	ND	ND	ND	18
FlK-1	Mesoderm marker	++	−	ND	ND	ND	ND	18
CD68	Highly expressed by human monocytes and tissue macrophages	+++	ND	ND	ND	ND	ND	20

The table given next presents the difference of gene expression, for various markers, between the different MSCs sources. The difference of expression between the sources is presented by+++, ++, +, −, and ND corresponds to a nondetermined comparison.

PPIA, cyclophilin A, Homo sapiens peptidylprolylisomerase A; ITGB1, integrin, b1:fibronectin receptor, b polypeptide; WNT4, wingless-type MMTV integration site family, member 4; SHH, sonic hedgehog homolog (Drosophila); SNAI2, snail homolog 2 (Drosophila); SNAI2, snail homolog 2 (Drosophila); TGFBR1, transforming growth factor, β receptor I; TERT, telomerase reverse transcriptase; ESG1:ESTs, weakly similar to embryonal stem cell specific gene 1; Cx43, connexin-43; ACTA, alpha-actin; ACTB, beta actin; CDH2, cadherin 2; OPN, osteopontin; LIFR, leukemia inhibitory factor receptor: SOX2, sex-determining region Y (SRY)-box; ND, nondetermined.

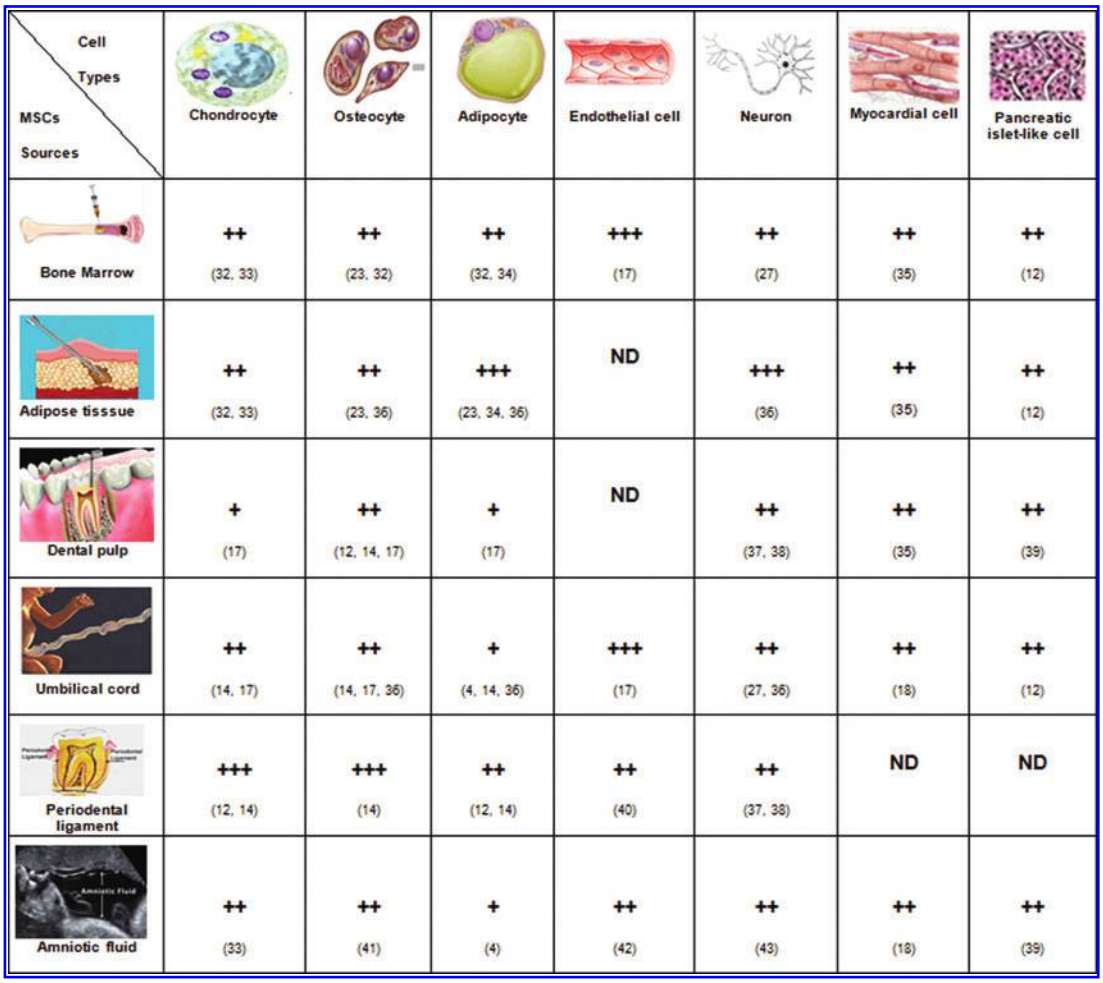

FIG. 1. Differentiation potential of WJ-MSCs compared with MSCs from other sources. This figure shows differences in the differentiation potential of MSCs from many sources toward a specific cell type, which is indicated by + + +, + +, +, −, and ND corresponds to a nondetermined comparison. For example, PDL-MSCs have the greatest potential to differentiate into chondrocytes, while DP-MSCs have the lowest differentiation potential toward this type of cells. DP, dental pulp; WJ-MSC, Wharton's jelly-mesenchymal stromal cell. Color images available online at www.liebertpub.com/teb

Very recently, Yu *et al.* have shown that WJ-MSCs are not good alternatives for periodontal tissue generation compared with PDL-MSCs which have a much better osteo/dentinogenic differentiation potential.[12,14] Various studies have demonstrated the capacity of WJ-MSCs to differentiate into pancreatic islet-like cells.[46–48] Kim *et al.* have compared this potential with other sources of MSCs and did not show significant differences between BM-MSCs and AT-MSCs.[46]

Some authors have focused on the application of MSCs in vascular engineering and more particularly their capacity to differentiate into EC, or to acquire pericyte markers when co-culturing with EC. Chen *et al.* were pioneers in studying the endothelial differentiation potential of BM-MSCs and WJ-MSCs. Both sources of MSCs were able to differentiate into EC but WJ-MSCs appear to have a greater differentiation potential, as derived EC-like exhibited a higher expression of endothelial markers.[5]

For a long time, it has been considered that the regenerative potential of MSCs is due to their plasticity and differentiation capacity. However, the direct link between their differentiation potential and their beneficial effects has never been proved. Indeed, recent studies suggest that the benefits of MSCs transplantation may be associated to a paracrine modulatory effect rather than the replacement of affected cells, at the site of injury, by differentiated stem cells.[49,50] Emerging data suggest that stem cells could be then considered as a reservoir of trophic factors which are released when needed to modulate and repair surrounding damaged tissues, which leads to a paradigm shift in regenerative medicine. Understanding the cell secretome has attracted much attention, and it has been demonstrated that trophic factors could have many effects such as modulation of inflammatory reactions, immunomodulation, anti-apoptotic and pro-angiogenic capacities, and many others (reviewed in Doorn *et al.*[51]). Vallone *et al.* have highlighted in their review the exact mechanisms that would lead MSCs to damaged tissues after transplantation, where they will exert their remedial actions.[52] Katsuda *et al.* have also described a possible therapeutic mechanism of AT-MSCs, in Alzheimer disease, through a paracrine pathway. Vesicules secreted by these cells could carry soluble factors that may treat this pathology. Results of this study will be discussed

in the final part of this review. Therapeutic effects of BM-MSCs in regenerative medicine (heart disease for example) through paracrine/autocrine mechanisms have been reviewed by Pourrajab *et al.*[53]

The controversy between the implication of the differentiation potential and the paracrine mechanisms of MSCs in their beneficial therapeutic actions is shown in Figure 2.

During the next few sections of this review, we will highlight the effects of WJ-MSCs secretome involved in many processes such as immunomodulation, homing to damaged tissues and others.

WJ-MSCs as Immunoprivileged Cells

Immunological features of MSCs

In the last decade, MSCs have gained considerable attention as candidates for tissue engineering, as modulators of immune responses in graft-versus-host disease, and as autoimmune diseases,[54] as these cells, once administered therapeutically, may be able to evade the immune system of the host. They are currently being assessed as a novel anti-inflammatory therapeutic agent in numerous clinical trials.[55] Two outstanding features of MSCs are relevant to their immunomodulatory effects:

Immunosuppression. MSCs-mediated immunosuppression describes the fact that MSCs are able to suppress several functions (proliferation, production of soluble factors, and cellular cytotoxicity) exerted by diverse immune cells such as T-, B-, and natural killer (NK) cells. It has been shown that immunosuppression is mediated by both cell–cell contact and paracrine signals via soluble factors.

Immunoprivilege. MSCs themselves are somehow protected from immunological defense mechanisms.[56] Indeed, MSCs lack expression of major histocompatibility complex (MHC) class II, giving MSCs the potential to escape recognition by alloreactive $CD4^+$ T cells but express MHC Class I molecules. This expression enables them to escape from NK cell lysis, In addition, MSCs do not express co-stimulatory molecules required for effector T-cell induction.[57]

Even if BM-MSCs, considered the gold standard in MSC therapy, and UC-MSCs share many similarities, emerging data suggest that WJ-MSCs could be less immunogenic than BM-MSCs, making them a good candidate for allogeneic transplantation.

MSCs-mediated immunosuppression

MSCs show an absence or a low expression of MCH class II and co-stimulatory molecules, so they can be considered immunoprivileged cells, but they also interfere with different pathways of the immune response.[58] Their ability to modulate the immune system was first recognized after the fact that they could evade immunosurveillance after cell transplantation.[59] Especially, human MSC populations such as BM-, AT-, or UC-derived MSCs selectively alter immune cell function by suppressing T-cell proliferation, B-cell proliferation, and terminal differentiation,[60] inhibiting NK cell proliferation and cytotoxicity, steering monocytes and dendritic cells (DCs) to an immature DC state.[61]

MSCs and immune cell population. Adaptive immunity: *MSCs and T cells:* T cells recognize antigens and are critical for cell-mediated immune response. They mature within the thymus into one of different subtypes with diverse roles. These cells are involved in the maintenance of self-tolerance, activation of other lymphocytes, lysis of infected cells, and interaction with cells of the innate immune system.

Currently, interactions of MSCs with T cells have been extensively studied. Graft versus Host Disease models presented the first evidence that MSCs can regulate immunosuppression *in vivo*.[62] MSCs could reduce allograft rejection, which is partly mediated by T cells.[63,64] Shortly afterward, T-cell immunosuppression mediated by MSCs was demonstrated *in vitro*. MSCs probably inhibit, via their induced or constitutively expressed secreted factors, T-lymphocyte activation and proliferation induced by mitogens and alloantigens[65–68] as well as T-cell activation with CD3 beads.[66,69] MSCs have been shown to equally inhibit $CD4^+$, $CD8^+$, $CD2^+$, and $CD3^+$ subsets.[70] In addition, T-lymphocytes inhibited by BM-MSCs do not enter apoptosis, as they actively proliferate on re-stimulation with cellular and humoral activators.[65] Many other studies have shown the ability of BM-MSCs to induce the expansion of functional regulatory T cells (Tregs).[70,71] Recently, it has been shown that adhesion molecules ICAM1 and VCAM1,

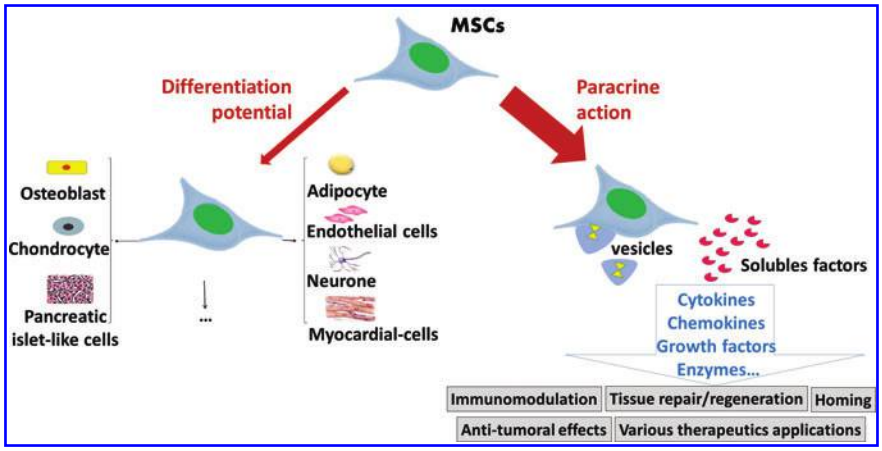

FIG. 2. Summary of the potential therapeutic roles of MSCs. Beneficial effects of MSCs have been attributed to their differentiation potentials. However, attention has been shifted to their paracrine effects (via vesicles and soluble factors) rather than their plasticity. The role that MSCs will play is determined by the microenvironment where they reside. MSCs, mesenchymal stromal cells. Color images available online at www.liebertpub.com/teb

which are required for a direct adhesion of MSCs to T cells, are critical for subsequent MSC-mediated immunosuppression, and are inducible by the parallel presence of interferon-gamma (IFN-γ) and inflammatory cytokines.[72] Another possible mechanism underlying the BM-MSC-mediated suppression of T cells is to prevent their entry into the S phase of the cell cycle by mediating irreversible G0/G1 phase arrest through the inhibition of cyclin D2 expression.[69,73] Similarly, it has been shown that the addition of DP-MSCs to phytohemagglutinin-stimulated T cells mediated an inhibition of their response.[74] Increased expression of immunodulatory soluble factors (hepatocyte growth factor [HGF]-β1, ICAM-1, IL-6, IL-10, transforming growth factor-β1 [TGF-β1], VCAM1, and vascular endothelial growth factor (VEGF)) secreted by human DP-MSCs was detected in a co-culture system with decreased expression levels of some pro-inflammatory cytokines and increased levels of some anti-inflammatory ones. Induction of Treg markers by human DP-MSCs was also demonstrated.[75] A very recent study has examined the *in vivo* and *in vitro* immunomodulatory effects of human supernumerary tooth-derived mesenchymal stem cells (SNT-MSCs). It has been shown that, in *in vitro* co-cultures, these cells suppressed the viability of T cells and also the differentiation of Th17 cells. *In vivo* transplantation of SNT-MSCs in systemic lupus erythematosus model MRL/Ipr mice suppressed increased levels of peripheral Th17 cells and IL-17 as well as *ex-vivo* differentiation of Th17 cells.[76]

Fetal MSCs have been reported to have similar inhibitory effects on T-lymphocytes. It has been shown that mitogen-induced T-cell proliferation in an allogeneic model transplant, as well as in a xenograft model, was effectively suppressed by WJ-MSCs with levels comparable to BM-MSCs immunosuppression.[77] In addition, IFN-γ and/or tumor necrosis factor-alpha (TNF-α) produced by activated T cells stimulate the production of indoleamine 2,3-dioxygenase (IDO) by MSCs, which, in turn, inhibited T-cell proliferation.[78] Tipnis *et al.* have reported that the expression of B7-H1, a negative regulator of T-cell activation constitutively expressed by WJ-MSCs, is increased after IFN-γ treatment. In addition, IFN-γ treatment induced IDO secretion by WJ-MSCs, which inhibited T-cell proliferation.[79] These results were confirmed very recently by Manochantr *et al.* showing that MSCs from amnion, placenta, and WJ can potentially substitute BM-MSCs in several therapeutic applications. Indeed, these cells inhibited alloreactive T-lymphocytes in the mixed lymphocyte reaction in a similar degree as BM-MSCs.[80]

MSCs and B cells: The reasearch on T-cell immunosuppression mediated by MSCs has attracted most of the attention in clinical applications and has been widely studied. However, B cells and humoral immune responses are more and more known as important mediators of chronic allograft rejection. Indeed, data about the influence of MSCs on B cells growth, differentiation, and production of immunoglobulins (Ig) are still scarce and controversial.[81]

B cells play an essential role in adaptive immunity. These cells develop in the BM strictly after a close interaction between B-cell progenitors and stromal cells that produce cytokines which are capable of supporting B-cell survival and proliferation.[82] They are directly responsible for the humoral immune response via the secretion of antibodies against pathogenic or foreign antigens. A subset of B lineage differentiates into memory B cells, which can mediate a rapid response on secondary exposure to that same antigen.

Corcione *et al.* demonstrated that BM-MSCs inhibited the proliferation of B cells and significantly decreased the production of IgM, IgG, and IgA[83]; the same effect has been reported by Che *et al.* showing that UC-MSCs significantly suppressed the proliferation, differentiation, and immunoglobulin secretion of B cells *in vitro.*[84] To understand the results of Che *et al.*, it is essential to know that ''B-lymphcote-induced matruration protein-1'' (Blimp-1), ''X-box binding protein-1'' (Xbp-1), ''B-cell lymphoma-6'' (Bcl-6) and ''paired box gene-5'' (PAX-5) are known as the main regulators of B-cell differentiation to immunoglobulin-secreting cells. PAX-5 and Bcl-6 are required to keep B-cell phenotypes. Blimp-1 inhibits the expression of both PAX-5 and Bcl-6 in order to let B cells differentiate. BCR signaling involves the MAPK signaling pathway and increases the transcriptional activity that is mediated by the transcription factor activator protein-1 (AP-1), which leads to Blimp-1 expression. Che *et al.* have shown a suppression of Blimp-1 expression and an induction of PAX-5 in the co-cultures of UC-MSCs and B-cells. Thay have also found that Akt and p38 MAPK were inhibited by WJ-MSCs.[84]

However, these results have been contradicted by other groups. Rasmusson *et al.* have shown an increase of B-cell immunoglobulin secretion when co-cultured with BM-MSCs; this effect varied depending on the type of stimulus used to trigger B cells.[85] Likewise, Traggiai *et al.* have reported that BM-MSCs could promote B-cell expansion and differentiation after treatment with an agonist of Toll-like receptor 9.[86] A recent study has demonstrated that UC-MSCs promoted proliferation and differentiation of B cells both *in vitro and in vivo* partially through prostaglandin E2 (PGE2) axis.[82]

Contradictions in the effects of MSCs on B cells could be associated to the differences in the B-cell source, the manner of their purification and stimulation, the culture conditions, and many other factors. However, the microenvironment plays a decisive role in determining the role that the MSCs will play.

Innate response:

MSCs and NK cells: NK cells are major effector cells of innate immunity, because they lack antigen-specific cell surface receptors.[87] They mediate antibody-dependent cellular cytotoxicity as well as ''spontaneous'' killing of infected or transformed cells through the release of perforin and granzyme from cytotoxic granules.[88]

MSCs and NK cells have been shown to interact *in vitro*. The outcome of this interaction may depend on the state of NK-cell activation and/or the cytokines present in the culture medium. IFN-γ-activated MSC-escaped NK cells mediated lysis through the induction of HLA-E and NK inhibitory ligands.[89,90] Previous studies have indicated that cytokine-induced proliferation of NK cells leads to the up-regulation of HLA class I on MSCs.[90] In response to this up-regulation, HLA class I molecules, including human leukocyte antigen-G5 (HLA-G5), expressed by MSCs, bind

to the inhibitory receptor ILT2 expressed on NK cells.[91] Furthermore, other studies have shown that the suppression of NK cell functions is mediated by a down-modulation of some activating NK cell receptors (NKp30, NKp44, and NKG2D) and by the inhibition of NK cell lytic granule formation.[92] There is growing evidence that IDO, PGE2, and TGF-β1 may control MSC-mediated inhibition of NK-cell function.[93]

Boissel *et al.* evidenced that NK cells had a higher expansion when cultured with allogeneic and autologous WJ-MSCs as feeders in the presence of NK growth factors. WJ-MSCs feeders were rejected during the first week of co-culture. Expanded NK cells maintained an elevated cytotoxic profile and may be genetically manipulated.[88] In a recent study, Zhao *et al.* have been interested in elucidating the effect of UC-MSCs on NK cell-mediated cytotoxicity against DCs and the mechanism involved. They found that UC-MSCs can enhance this effect possibly by inhibiting DCs maturation and up-regulating the ligands for killer activator receptor on the surface of the DCs.[94] When comparing the immunosuppressive activity of MSCs derived from UC, AT, and BM on lymphocytes, Ribeiro *et al.* have shown that all the three types of MSCs exhibited a strong inhibitory effect on $CD56^{dim}$NK cell subset activation (cytotoxic NK cells). UC-MSCs were the only cells that were unable to inhibit the activation of $CD56^{bright}$ NK cell subset (a subset that has the capacity to produce abundant cytokines after activation but has a low natural cytotoxicity). Among all these MSCs, AT-MSCs had a higher inhibitory capacity. A down-regulation of perforin and TNF-α ARNm by MSCs from the three sources was observed, while only AT- and BM-MSCs induced a minor reduction of granzyme B ARNm.[95]

MSCs and DCs: DCs play a key role in the initiation of primary immune responses and tolerance, depending on the activation and maturation stage of DCs. Locally produced inflammatory cytokines or microbial components promote the maturation of DCs from a processing to a presenting stage, characterized by the up-regulation of MHC-class II and co-stimulatory molecules (CD80 and CD86), production of IL-12, and migration to lymphoid tissue. DCs maturation is a prerequisite to induce immunogenic T-cell responses, whereas tolerance is observed when antigens are presented by immature or semi-mature DCs. Therefore, DC maturation plays a key role in initiating T-cell responses.

BM-MSCs were shown to block the generation of functional antigen-presenting cells, including myeloid DCs from both monocytes and $CD34^+$ cell precursors.[96–98] Most results supported the notion that DCs at early stages of differentiation are sensitive to their inhibitory effects, while at later stages, they are resistant. However, WJ-MSCs inhibited DC maturation and activation even when the contact happended during the mature or immature stage. Both cell contact via surface ligands (B7H1) and soluble factors (IDO) enhanced the efficiency of suppression.[79] Very recently, Saeidi *et al.* showed that UC-MSCs and BM-MSCs strongly inhibited the differentiation and maturation of DCs with a more inhibitory effect on CD1a, CD83, CD86 expression, and DC endocytic activity. These cells also severely up-regulate CD14 expression. Results have indicated that UC-MSCs and BM-MSCs exerted their inhibitory effect on differentiation, maturation, and function of DCs through the secreted factors and free of any cell-to-cell contacts.[99]

Immunomodulatory properties of WJ-MSCs in innate and adaptive responses are resumed in the figure given next (Fig. 3).

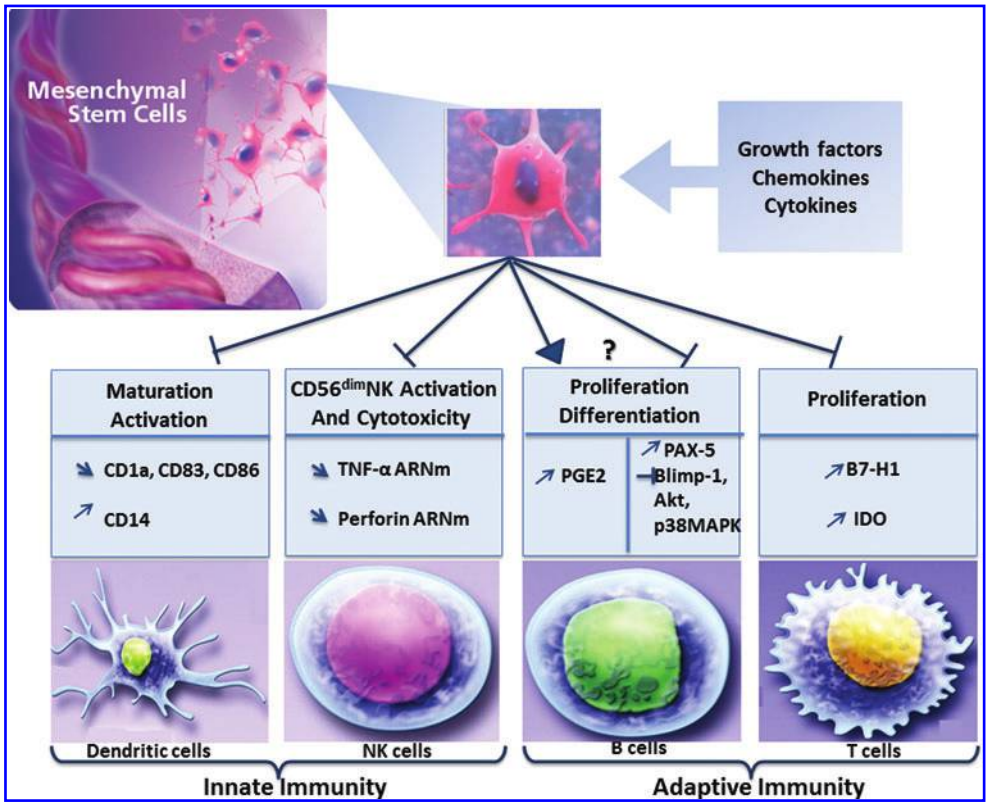

FIG. 3. Immunomodulatory effects of the WJ-MSCs on innate and adaptive immunity. The effects can be summarized as follows: inhibition of the maturation and activation of dendritic cells as well as the proliferation of T cells, activation of the expansion and cytotoxicity of NK cells. Effects of WJ-MSCs on B cells are still contradictory; they can stimulate or block the proliferation and differentiation of B cells and the secretion of immunoglobulin (Ig). NK, natural killer; WJ, Wharton's jelly. Color images available online at www.liebertpub.com/teb

MSCs and immumodulatory paracrine factors. Multiple reports have evidenced, first *in vitro* and then *in vivo*, the ability of MSCs to express molecules that interact with both innate and adaptive immunity, both through soluble factors[65,100] and in a cell contact-mediated fashion probably through the interaction of membrane receptors, adhesion molecules, or the cellular exchange of membrane vesicles.[101] It is still a matter of debate whether the regulatory effects are cell-to-cell contact -dependent, or whether soluble factors are sufficient.[102] The MSCs immune modulating effects will also depend on the ratio between MSCs and immune cells, and the state and stage of immune cell activation or maturation. Several factors that contribute to the MSCs-mediated effects have been identified, in particular growth factors, cytokines, chemokines, and hormones, all of which exert paracrine effects on immune cells and enable homing, migration, and their attachment to injured cells. Soluble factors implicated in MSCs-mediated immune modulation include nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), heme oxygenase (Hmox1), secretion of anti-inflammatory cytokines such as IL-10, TGF-β, HGF, IL-6, and PGE2.[68,70,103,104] A study comparing the immunomodulatory properties of MSCs derived from many sources showed that despite their similar cytokine profiles, WJ-MSCs only secrete IL-12, IL-15, and platelet-derived growth factor-AA (PDGF-AA). They did not secrete VEGF similar to other adult MSC sources.[78] The precise meaning of these differences, however, needs to be understood in WJ-MSCs/immune cell co-cultures.[105]

Specialized immune tolerance implicated at the maternal-fetal interfaces depends on the expression of many molecules, including galectin-1, B7 proteins, HLA-G,[106] and the expression of immune suppressive cytokines such as leukemia inhibitory factor (LIF).[107,108] Since WJ-MSCs are isolated from a peri-natal source, they could exhibit immune evasion mechanisms that are dominant at the fetal-maternal interface. In fact, Najar *et al.* showed that higher constitutive as well as IFN-γ inducible levels of LIF are expressed by WJ-MSCs than by BM-MSCs and the suppression of lymphoproliferation can be rescued by blocking LIF in co-cultures.[109] Furthermore, Prasanna *et al.* reported higher levels of both constitutive and IFN-γ inducible HGF in WJ-MSCs compared with BM-MSCs.[110]

Nonclassical type I HLA molecules are an interesting as yet only partly explored field in MSCs immune function. Several reports showed that BM-MSCs and WJ-MSCs express the HLA-G molecule, at both mRNA and protein level, and its soluble form HLA-G5.[29,77,111] Weiss *et al.* also showed that WJ-MSCs constitutively express high levels of the immune suppressive HLA-G6 isoform, while BM-MSCs express the HLA-G5 isoform constitutively and its expression is not induced by IFN-γ.[77] HLA-G5 secretion has been directly implicated in the induction of regulatory cells ($CD4^+CD25^+FoxP3^+$ Tregs) that are characterized as key suppressors of effector responses to alloantigens.[112] HLA-G5 secretion has also been linked with the suppression of NK cell production of IFN-γ in BM-MSCs co-cultures.[113] Since the inhibition of maternal alloreactivity is due to the expression of high levels of HLA-G by the fetus, the exact role of immune-suppressive HLA-G isoforms, such as HLA-G6 expressed by WJ-MSCs, needs to be evaluated in detail.[105] Recently, the expression of HLA-E and HLA-F in the WJ-MSCs has been reported; both are implicated in tolerogenic processes occurring at the fetal-maternal interface, along with HLA-G.[114]

Homing of MSCs

Mantaining the function and the integrity of the human body, which is often subjected to injuries, is essentially due to tissue repair. Shortly after an injury, different types of immune cells (neutrophils, monocytes, and lymphocytes) are conducted to the site of damage. These cells are responsible for the secretion of various growth factors and cytokines that will attract other residing or circulating cells such as MSCs. Endogenous MSCs present a pool of regenerative cells, participate in tissue repair, and communicate with other cells in response to signals of cellular damage.[115] Their ''homing'' can be defined as the arrest of MSCs within the vasculature of a tissue than crossing the endothelium.[116]. Thus, the homing of endogenous MSCs is being considered a therapeutic benefit, and studies are evaluating new methods for recruiting a sufficient number of MSCs to exert their regenerative capacity. In cases where the reservoir of MSCs is depleted because of several diseases or the age, exogenous MSCs could be administrated to compensate the lack of endogenous MSCs (reviewed in Marquez-Curtis and Janowska-Wieczorek[115] and Sohni and Verfaillie[117]). It has been reported that *in-vitro*-expanded MSCs preferentially home to sites of tissue damage, where they enhance wound healing, support tissue regeneration, and restore the BM microenvironment after damage by myeloablative chemotherapy or integrate into tumors.[118]

Since the precise molecular mechanisms by which MSCs migrate into sites of injury are not yet fully defined, the migration of leukocytes into sites of inflammation has been taken as a model.[119] Indeed, on delivery into the blood stream, the MSCs keep close contact with EC whose role is being extensively studied in MSCs migration. They engraft into the endothelium, and eventually pass and leave the endothelium.[120] The migration of MSCs is mediated by a wide variety of molecules that are expressed by MSCs, including growth factors, chemokines, and receptors, and by chemotactic factors produced by immune cells.[116] It has been demonstrated that human MSCs showed significant chemotaxis responses to several factors (including PDGF, VEGF, IGF-1, IL-8, bone morphogenetic protein BMP-4, and BMP-7)[121] and express a variety of chemokine receptors (such as CCR1, CCR4, CCR7, CXCR5, and CCR10) which might be involved in their migration into injured tissues along a chemokine gradient.[122]

In addition, specific proteolytic enzymes are required so the cells can traverse the protein fibers of the extracellular matrix (ECM) and reach the target sites.[118,123] In particular, the matrix metalloproteinases (MMPs), consisting of more than 24 zinc-dependent endopeptidases, are physiologically necessary for stem cell migration, degradation, and remodeling of ECM components, and are crucial for developmental events such as morphogenesis, cell proliferation, apoptosis, and differentiation.[124–127]

Ries *et al.* were the first to show that human BM-MSCs use constitutively expressed MMP-2 (gelatinase A), membrane-type matrix metalloproteinase-1 (MT1-MMP), and tissue inhibitor of metalloproteinase 2 (TIMP-2) to migrate

through human recombinant basement membranes. Inflammatory cytokines such as TGF-β1, IL-1β, and TNF-α are able to exert chemoattractive potential on hBM-MSCs and to up-regulate MMP-2, MT1-MMP, and/or MMP-9, enabling cellular trafficking of MSCs across human ECM barriers.[118] MMP-2 has been also detected in WJ-MSCs in association with MMP-9 (gelatinase B), MMP-8, and MMP-13 (respectively collagenase-1 and -2), as well as in different regions of full-term human umbilical cord and in cultured HUVEC. The wide expression of these enzymes in the umbilical cord has been attributed to their role in the degradation and remodeling of ECM and in other physiological processes.[128]

Recently, Balasubramanian *et al.* have compared chemokine and receptor gene expression between WJ- and BM-MSCs. Their results have shown that Chemokine (C-C motif) receptor 3 (CCR3) was more expressed in WJ-MSCs than in BM-MSCs; whereas the latter have presented a higher expression of CCR1, CCR7, CCRL2, Chemokine (C-X3-C motif) receptor 1 (CX3CR1), and CXCR5. MSCs from both sources had a similar expression of CCR5, CCR6, and CCRL1.

In addition, Chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL2, CXCL5, CXCL6, and CXCL8 (members of the CXC chemokine family) were up-regulated in WJ-MSCs in comparison with BM-MSCs. These chemokines are known as potent promoters of angiogenesis and mediate their activity by binding CXCR2 receptor on the endothelium (Fig. 4). On the contrary, CXCL12 and CXCL13, also two members of the CXC chemokine family and known to contribute to immune and nonimmune cell homing, were up-regulated in BM-MSCs. WJ-MSCs have shown a higher expression of IL-1A (enhance the expression of CXCL8) and TNF-α (angiogenic factor) than BM-MSCs; while IL16 (has an immunomodulatory role in asthmatic inflammation) and CCRL12 (plays a role in the control of airway inflammatory response and in lung DC trafficking) were more expressed in BM-MSCs. Moreover, results have reported a stronger expression, in WJ-MSCs, of many growth factors linked with angiogenesis such as VEGF-D, PDGF-AA, TGF-β2, β-FGF, and HGF.[116] Their chemokine gene profile suggests that WJ-MSCs may be useful in the healing and treatment of ischemic lesions such as the ischemic myocardium, with cerebral ischemia for example. Moreover, they could be suggested as a treatment to reduce or prevent fibrosis and scarring in tissue lesions, as it has been shown that they secreted bFGF and HGF (known to have an anti-fibrotic effect).

Thus, *ex vivo*-expanded human MSCs with cytokines may be a useful method, in clinical applications, to increase their migration/homing potential after transplantation into patients, as well as the administration of cytokines to mobilize MSCs to sites of injury.

Anti-tumourigenic Effects of WJ-MSCs

MSCs have the capacity to migrate to tumor sites and modulate their microenvironment. Thus, they have an impact on tumour behavior.[129] A great deal of evidence suggests that solid tumors generate a microenvironment similar to that associated with wound healing, as they apply physical and chemical stress to neighboring tissues. Tumors can, therefore, be considered sites of tissue damage, which induces the migration of MSCs.[130]

Human MSCs have been intensively studied for their potential use in cancer treatment. Their use has been limited, however, by a general concern related to their biosafety.[131] Many studies have reported pro- or anti-tumorigenic effects of MSCs on the progression of primary and metastatic tumours. These contradictory results could be associated with differences in the MSC sources used, the type of tumour

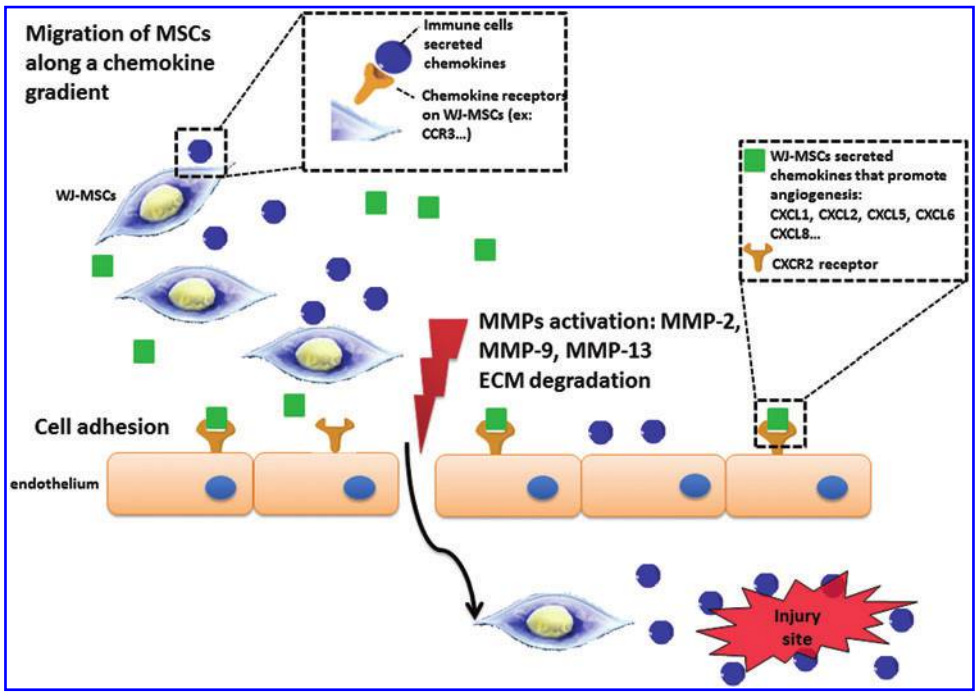

FIG. 4. Proposed mechanisms involved in the homing of WJ-MSCs to sites of tissue injury and their angiogenesis capacity. MSCs home to site of injury along gradients created by inflammatory chemokines and several factors. They express a variety of chemokine receptors that might be involved in their migration into injured sites. The expression of a set of MMPs by WJ-MSCs contributes to the extracellular matrix (ECM) degradation, which enables them to cross the ECM and reach the site of injury. They also express a variety of chemokines that are known as angiogenesis mediators and exert their function by binding to their receptor CXCR2 on the endothelium. MMPs, metalloproteinases. Color images available online at www.liebertpub.com/teb

model, the method of administration, or other unknown factors.[130,132]

The tumor stroma consists of a complex ECM in which inflammatory and immune cells, fat cells, fibroblasts, and blood vessels reside. It plays a crucial role in tumor progression, angiogenesis, and metastasis through its effects on tumor-host interactions. Tumor-associated fibroblasts (TAFs) are activated fibroblasts in the tumor stroma.[131] Several reports have hypothesized that BM-MSCs selectively proliferate to tumors and contribute to the formation of tumor-associated stroma by transforming into TAFs. They also promote tumor growth and metastasis by enhancing migration and angiogenesis and inhibiting apoptosis of tumor cells.[133–137]

On the other hand, the immunosuppressive effects of MSCs can impair the function of a variety of immune cells (directly or through paracrine signals). This may be an important mechanism enabling MSCs to promote tumor growth or to increase the incidence of tumor formation. For instance, by increasing Tregs and reducing the activity of NK cells and cytotoxic T lymphocytes (CTL) (known to kill tumour cells), BM-MSCs can protect breast cancer cells.[138] They also have been linked to osteocarcinomas,[139] prostate tumors,[140,141] breast tumors,[138,140,142] colon cancer,[137] and others. A recent study has also demonstrated a fusion between MSCs and gastrointestinal epithelial cells, suggesting the formation of a more cancer-prone cell type.[143]

Very recently, in order to examine the possible anticancer therapeutic applications of MSCs from different sources, Akimoto *et al.* have studied the inhibitory effects of MSCs from umbilical cord blood (UCB) and AT on ''glioblastoma multiforme (GBM)'' (the most aggressive type of primary brain tumor in humans). They found that, both *in vitro* and *in vivo*, GBM growth was inhibited by UCB-MSCs but promoted by AT-MSCs. UCB-MSCs induced apoptosis through the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is more strongly expressed by UBC-MSCs than by AT-MSCs.[144] Furthermore, it has been shown that naïve WJ-MSCs are able to produce factors suppressing cancer cell growth and inducing apoptosis, and so may be a novel tool for cancer therapy in contrast to MSCs from some other sources.[145] Other reports have likewise shown that WJ-MSCs can abrogate certain solid tumors.[146–149] These cells decreased the growth of human breast cancer *in vitro* and stopped its growth when intravenously injected in an SCID mouse model.[150] Later, Fan *et al.* showed that WJ-MSCs do not induce teratomas in immunodeficient SCID mice, nor do they induce tumors when transplanted into diseased animal models.[151] In a recent study, Subramanian *et al.* have examined whether WJ-MSCs, such as BM-MSCs, transform to the TAF phenotype in the presence of ovarian and breast cancer conditioned medium. Results have shown no expression of tumor-associated markers for hWJ-MSCs with a low expression of TAF-related genes, confirming that these cells are not associated with enhanced growth of solid tumors.[3] In order to determine whether WJ-MSC-mediated inhibition of cancer cell growth was not specific to breast cancer cells, the same group compared the effects of WJ-MSC extracts and cell lysate on three other types of solid tumors: breast adenocarcinoma, ovarian carcinoma, and osteocarcinoma. They observed the same effects, which were probably mediated via agents in WJ-MSC extracts.[152] In addition, Ma *et al.* have shown that WJ-MSCs significantly inhibited the growth of breast cancer stem cells *in vitro* and *in vivo*, probably by inducing a cell cycle arrest and tumor cell apoptosis and inhibiting the activities of phosphoinositide 3-kinase (PI3K) and AKT (also known as protein kinase B).[149] A more recent study has reported that WJ-MSC conditioned medium as well as its cell lysate inhibits mammary carcinoma and osteosarcoma cell growth via apoptosis and autophagy *in vitro* and in xenograft mice.[153] In another study evaluating the tumorigenesis potential of WJ-MSCs in comparison with ESCs, animals injected with ESCs developed teratomas with increased levels of pro-inflammatory cytokines; whereas those injected with WJ-MSCs developed no tumors or inflammatory reactions at the injection sites and exhibited increased production of anti-inflammatory cytokines.[154] A very recent study on the effects of WJ-MSCs on intrahepatic cholangiocarcinoma (ICC, a common form of primary liver cancer) has shown that these cells can inhibit the proliferation and induce the apoptosis of human ICC cells. Apoptosis of tumour cells is related to the inhibition of PI3K/Akt and the Wnt/β-catenin signaling pathways.[155] The effects of WJ-MSCs have also been studied in hematopoietic tumours. Results obtained by Tian *et al.* have provided a new insight on how these cells may modulate leukemic tumour growth *in vitro.* According to this study, p38 MAPK, a suppressor of tumor development, was required for leukemic tumor suppression by WJ-MSCs.[156]

These studies, taken together, indicate that WJ-MSCs are nontumorigenic, anti-tumorigenic, and hypoimmunogenic; do not transform to the TAF phenotype that is associated with enhanced growth of solid tumors; and suppress hematopoietic tumor development. WJ-MSCs appear to be a safe and promising tool for future cancer therapy and clinical applications, but more pieces of evidence are needed to further characterize their anti-tumorigenic mechanisms and to confirm this hypothesis.

Therapeutic Applications

Treatment of autoimmune diseases

The immune properties of WJ-MSCs suggest that they may be a therapeutic option to treat autoimmune diseases such as type 1 diabetes or Crohn's disease (CD).

Type 1 diabetes. Diabetes is a metabolic disease listed among the leading causes of death in some countries. It is characterized by absolute or relative insulin deficiency. Type 1 diabetes is characterized by an absolute insulin decrease due to T-cell-mediated destruction of insulin-producing pancreatic β cells.[157] This autoimmune destruction of pancreatic islet β-cells reduces the patient's ability to regulate blood glucose, leading to a high frequency of vascular complications that compromise the quality and expectancy of life.[158]

Transplantation of pancreatic islet cells (PICs) as a potential cure for type I diabetes has been hampered by immune rejection and recurrent attacks against islets by the underlying autoimmunity. Studies have shown the capacity of WJ-MSCs to differentiate into mature islet-like cell clusters. These islet-like cell clusters have been shown to contain human C-peptide and to release insulin *in vitro* and

in vivo in response to physiological glucose levels. Real-time PCR analysis has shown the enhancement of insulin and other pancreatic β-cell-related genes, such as pancreatic and duodenal homeobox 1 (pdx1), homeobox HB9 ou MNX1 (hlxb9), NK2 homeobox 2 (nkx2.2), NK6 homeobox 1 (nkx6.1), and glucose transporter 2 (glut-2) in these cells.[159] Various publications have confirmed the pancreatic islet-like cell differentiation potential of WJ-MSCs.[47,48,160] Kim *et al.*, comparing the capacities of MSCs from various sources (WJ, BM, AT, and periosteum) to differentiate into PICs, have confirmed that all cell lines were well differentiated with an increased insulin mRNA expression, but only PICs derived from periosteum progenitor cells showed insulin secretion to a high glucose concentration.[160]

More recently, Hu *et al.* have studied the therapeutic potential of WJ-MSCs in patients with type 1 diabetes, evaluating the effects of these cells over a longer treatment time. They followed two groups of patients, the first of which received a basic treatment combined with WJ-MSC implantation; while the second received a basic treatment combined with normal saline therapy. Patients were followed for two years after the operations. During the follow-up period, patients treated with WJ-MSCs showed better Hba1 and C-peptide expression levels than patients in the second group. Although the precise mechanisms involved are unknown, WJ-MSC therapy appears to have a promising effect on type 1 diabetes patients and to be a good strategy for treatment of this disease.[158]

Type 2 diabetes. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and is characterized by insulin resistance and pancreatic β-cell dysfunction. Hu *et al.* studied the effect of intravenous infusion of human WJ-MSCs as therapy, administering them alone and in combination with sitagliptin (a dipeptidyl-peptidase IV inhibitor known to increase insulin release and decrease glucagon levels by having an impact on α and β cells in the pancreatic islets) in a T2DM rat model. Compared with the control groups (a diabetic control group and a sitagliptin-only group), rats treated with WJ-MSCs only and those treated with a combination of WJ-MSCs and sitagliptin exhibited increased numbers of β cells. Glucagon level was decreased in the sitagliptin-only group and the WJ-MSCs + sitagliptin group compared with the WJ-MSCs-only group and the diabetic control group. These results suggest the therapeutic potential of WJ-MSCs in β-cell regeneration.[161]

Crohn's disease. CD is an inflammatory chronic disease caused by a dysregulation of immune tolerance and characterized by an idiopathic inflammation of the gastrointestinal tract. Frequent complications in CD are abscess and stricture formation, intestinal obstruction, and fistulas (abnormal connective passages from the epithelial lining of the intestines to another organ or to the skin caused by inflammation). Anti-TNF-α therapy is the first choice in the treatment of patients with perianal fistulas. Even with this treatment, however, perianal fistulas often lead to physical and emotional distress, and only 46% of cases heal completely.[162]

Therapeutic effects of AT-MSCs and BM-MSCs on CD have already been proved.[163,164] For instance, Garcia-Olma *et al.* performed a clinical study on patients suffering from Crohn's enterocutaneous fistulas in which they compared the therapeutic effects of autologous expanded AT-MSCs and unexpanded cells corresponding to the stromal vascular fraction (SVF) when cells of each type were implanted in the fistulas. Three out of four cases treated with expanded AT-MSCs were healed, compared with only one out of four cases treated with SVF. The authors have suggested that the use of expanded AT-MSCs would be more advantageous, and that the immunosuppressive properties of these cells were responsible for their healing effects in the treatment of CD. Other studies in progress will enable us to better understand the link between the expansion of AT-MSCs and their beneficial effects.[163] In another study, Ciccocioppo *et al.* examined the effect of *ex-vivo*-expanded BM-MSCs in CD. All 10 cases in which BM-MSCs were injected into the fistula exhibited signs of healing. In addition, a pro-apoptotic effect of BM-MSCs on mucosal T cells has been observed.[164]

Recently, studies have focused on two granulomatous disorders: intestinal tuberculosis (ITB) and CD. Both diseases present similar clinical signs and are difficult to distinguish. The current challenge is the early identification of the correct disease in order to treat it efficiently and quickly to avoid complications or death. Working from the fact that recruited MSCs within granulomas in ITB can evade the host immune response, Banerjee *et al.* have been pursuing the possibility of analyzing MSC markers in the two types of granulomas (i.e., those derived from patients with CD and those derived from patients with ITB). Their results have shown that the mesenchymal marker CD73 is expressed only in MSCs within tuberculous granulomas, identifying CD73 as a possible marker of ITB. This would explain the essential pathogenic mechanisms in ITB as being based on the recruitment of MSCs with high CD73 expression. These observations suggest that MSCs with increased CD73 expression could be a future candidate for therapeutic intervention in CD. Given their phenotypic profile, WJ-MSCs could have real potential for therapeutic applications in CD.[165]

Treatment of neurodegenerative diseases

Neurodegenerative diseases are chronic and progressive disorders of the central nervous system (CNS) which are characterized by a steady loss of neurons in the region of the brain and spinal cord that affects the mental and motor abilities of affected people. According to the World Health Organization, in 2040, the devastating diseases known as Alzheimer's and Parkinson's will represent the second leading cause of death worldwide. Multiple sclerosis (MS) is another neurodegenerative disease.

Parkinson's disease. Parkinson's disease (PD) is a neurodegenerative disorder that is more common in the elderly. Its symptoms (tremor, rigidity, bradykinesia, and postural instability) are caused primarily by the degeneration of dopamine (DA) neurons in the substantia nigra.[166] Current therapies mostly relieve symptoms but do not restore the function of the lesioned side of the brain or the efficacy lost due to disease progression. Embryonic stem cells have been investigated as a renewable source of DA-producing cells.

However, technical and ethical obstacles have limited the application of this therapy.[167]

Several groups have been interested in determining the effect of MSCs on the CNS. Ribeiro *et al.* have shown that AT-MSCs and WJ-MSCs are able to release trophic/neuroregulatory factors that could improve the metabolic viability of hippocampal neurons *in vitro*. These two types of MSCs do not have similar functionality, however, because their secretomes act differently on cell viability and on the densities of hippocampal neurons. Indeed, AT-MSCs require exogenous factors such as bFGF to be added in the primary cultures of hippocampal neurons in order to influence the metabolic viability and neuronal cell densities; whereas WJ-MSCs are able to promote neuronal survival without the addition of exogenous factors.[168]

Weiss *et al.* have characterized WJ-MSCs and compared them with MSCs derived from other sources. In their study, they tested the therapeutic effects of WJ-MSCs in parkinsonian rats. Their initial results demonstrated that WJ-MSCs express growth factors and angiogenic factors, suggesting that they may be useful for the treatment of neurodegenerative diseases. Indeed, the characterization of WJ-MSCs reveals that they produce glial cell line-derived neurotrophic factor located in the cytoplasm. WJ-MSCs also express nestin, a marker of primitive neural stem cells.[4] After neural induction of WJ-MSCs, the expression of nestin was lower in differentiated cells than in undifferentiated cells; whereas the expression of tyrosine hydroxylase (a mature neural marker of catecholaminergic neurons) was greater in differentiated cells. Moreover, it has been shown that WJ-MSCs express some genes encoding for proteins with a neurotrophic effect: CNTF (ciliary neurotrophic factor), VEGF, FGF20, and TRKC (neurotrophic tyrosine kinase). In addition, when they are transplanted into parkinsonian rats, WJ-MSCs can partially reverse the parkinsonian behavioral phenotype.[27] Yan *et al.* have managed to differentiate WJ-MSCs into neural-like cells *in vitro* and have subsequently tested the therapeutic potential of differentiated cells by implanting them into the striatum and substantia nigra of methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) lesioned hemi-parkinsonian rhesus monkeys. PD monkeys transplanted with the induced cells showed an improvement in behavioral measures. Furthermore, pathological and immunohistochemical data have indicated the presence of neuronal-like cells in the right brain hemisphere of PD monkeys, suggesting that they may be dopaminergic neurons.[167] Nearly identical results were obtained when WJ-MSCs were replaced by AT-MSCs.[169] The beneficial effect of BM-MSCs on a parkinsonian rat model was shown by Ye *et al.*[170]

Multiple Sclerosis. Multiple sclerosis (MS) is a progressive neurodegenerative disorder of the CNS that is characterized by chronic inflammation, demyelination, and neuronal damage. Currently, there is no medical cure for MS, mainly owing to an incomplete understanding of its pathophysiology.[171]

Recently, Payne *et al.* have assessed the therapeutic efficiency of BM-MSCs, AT-MSCs, and WJ-MSCs against MS, using recombinant myelin oligodendrocyte glycoprotein (rMOG)-induced experimental autoimmune encephalomyelitis (EAE), a model of MS in which both T- and B-cells contribute to the disease pathogenesis. They have demonstrated that BM-MSCs exerted more potent immunomodulatory effects *in vitro* compared with AT-MSCs and WJ-MSCs. Unexpectedly, however, BM-MSCs did not impact the disease course, although the transplantation of AT-MSCs ameliorated clinical signs in two animal models of EAE. Furthermore, only AT-MSCs and WJ-MSCs expressed integrin-α4 (CD49d); BM-MSCs, which may not be able to adhere to VCAM1, a critical step in the extravasation of cells into the CNS during EAE, did not express integrin-α4.[172] In their recent work, Liu *et al.* showed that WJ-MSCs could potentially play a therapeutic role in MS and could be an alternative to BM-MSCs, which have been extensively studied with regard to the treatment of MS.[173–175] Thus, it is believed that these cells could restore behavioral functions and attenuate the histopathological deficits of EAE mice over the long term (50 days).[176] These results confirm those of Liang *et al.*, who transplanted WJ-MSCs to a patient with refractory progressive MS and subsequently observed stabilization of the disease.[177]

Alzheimer's disease. Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder that is characterized by a loss of memory and a deterioration of cognitive ability. Cumulative evidence supports the hypothesis that the accumulation of amyloid-β peptide (Aβ) in the brain and oxidative stress play critical roles in AD pathogenesis.

Very recently, Liang *et al.* have attempted to differentiate WJ-MSCs into cholinergic-like neurons. Cholinergic neurons are neurons of the autonomic nervous system and are one of the causes of cognitive disorders such as AD. To induce differentiation, Liang *et al.* used a neural stem cell conditioned medium supplemented with bone morphogenetic protein 4 (BMP4) and fibroblast growth factors 8 (FGF8). First, they observed morphological changes of WJ-MSCs after culture in conditioned medium. These cells, which under normal conditions have a bipolar spindle-like morphology, changed into a bulbous shape with thin extensions touching each other to a certain extent after 16 days of differentiation. These observations already suggest a structural organization into axons. Moreover, they confirmed their hypothesis by showing an expression of cholinergic neuron markers, including choline acetyltransferase and NF by immunofluorescence and RT-PCR 20 days after the beginning of WJ-MSC induction. These *in-vitro* results demonstrate that WJ-MSCs can be induced into cholinergic-like neurons, which suggests that WJ-MSCs may be a very good candidate for the treatment of AD.[178] Patients suffering from AD show a decrease in the expression and activity levels of neprilysin (neural endopeptidase [NEP]), which is one of the several proteases involved in the proteolysis of Aβ. Thus, NEP has been intensively studied as a potential therapeutic target for AD. Since MSCs have the ability to synthesize vesicles (generated from the membrane), which also have a real therapeutic potential, Katsuda *et al.* have studied vesicles derived from AT-MSCs and identified their effects in co-cultures with cells over-producing Aβ. They observed a decrease in the amount of Aβ in the presence of AT-MSC vesicles. This phenomenon is explained by the initial expression on AT-MSC membranes of the NEP that is later present as an active form in the vesicles. Furthermore, they observed that AT-MSCs expressed NEP at a higher

level than BM-MSCs did, suggesting that AT-MSCs could be a better candidate than BM-MSCs for the treatment of AD.[179]

In another recent *in-vivo* study, Yang *et al.* sought to determine the therapeutic impact and mechanisms of action of neuron-like cells differentiated from WJ-MSCs in AD. They induced the differentiation of human WJ-MSCs into neuron-like cells using tricyclodecan-9-yl-xanthogenate (D609), then transplanted them into a transgenic AD mouse model. The resulting beneficial effects were linked to an ''alternatively activated'' microglia (M2-like microglia). Treated mice showed increased M2-like microglial activation, associated with an increase in the expressions of IL-4 (an anti-inflammatory cytokine) and NEP and a decrease in the expressions of IL-1β and TNF-α (pro-inflammatory cytokines). Hence, according to this study, transplantation of neuron-like cells differentiated from WJ-MSCs might be a promising cell therapy for AD.[180] Later, the same group studied the therapeutic impact of systemic administration of WJ-MSCs in a transgenic AD mouse model, and found that WJ-MSC infusion improved spatial learning and alleviated memory decline by reducing oxidative stress. All these results, taken together, suggest that WJ-MSCs in their differentiated (neuron-like cells) and undifferentiated forms may have beneficial effects in the prevention and treatment of AD.

Conclusion

The number of diseases that in their final stages require organ transplant or cellular therapy is increasing. Many of them are derived from or are accompanied by an unbalance in the organ inflammatory or immune state. In this regard, the use of a cellular therapy vehicle that can provide both organ recellularization and restoration of a physiological microenvironment without being rejected by the patient would benefit patients significantly.

To date, BM-MSCs have been considered the gold standard among therapeutic MSC-based therapies, yet BM-MSCs eventually degrade, exhibiting loss of proliferation and senescence. In the search for an alternative therapy lacking this flaw, different sources of MSCs have been explored. MSCs from AT, dental pulp, and AF have variable proliferation potentials and multilineage differentiation capacities, suggesting that the source of MSCs should be chosen carefully depending on the clinical applications targeted. Over the last few years, MSCs derived from WJ have gained much attention in regenerative medicine. The overview of the literature presented in this review has described their high differentiation potential as well as their important trophic, immunomodulatory, and anti-tumorigenic effects, which should be confirmed in different animal models. Taken together, all these works clearly show that the immunological features of various types of MSCs may affect their applications in regenerative medicine in ways which may be essential. Since these two properties rely, in turn, on paracrine effects, it is essential to further study the composition of MSCs secretome. This task will probably represent a major part of the relevant publications over the next 10 years.

The great question with regard to MSC therapy that still remains to be answered concerns its biosafety. Clinical trials are still needed to evaluate this particular aspect, especially in new sources of MSCs such as WJ-MSCs, even if they seem to be promising tools for the treatment of incurable degenerative diseases.

Disclosure Statements

No competing financial interests exist.

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