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## Umbilical cord mesenchymal stem cells: the new gold standard for mesenchymal stem cell-based therapies?

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3 **Umbilical mesenchymal stem cells: the new gold standard for mesenchymal stem cell**  
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5 **based therapies?**  
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**Key words:**

Mesenchymal stem cells, umbilical cord matrix, differentiation potential, immunological features, homing, anti-tumorogenic, autoimmune diseases, neurodegenerative diseases

**Abstract**

Mesenchymal Stem Cells or Mesenchymal Stromal Cells (MSCs) were first described by Friedenstein and his colleagues as cells growing on plastic surfaces, able to undergo self-renewal and to differentiate into osteoblasts, chondrocytes and adipocytes. Recent studies showed that these cells can be isolated from many adult tissues and differentiated into other mesoderm-derived cytotypes, nervous cells and endoderm-derived cells, therefore expanding their potential clinical use. MSCs are known for their both in vitro and in vivo immune regulatory activities, which are mediated by a complex interconnected network that combines microenvironment, soluble factors such as cytokines and signaling pathways. These properties allow the inhibition of different immune cell subpopulations of innate and adaptive immunity.

This review focuses on MSCs from the umbilical cord and compares them to the different sources available for MSCs regarding their phenotype and differentiation capabilities. The advantages of umbilical cord-MSCs, especially considering their transcriptomic profile, homing capacity, anti-tumorogenic effect and immunomodulatory properties, will be described. The final part of the review considers the therapeutic use of umbilical cord MSCs as allogeneic source of stem cells.

## INTRODUCTION

Mesenchymal stem cells (MSCs) are attractive stem cells due to their capacity of long-term *ex vivo* proliferation, multilineage differentiation potential and immunomodulatory properties. Even if adult human bone marrow (BM) is the most common MSCs source, the number of cells useful for cell therapy or regenerative medicine applications is extremely low, and autologous MSCs are not suitable for many clinical applications. Hence, the search for an allogeneic source of MSCs became critical. Umbilical cord Wharton's jelly (WJ) provides a new source of MSCs with higher accessibility and fewer ethical constraints than BM. MSCs can be non-invasively isolated from umbilical cord's Wharton's Jelly (WJ) (1) which is an extra-embryonic tissue easily obtained after birth. It has been initially described by Thomas Wharton in 1656, whose name was given to this connective tissue (2). These umbilical 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 (3). Moreover, WJ-MSCs have faster proliferation rates, greater expansion capability compared with adult MSCs with wide multipotency and no induction of teratomas and harbor strong immunomodulatory capacities (4, 5).

This paper gives an overview of the main features of WJ-MSCs in comparison to others sources. It also discusses more particularly their role as **(a)** immunomodulators and the mechanisms involved in their immunosuppressive properties, as **(b)** anti-tumor agents, **(c)** migratory curative cells with **(d)** special emphasis on their clinical and therapeutic applications.

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## I. Main Features of WJ-MSCs

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### 1. 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 (6, 7).

The proliferation capacity of cells is important regarding their potential application in cell therapy and tissue engineering. WJ-MSCs proliferation capacity seems to be different from MSCs from other sources. Indeed, for instance, Yu *et al.* have shown that over a period of seven days after seeding, the 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 (8). Compared to BM-MSCs, WJ-MSCs grow much faster for the early passages and have a cell doubling time (24h) almost twice shorter than BM-MSCs (40h) over 1<sup>st</sup> 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 to BM and adipose tissue (AT)-MSCs (9). However, WJ-MSCs have a greater ability to form CFU-F colonies *in vitro* than BM-MSCs and their formation frequency depends on seeding cell density (3, 10).

Other studies focusing on MSCs derived from DP-MSCs showed that cells from both sources (WJ and teeth) initially grew slowly but their proliferation rates were increased after the first subculture (11). However, WJ-MSCs growth is influenced by the number of culture

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3 passages *in vitro*, as amplifying these cells until passage 10 will result in a slower cell growth  
4  
5 compared to the same cell culture at passage 5. (12).  
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7 A very recent study has evaluated the proliferation kinetics and phenotypic  
8 characteristics of MSCs derived from WJ and AT during prolonged *in vitro* expansion and found  
9 that WJ-MSCs are isolated with high efficiency and bear a substantially increased proliferation  
10 capacity, whereas AT-MSCs exhibit a reduced proliferation potential showing typical signs of  
11 senescence at an early stage (13).  
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## 23 **2. Markers expression at protein level**

24 A large number of studies have analyzed the surface markers of WJ-MSCs and  
25 compared their expression profiles to other sources of MSCs such as bone marrow, teeth or  
26 amniotic fluid. The following table summarizes the phenotypic profiles of these MSCs mentioned  
27 in the literature (Table 1).  
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33 WJ-MSCs, as all MSCs, positively express mesenchymal surface markers such as CD90  
34 (Thy-1), CD73 (NT5E), CD44 (HA receptor), CD105 (endoglin), CD29 (integrin  $\beta$ 1) and do not  
35 express (1) hematopoietic surface markers such as CD34, CD133, CD38, CD14, CD11b, CD19  
36 and CD45, (2) endothelial cell markers such as CD31 and, (3), immunological markers such as  
37 HLA-DR (human leukocyte differentiation antigen class II) and CD86. As MSCs from dental  
38 pulp, bone marrow, periodontal ligament, teeth and amniotic fluid, WJ-MSCs express CD13,  
39 CD56, CD61, CD166 and CD 59 (Table 1).  
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49 In early passages (P4-P8), WJ-MSCs maintain their morphology and surface markers  
50 expression (CD10+, CD13+, CD29+, CD44+, CD90+, CD105+ and CD14-, CD31-, CD33-,  
51 CD34-, CD45-, CD56-, CD133-, HLA-DR-). Unlike BM-MSCs, few populations of WJ-MSCs  
52 express endoglin (SH2, CD105) and CD49e at passage 8 (18). For WJ-MSCs, Lu *et al.* have  
53 specifically highlighted a much lower expression of CD106 which is probably a specific marker  
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3 for identifying peripheral MSCs from BM-MSCs. This low expression has also been identified in  
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5 AT-MSCs. HLA-ABC was very weakly expressed by WJ-MSCs suggesting that these cells could  
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7 be excellent candidates for allogeneic cell therapy (19). Recent studies have shown that WJ-  
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9 MSCs express mesodermal markers (vimentin, smooth muscle specific actin), CD68 (a  
10  
11 macrophage marker), Nestin (molecule precursor of neurofilamentproteine), GATA-4 (an  
12  
13 important factor in early myocardial development), but also primitives markers such as Oct-4  
14  
15 (12), Oct-1, Sox-2, Nanog and line28 which are embryonic stem cell markers (3, 18, 20, 21).  
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### 20 21 **3. Transcriptomic profile**

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23 Emerging data has compared the transcriptomic profile of WJ-MSCs to MSCs from other  
24  
25 sources. The following table gives an overview of the main comparisons (table 2).  
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28 Some works show a highly expression embryogenic genes such as LIFR, ESG1, SOX2,  
29  
30 TERT NANOG, POUF1, OCT4, LIN28, DNMT3B, GABRB3 by WJ-MSCs, suggest that WJ  
31  
32 could be a more primitive source of MSCs (3, 5, 22-24). Furthermore, as shown in table 2, WJ-  
33  
34 MSCs express genes encoding for proteins associated with morphogenesis: SHH, neuregulin-1  
35  
36 and 4, SNA2, and WNT4 (18).  
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#### 40 41 **WJ-MSCs, compared to MSCs from other tissues, differentially express genes involved in** 42 43 **bone development:**

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45 Transcription factors involved in osteoblast differentiation such as RUNX2 were found to  
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47 be expressed at comparative levels in BM-MSCs, skin-MSCs, AT-MSCs and WJ-MSCs.  
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49 However, table 2 shows that skin-MSCs are characterized by a significantly increased  
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51 expression of genes (BMP4, BMP2) associated with bone and cartilage development in  
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53 comparison to the other MSCs.  
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3 **WJ-MSCs reveal a significant expression of genes involved in liver and cardiovascular**  
4 **development:**  
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8 The transcriptomic profile of WJ-MSCs and AF-MSCs reveals the basal expression of  
9 several mature myocardial genes: GATA-4, c-TnT, Cx43, which could be associated to the  
10 potential of differentiation into myocardial cells. Interestingly, a high expression of genes  
11 encoding for GATA-binding protein 6 (*GATA6*), heart and neural crest derivatives expressed 1  
12 (*HAND1*), *ICAM1* and *VCAM1* was detected in WJ-MSCs (table 2). WJ-MSCs were shown to  
13 express genes involved in cardiovascular system development, including angiogenesis,  
14 cardiogenesis, development of endothelial tissue, endothelial cell development, and  
15 vasculogenesis (Table 2). In addition, other genes involved in cardiovascular development,  
16 including endoglin (*ENG*), *GJA1*, *VCAM1*, and *GATA6* (Table 2) were significantly increased in  
17 BM-MSCs (25).  
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29 The transcriptomic profile reveals also that WJ-MSCs have a significantly increased  
30 expression of genes (*AFP*, *DKK1*, *DPP4*, *DSG2*) associated with liver development compared to  
31 BM-MSCs, AT-MSCs and skin-MSCs. These observations suggest that WJ-MSCs could be the  
32 most promising mesoderm-derived stem cells able to differentiate into hepatocyte-like cells.  
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40 **WJ-MSCs express genes involved in neural development:**  
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42 Based on gene expression profiling, BM-MSCs constitutively seem to express genes  
43 related to inflammation and immunodulation, whereas genes implicated in tissue development  
44 were highly expressed in AT-MSCs while WJ-MSCs and DP-MSCs revealed an inherent bias  
45 towards the neuro-ectoderm lineage (9). De Kock *et al.* studied the whole gene expression  
46 profiles of 4 human mesoderm-derived stem cell populations: AT-MSCs, BM-MSCs, skin-MSCs  
47 and WJ-MSCs. They showed differences in gene expression between distinct stem cell types.  
48 Skin-MSCs pre-dominantly expressed genes involved in neurogenesis (*NES*), skin and bone  
49 (*RUNX2*, *BMP4*).  
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3 Such a transcriptomic profile reveals a closer proximity between WJ-MSCs and BM-  
4 MSCs than between other combinations. However, WJ-MSCs genomic profile suggests that WJ  
5 appears to be the most appropriate source to use in cardiovascular area.  
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#### 10 11 12 **4. Differentiation potential** 13

14 A summary of the various comparisons between sources of MSCs already made in the  
15 literature is shown in figure 1. WJ-MSCs have, as BM-MSCs, low capacity to differentiate into  
16 adipocytes cells (3). Bai *et al.* have shown that AF-MSCs and WJ-MSCs could differentiate into  
17 myocardial-like cells with an important expression of myocardial genes such as GATA-4, c-TnT,  
18  $\alpha$ -actin, Cx43 after myocardial induction (12).  
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25 More recently Chen *et al.* worked on *in vitro* differentiation analysis of MSCs isolated  
26 from DP and WJ. They show that all isolated MSCs from different origins exhibited the capacity  
27 to differentiate into osteoblasts, chondrocytes, and adipocytes. However, they noted some  
28 differences between the 3 different types of MSCs. Osteogenic differentiation potential was the  
29 same but adipogenic and chondrogenic differentiations of WJ-MSCs were more important than  
30 the differentiation of DP-MSCs (11). Meanwhile, Jo *et al.* studied *in vivo* osteogenic  
31 differentiation of MSCs isolated from different sources. They found no differences detectable in  
32 osteogenesis between adult AT-MSCs, BM-MSCs and WJ-MSCs (26). According to Zhang *et*  
33 *al.*, bone formation was significantly higher for BM-MSCs compared to WJ-MSCs. Conversely,  
34 Jo *et al.* showed a significantly higher osteogenesis in the WJ-MSCs compared with the BM-  
35 MSCs. The *in vitro* comparison of umbilical cord derived-MSCs and BM-MSCs by Baksh *et al.*  
36 concurs with Jo *et al.* findings (17, 27). Differences between mentioned studies are probably  
37 due to the different experimental conditions. These results suggest that MSCs of various tissue  
38 origins have specific characteristics of differentiation or require different conditions for  
39 osteoinduction.  
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3 Very recently, Yu *et al.* showed that WJ-MSCs are not a good alternative for periodontal  
4 tissue generation compared to PDL-MSCs which have a much better osteo/dentinogenic  
5 differentiation potential (6, 8). Various studies have shown the capacity for WJ-MSCs to  
6 differentiate into pancreatic islet-like cell (28-30). Kim *et al.* compared this potential with other  
7 sources of MSCs and didn't show significant differences between BM-MSCs and AT-MSCs (28).  
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10  
11 Some authors have focused on the application of MSCs in vascular engineering and  
12 more particularly their capacity to differentiate into endothelial cells (EC). Chen *et al.*, were  
13 pioneer in studying the endothelial differentiation potential of BM-MSCs and WJ-MSCs. Both  
14 sources of MSCs were able to differentiate into EC but WJ-MSCs appear to have a greater  
15 differentiation potential as derived EC-like have a higher expression of endothelial markers (4).  
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## 27 28 29 **II. WJ-MSCs as immunoprivileged cells**

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### 30 31 32 33 34 **1. Immunological features of MSCs**

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36 MSCs have in the last decade gained considerable attention as candidates for tissue  
37 engineering, as modulators of immune responses in graft-versus-host-disease and autoimmune  
38 diseases (31), since these cells, once administered therapeutically may be able to evade the  
39 immune system of the host. They are currently being assessed as a novel anti-inflammatory  
40 therapeutic agent in numerous clinical trials (32). Two outstanding features of MSCs are  
41 relevant to their immunomodulatory effects:  
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50 **- Immunosuppression:** MSCs-mediated immunosuppression describes the fact that MSCs are  
51 able to suppress several functions (proliferation, production of soluble factors and cellular  
52 cytotoxicity) exerted by diverse immune cells such as T-, B- and natural killer (NK) cells. It has  
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3 been shown that immunosuppression is mediated by both cell-cell contact and paracrine signals  
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5 via soluble factors (figure 2).  
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8 - **Immunoprivilege:** MSCs themselves are somehow protected from immunological defense  
9 mechanisms (33). Indeed, MSCs lack expression of major histocompatibility complex (MHC)  
10 class II giving MSCs the potential to escape recognition by alloreactive CD4+ T cells but  
11 express MHC Class I molecules. This expression allows them to escape from NK cell lysis (34).  
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13 Also, MSCs do not express co-stimulatory molecules required for effector T cell induction.  
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18 Even if BM-MSCs, considered as the gold standard in MSC therapy, and WJ-MSCs  
19 share many similarities, emerging data suggest that the latter could be less immunogenic than  
20 BM-MSCs, making them a good candidate for allogeneic as well as xenogeneic transplantation  
21 (Figure 2).  
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## 29 **2. MSCs-mediated immunosuppression**

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31 MSCs show an absence or a low expression of MCH class II and co-stimulatory  
32 molecules, so they can be considered as immunoprivileged cells, but they also interfere with  
33 different pathways of the immune response (35). Their ability to modulate the immune system  
34 was first recognized after the fact that they could evade immunosurveillance after cell  
35 transplantation (36). Especially, human MSC populations such as BM-, AT-, or UC-derived  
36 MSCs selectively alter immune cell function by suppressing T cell proliferation, B cell  
37 proliferation and terminal differentiation (37), inhibiting NK cell proliferation and cytotoxicity,  
38 steering monocytes and dendritic cells (DCs) to an immature DC state (38).  
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### 50 *a. MSCs and immune cell population*

#### 51 **a.1 Adaptive Immunity**

##### 52 **➤ MSCs and T cells**

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3 T cells recognize antigens and are critical for cell-mediated immune response. They  
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5 mature within the thymus into one of different subtypes with diverse roles. These cells are  
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7 involved in the maintenance of self-tolerance, activation of other lymphocytes, lysis of infected  
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9 cells, and interaction with cells of the innate immune system.  
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12 Currently, interactions of MSCs with T cells have been extensively studied. Graft versus  
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14 Host Disease models presented the first evidence that MSC can regulate immunosuppression *in*  
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16 *vivo* (39). MSCs could reduce allograft rejection, which is partly mediated by T cells (40, 41).  
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18 Shortly after, T cell immunosuppression mediated by MSCs was demonstrated *in vitro*. MSCs  
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20 inhibit probably *via* their induced or constitutively expressed secreted factors, T-lymphocyte  
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22 activation and proliferation induced by mitogens and alloantigens (42-45) as well as T-cell  
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24 activation with CD3 beads (43, 46). MSCs have been shown to equally inhibit CD4+, CD8+,  
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26 CD2+ and CD3+ subsets (47). In addition, T-lymphocytes inhibited by BM-MSCs do not enter  
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28 apoptosis, since they actively proliferate on re-stimulation with cellular and humoral activators  
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30 (42). Many other studies have shown the ability of BM-MSCs to induce the expansion of  
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32 functional regulatory T cells (Tregs) (47, 48). Recently, it has been shown that adhesion  
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34 molecules ICAM-1 (inflammatory cytokine-induced intercellular adhesion molecule-1) and  
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36 VCAM-1 (vascular cell adhesion molecule-1), which are required for a direct adhesion of MSCs  
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38 to T cells, are critical for subsequent MSCs mediated immunosuppression, and are inducible by  
39  
40 the parallel presence of IFN- $\gamma$  and inflammatory cytokines (49). Another possible mechanism  
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42 underlying the BM-MSCs mediated suppression of T-cells is to prevent their entry into the S  
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44 phase of the cell cycle by mediating irreversible G0/G1 phase arrest through the inhibition of  
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46 cyclin D2 expression (46, 50). Similarly, it has been shown that addition of DP-MSCs to  
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48 phytohemagglutinin (PHA)-stimulated T cells mediated an inhibition of their response (51).  
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50 Elevated expression of immunodulatory soluble factors (HGF- $\beta$ 1, ICAM-1, IL-6, IL-10, TGF- $\beta$ 1,  
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52 VCAM-1, VEGF) secreted by human DP-MSCs was detected in co-culture system with  
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54 decreased expression levels of some pro-inflammatory cytokines and increased levels of some  
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3 anti-inflammatory ones. Induction of Treg markers by human DP-MSCs was also demonstrated  
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5 (52). A very recent study has examined the *in vivo* and *in vitro* immunomodulatory effects of  
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7 human supernumerary tooth-derived mesenchymal stem cells (SNT-MSCs). It has been shown  
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9 that, in *in vitro* co-cultures, these cells suppressed the viability of T-cells and also the  
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11 differentiation of Th17 cells. *In vivo* transplantation of SNTSCs in systemic lupus erythematosus  
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13 model MRL/lpr mice suppressed increased levels of peripheral Th17cells and IL-17 as well as  
14  
15 *ex-vivo* differentiation of Th17 cells (53).  
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18 Fetal MSCs have been reported to have similar inhibitory effects on T-lymphocytes. It  
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20 has been shown that, mitogen-induced T-cell proliferation in an allogeneic model transplant as  
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22 well as in a xenograft model was effectively suppressed by WJ-MSCs with levels comparable to  
23  
24 BM-MSCs immunosuppression (54). Also, IFN- $\gamma$  and/or TNF- $\alpha$  produced by activated T cells  
25  
26 stimulate production of IDO (indoleaminedesoxxygenase) by MSCs, which, in turn, inhibited T-  
27  
28 cell proliferation (55). Tipnis *et al.* have reported that the expression of B7-H1, a negative  
29  
30 regulator of T-cell activation constitutively expressed by WJ-MSCs, is increased after IFN- $\gamma$   
31  
32 treatment. In addition, IFN- $\gamma$  treatment induced IDO secretion by WJ-MSCs which inhibited T-  
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34 cell proliferation (56). These results were confirmed very recently by Manochante *et al.* showing  
35  
36 that MSCs from amnion, placenta, WJ and UC can potentially substitute BM-MSCs in several  
37  
38 therapeutic applications. Indeed, these cells inhibited alloreactive T-lymphocytes in the mixed  
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40 lymphocyte reaction in a similar degree as BM-MSCs (57).  
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47 ➤ MSCs and B cells  
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49 B cells play an essential role in adaptive immunity. These cells develop in the bone  
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51 marrow strictly after close interaction between B cells progenitors and stromal cells that produce  
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53 cytokines capable of supporting B cell survival and proliferation (59). They are directly  
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55 responsible for the humoral immune response *via* the secretion of antibodies against pathogenic  
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3 or foreign antigens. A subset of B lineage differentiates into memory B cells, which can mediate  
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5 a rapid response upon secondary exposure to that same antigen.  
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8 Data about influence of MSCs and B cells growth, differentiation and production of  
9 immunoglobulins (Ig) is still scarce and controversial (58). Corcione *et al.* demonstrated that  
10 BM-MSCs inhibited the proliferation of B cells and decreased significantly the production of IgM,  
11 IgG and IgA (60); the same effect has been reported by Chen *et al.* showing that UC-MSCs  
12 significantly suppressed the proliferation, differentiation, and immunoglobulin secretion of B cells  
13 *in vitro* (61). However, these results have been contradicted by other groups. Thus, an  
14 increased proliferation and IgG production of B cells after co-culturing with BM-MSCs has been  
15 reported (62, 63), and a recent study demonstrated that UC-MSCs promoted proliferation and  
16 differentiation of B cells *in vitro and in vivo* partially through prostaglandin E2 (PGE2) axis (59).  
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## 28 **a.2 Innate response**

### 29 ➤ MSCs and NK cells

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34 NK cells are major effector cells of innate immunity because they lack antigen-specific  
35 cell surface receptors (64). They mediate antibody-dependent cellular cytotoxicity as well as  
36 “spontaneous” killing of infected or transformed cells through release of perforin and granzyme  
37 from cytotoxic granules (65).  
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43 MSCs and NK cells have been shown to interact *in vitro*. The outcome of this interaction  
44 may depend on the state of NK-cell activation and/or the cytokines present in the culture  
45 medium. IFN- $\gamma$  activated MSCs escaped NK cells mediated lysis through induction of HLA-E,  
46 up-regulation of MHC class I and NK inhibitory ligands (66, 67). In response to this up-  
47 regulation, MHC class I molecules including human leukocyte antigen-G5 (HLA-G5), expressed  
48 by MSCs, bind to the inhibitory receptor ILT2 expressed on NK cells (68). Furthermore, other  
49 studies have shown that suppression of NK cell functions is mediated by a down-modulation of  
50 some activating NK cell receptors (NKp30, NKp44 and NKG2D) and by inhibition of NK cell lytic  
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3 granule formation (69). There is growing evidence that IDO, PGE2 and TGF- $\beta$ 1 may control  
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5 MSC-mediated inhibition of NK-cell function (70). However, these results may be different  
6  
7 depending on the MSC source in the studies.  
8

9  
10 Boissel *et al.* evidenced that NK cells had higher expansion when cultured with  
11  
12 allogeneic and autologous WJ-MSCs as feeders in the presence of NK growth factors. WJ-  
13  
14 MSCs feeders were rejected during the first week of co-culture. Expanded NK cells maintained  
15  
16 an elevated cytotoxic profile and may be genetically manipulated (65). In a recent study, Zhao *et*  
17  
18 *al.* has been interested in elucidating the effect of UC-MSCs on NK cells-mediated cytotoxicity  
19  
20 against DCs and the mechanism involved. They found that UC-MSCs can enhance this effect  
21  
22 possibly by inhibiting DCs maturation and up-regulating the ligands for killer activator receptor  
23  
24 on the surface of the DCs (71).  
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### 31 ➤ MSCs and DCs 32 33

34 DCs play a key role in the initiation of primary immune responses and tolerance,  
35  
36 depending on the activation and maturation stage of DCs. Locally produced inflammatory  
37  
38 cytokines or microbial components promote the maturation of DCs from a processing to a  
39  
40 presenting stage, characterized by up-regulation of MHC-class II and co-stimulatory molecules  
41  
42 (CD80 and CD86), production of IL-12, and migration to lymphoid tissue. DCs maturation is a  
43  
44 prerequisite to induce immunogenic T cell responses, whereas tolerance is observed when  
45  
46 antigens are presented by immature or semi-mature DCs. Therefore, DC maturation plays a key  
47  
48 role in initiating T cell responses.  
49  
50

51 BM-MSCs were shown to block the generation of functional antigen-presenting cells,  
52  
53 including myeloid DCs from both monocytes and CD34+ cell precursors (72-74). Most results  
54  
55 supported the notion that DCs at early stages of differentiation are sensitive to their inhibitory  
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3 effects, while at later stages are resistant. However, WJ-MSCs inhibited DC maturation and  
4  
5 activation even when the contact happened at the mature or immature stage. Both cell contact  
6  
7 *via* surface ligands (B7H1) as well as soluble factors (IDO) enhanced the efficiency of  
8  
9 suppression (56). Very recently, Saeidi *et al.* showed that UC-MSCs and BM-MSCs strongly  
10  
11 inhibited differentiation and maturation of DCs with more inhibitory effect on CD1a, CD83, CD86  
12  
13 expression and dendritic cell endocytic activity. These cells also severely up-regulate CD14  
14  
15 expression. Results have indicated that UC-MSCs and BM-MSCs exerted their inhibitory effect  
16  
17 on differentiation, maturation and function of DCs through the secreted factors and free of any  
18  
19 cell-to-cell contacts (75).  
20  
21

22 Immunomodulatory properties of WJ-MSCs on innate and adaptive responses are resumed in  
23  
24 the figure below (figure 3).  
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#### 29 b. MSCs and soluble factors 30 31

32 Multiple reports have evidenced, first *in vitro* and then *in vivo*, the ability of MSCs to  
33  
34 express molecules that interact with both innate and adaptive immunity, both through soluble  
35  
36 factors (42, 76) and in a cell contact-mediated fashion probably through the interaction of  
37  
38 membrane receptors, adhesion molecules or the cellular exchange of membrane vesicles (77).  
39  
40 It is still a matter of debate if the regulatory effects are cell-to-cell contact-dependent, or if  
41  
42 soluble factors are sufficient (78). The MSCs immune modulating effects will depend also on the  
43  
44 ratio between MSCs and immune cells, and the state and stage of immune cell activation or  
45  
46 maturation. Several factors that contribute to the MSCs-mediated effects have been identified,  
47  
48 in particular growth factors, cytokines, chemokines and hormones, all of which exert paracrine  
49  
50 effects on immune cells and allow homing, migration and their attachment to injured cells.  
51  
52 Soluble factors implicated in MSCs-mediated immune-modulation include nitric oxide (NO),  
53  
54 Indoleamine 2,3-dioxygenase (IDO), Heme Oxygenase (Hmox 1), secretion of anti-inflammatory  
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3 cytokines like IL-10, transforming growth factor- $\beta$  (TGF- $\beta$ ), hepatocyte growth factor (HGF), IL-  
4  
5 6 and PGE2 (45, 47, 79, 80). A study comparing the immunomodulatory properties of MSCs  
6  
7 derived from many sources showed that despite their similar cytokine profiles, WJ-MSCs only  
8  
9 secrete IL-12, IL-15 and platelet derived growth factor-AA (PDGF-AA). They did not secrete  
10  
11 VEGF like other adult MSC sources (55). The precise meaning of these differences, however,  
12  
13 needs to be understood in WJ-MSCs/ immune cell co-cultures (81).  
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16  
17 Specialized immune tolerance implicated at the maternal-fetal interfaces depend on  
18  
19 expression of many molecules including galectin-1, B7 proteins, HLA-G (82), and expression of  
20  
21 immune suppressive cytokines like Leukemia inhibitory factor (LIF) (83, 84). Because WJ-MSCs  
22  
23 are isolated from a peri-natal source, they could exhibit immune evasion mechanisms dominant  
24  
25 at the fetal maternal interface. In fact, Najjar *et al.* showed that higher constitutive as well as IFN-  
26  
27  $\gamma$  inducible levels of LIF are expressed by WJ-MSCs than by BM-MSCs and the suppression of  
28  
29 lymphoproliferation can be rescued by blocking LIF in co-cultures (85). Furthermore, Prasanna  
30  
31 *et al.* reported higher levels of both constitutive and IFN- $\gamma$  inducible HGF in WJ-MSCs over BM-  
32  
33 MSCs (86).  
34  
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36  
37 Non-classical type I HLA molecules are an interesting as yet only partly explored field in  
38  
39 MSCs immune function. Several reports showed that BM-MSCs and WJ-MSCs express the  
40  
41 HLA-G molecule, both as mRNA and protein level, and its soluble form HLA-G5 (20, 54, 87);  
42  
43 whereas Weiss *et al.* showed that WJ-MSCs constitutively express high levels of the immune  
44  
45 suppressive HLA-G6 isoform while BM-MSCs express HLA-G5 isoform constitutively and its  
46  
47 expression is not induced by IFN- $\gamma$  (54). HLA-G5 secretion has been directly implicated in  
48  
49 induction of regulatory cells (CD4+CD25+FoxP3+ Tregs) which are characterized as key  
50  
51 suppressors of effector responses to alloantigens (88). HLA-G5 secretion has also been linked  
52  
53 with the suppression of NK cell production of IFN- $\gamma$  in BM-MSCs co-cultures (89). Inhibition of  
54  
55 maternal alloreactivity is due to expression of high levels of HLA-G by the fetus; however, the  
56  
57 exact role of immune-suppressive HLA-G isoforms in influencing immune suppression needs to  
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3 be evaluated in detail, it is possible that the HLA-G6 isoform has a specific role in WJ-MSCs-  
4 mediated immune suppression (81). Recently, the expression of HLA-E and HLA-F on the WJ-  
5 MSCs has been reported, both are implicated in tolerogenic processes occurring at the  
6 fetomaternal interface, together with HLA-G (90).  
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### 14 15 16 17 **III. Homing of MSCs** 18 19

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22 Homing is essentially the process by which cells migrate to and engraft in the tissue in  
23 which they will exert functional and protective effects (91). For common therapeutic purpose,  
24 systemic infusion of MSCs is regarded as the most practical mode of administration. It has been  
25 reported that *in-vitro*-expanded MSCs preferentially home to sites of tissue damage, where they  
26 enhance wound healing, support tissue regeneration and restore the BM microenvironment  
27 following damage by myeloablative chemotherapy or integrate into tumors (92). Since the  
28 precise molecular mechanisms by which MSCs migrate into sites of injury are not yet fully  
29 defined, migration of leukocytes into sites of inflammation has been taken as model (93).  
30 Indeed, upon delivery into blood stream, the MSCs keep close contact with endothelial cells.  
31 They engraft into the endothelium, and eventually pass and leave the endothelium (94). The  
32 migration of MSCs is mediated by a wide variety of molecules expressed by MSCs including  
33 growth factors, chemokines, and receptors and by chemotactic factors produced by immune  
34 cells (95). It has been demonstrated that human MSCs showed significant chemotaxis  
35 responses to several factors (including PDGF, VEGF, IGF-1, IL-8, bone morphogenetic protein  
36 BMP-4 and BMP-7) (96) and express a variety of chemokine receptors (such as CCR1, CCR4,  
37 CCR7, CXCR5 and CCR10) that might be involved in their migration into injured tissues along a  
38 chemokine gradient (97).  
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3 In addition, specific proteolytic enzymes are required so the cells can traverse the  
4 protein fibers of the extracellular matrix (ECM) and reach the target sites (92, 98). In particular,  
5 the matrix metalloproteinases (MMPs), consisting of more than 24 zinc-dependent  
6 endopeptidases, are physiologically necessary for stem cell migration, degradation and  
7 remodeling of ECM components, and are crucial for developmental events like morphogenesis,  
8 cell proliferation, apoptosis and differentiation (99-102). MMPs are physiologically necessary for  
9 stem cell migration. Ries *et al.* were the first to show that human BM-MSCs use constitutively  
10 expressed MMP-2, membrane-type matrix metalloproteinase-1 (MT1-MMP), and tissue inhibitor  
11 of metalloproteinase 2 (TIMP-2) to migrate through human recombinant basement membranes.  
12 It has been reported that the inflammatory cytokines TGF- $\beta$ 1, IL-1 $\beta$ , and TNF- $\alpha$  exerted  
13 chemoattractive potential on BM-MSCs and up-regulated MMP-2, MT1-MMP and/or MMP-9  
14 allowing cellular trafficking of MSCs across human ECM barriers (92).  
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29 The expression of a set of gelatinases (gelatinase-A and -B, MMP-2 and MMP-9),  
30 collagenases (collagenase-1 and -2; MMP-8 and MMP-13) has been detected in the WJ-MSCs  
31 (103, 104), as well as in the different regions of full-term human umbilical cord and in cultured  
32 HUVEC. The wide expression of these enzymes in the umbilical cord has been attributed to  
33 their role in the degradation and remodeling of ECM and in other physiological processes (104).  
34 A proposed mechanism for WJ-MSCs homing is shown in figure 4.  
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42 Thus, *ex vivo*-expanded human MSCs with cytokines may be a useful method, in clinical  
43 applications, to increase their migration/homing potential after transplantation into patients, as  
44 well as the administration of cytokines to mobilize hMSCs to sites of injury.  
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#### 52 **IV. Anti-tumorigenic effects of WJ-MSCs**

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5 MSCs have the capability to migrate to tumor sites and modulate their microenvironment  
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7 thus having an impact on the tumor behavior (105). Some evidences suggest that solid tumors  
8  
9 generate a microenvironment similar to that described in wound healing as they apply physical  
10  
11 and chemical stress to neighboring tissues. Tumors can therefore be considered as sites of  
12  
13 tissue damage which also induces the migration of MSCs to their sites (106).  
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17 Human MSCs have been studied intensively for their potential use in cancer treatment,  
18  
19 however their use has been met by a general concern related to the biosafety of using MSCs  
20  
21 (107). Reported effects of MSCs on progression of primary and metastatic tumors have been  
22  
23 both pro- and anti-tumorigenic, and these contradictory results could be associated to  
24  
25 differences in the sources of MSCs, the type of tumor model, the way of administration of  
26  
27 MSCs, or other unknown factors (106, 108).  
28

29  
30 The tumor stroma consists of a complex extracellular matrix wherein inflammatory and  
31  
32 immune cells, fat cells, fibroblasts and blood vessels reside. It plays a crucial role in tumor  
33  
34 progression, angiogenesis and metastasis by involving tumor-host interactions. Tumor  
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36 associated fibroblasts (TAFs) design activated fibroblast in the tumor stroma (107). Several  
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38 reports hypothesized that BM-MSCs selectively proliferate to tumors and contribute to the  
39  
40 formation of tumor-associated stroma by transforming into TAFs, and they promote tumor  
41  
42 growth and metastasis by enhancing migration, angiogenesis and by inhibiting apoptosis of  
43  
44 tumor cells (109-113).  
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47 On the other hand, immunosuppressive effects of MSCs can impair the function of a  
48  
49 variety of immune cells (directly or through paracrine signals) which may be an important  
50  
51 mechanism through MSCs promote tumor growth or increase incidence of tumor formation. For  
52  
53 instance, by increasing Tregs and reducing the activity of NK cells and cytotoxic T lymphocytes  
54  
55 (CTL) (known to kill tumor cells), BM-MSCs can protect breast cancer cells (114). BM-MSCs  
56  
57 were also shown to be linked to carcinomas (111, 115), prostate tumor (116, 117), breast  
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3 tumors (114, 116, 118), colon cancer (113), etc... A recent study has also demonstrated a fusion  
4  
5 between MSCs and gastrointestinal epithelial cells, suggesting the formation of a more cancer  
6  
7 prone cell type (119).  
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10 Similarly, tumorigenesis was described as the major obstacle of tissues derived from  
11  
12 hESCs and human induced pluripotent stem cells (iPSCs) for transplantation therapy (120).  
13  
14 Teratoma formation is due to the expression of some or all pluripotent genes of rogue  
15  
16 pluripotent undifferentiated hESCs and hiPSCs residing in the differentiated cell population  
17  
18 (121). Very recently, in order to examine the anticancer therapeutic application of MSCs from  
19  
20 different sources, Akimoto *et al* studied the inhibitory effects of MSCs from umbilical cord blood  
21  
22 (UCB) and adipose tissue on “glioblastoma multiforme (GBM)” (most aggressive primary brain  
23  
24 tumors in human). They found that, *in vitro* and *in vivo*, GBM growth was inhibited by UCB-  
25  
26 MSCs, but was promoted by AT-MSCs. UCB-MSCs induced apoptosis of GBM via tumor  
27  
28 necrosis factor-related apoptosis –inducing ligand (TRAIL) which was expressed more highly by  
29  
30 UCB-MSCs than by AT-MSCs (122). Furthermore, it has been shown that naïve UC-MSCs have  
31  
32 the ability to produce factors that can suppress cancer cell growth and induce apoptosis, and  
33  
34 may be a novel tool for cancer therapy over MSCs from other sources (123). Reports evidenced  
35  
36 that WJ-MSCs abrogate certain solid tumors (124-127). These cells decreased the growth of  
37  
38 human breast cancer *in vitro* and intravenous injections of these cells stopped its growth in  
39  
40 SCID mouse model (128). Later Fan *et al.* showed that WJ-MSCs do not induce (1) teratomas  
41  
42 in immunodeficient SCID mice and (2) tumors when transplanted in diseased animal models  
43  
44 (129). In a recent study, Subramanian *et al.* examined whether WJ-MSCs, like BM-MSCs,  
45  
46 transform to the TAF phenotype in the presence of ovarian and breast cancer conditioned  
47  
48 medium. Results showed absence of expression of tumor associated markers for WJ-MSCs  
49  
50 with low expression of TAF-related genes confirming therefore that these cells are not  
51  
52 associated with enhanced growth of solid tumors (1). In order to verify if the inhibition of cancer  
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54 cell growth by WJ-MSCs was not specific to only breast cancer cells, the same group compared  
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3 the effects of WJ-MSCs extracts and cell lysate on 3 other types of solid tumors: breast  
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5 adenocarcinoma, ovarian carcinoma and osteocarcinoma. Same effect as previously described  
6  
7 was observed and probably this effect is mediated *via* agents in WJ-MSC's extracts (130).  
8  
9 Then, Ma *et al* showed that WJ-MSCs significantly inhibited the growth of breast cancer stem  
10  
11 cells *in vitro* and *in vivo* by probably inducing a cell cycle arrest, a tumor cell apoptosis and a  
12  
13 suppression of phosphoinositide 3-kinase (PI3K) and AKT (also known as protein kinase B)  
14  
15 activities (127). A more recent study reported that WJ-MSCs conditioned medium as well as the  
16  
17 cell lysate inhibit mammary carcinoma and osteosarcoma cell growth *via* apoptosis and  
18  
19 autophagy *in vitro* and in xenograft mice model (131). In another study evaluating the  
20  
21 tumorigenesis potential of WJ-MSCs in comparison with hESC, it has been demonstrated that  
22  
23 animals receiving hESCs developed teratomas with an increased levels of pro-inflammatory  
24  
25 cytokines. However, when injected with WJ-MSCs, no tumors or inflammatory reactions were  
26  
27 developed at the injection sites and an increased production of anti-inflammatory cytokines was  
28  
29 detected (120). A very recent study interested by the effects of UC-MSCs on "intrahepatic  
30  
31 cholangiocarcinoma (ICC)" (common form of primary liver cancer), showed that these cells can  
32  
33 inhibit the proliferation and induce the apoptosis of human ICC cells. Apoptosis of tumor cells  
34  
35 was related to the inhibition of PI3K/Akt and the Wnt/ $\beta$ -catenin signaling pathways (132). Effects  
36  
37 of WJ-MSCs have also been studied in hematopoietic tumors. Results obtained by Tian *et al*  
38  
39 provide new insight how these cells modulate leukemic tumor growth *in vitro*, demonstrating that  
40  
41 the p38 MAPK- a suppressor of tumor development- is required for leukemic tumor suppression  
42  
43 by WJ-MSCs (133).  
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49 These studies taken together approve that WJ-MSCs are non-tumorigenic, anti-  
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51 tumorigenic, hypoimmunogenic, do not transform to the TAF phenotype that is associated with  
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53 enhanced growth of solid tumors, and suppress hematopoietic tumor development making them  
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55 a safe source for future cancer therapy and clinical applications.  
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## V. Therapeutical applications

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### 1. Treatment of autoimmune diseases

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 diseases.

#### a. Type 1 diabetes

Diabetes is a metabolic disease listed among the leading causes of death in some countries. It's characterized by absolute or relative insulin deficiency.

Type 1 diabetes is defined by an absolute insulin decrease due to T cell mediated destruction of insulin-producing pancreatic  $\beta$ -cells (134). This autoimmune destruction of pancreatic islet  $\beta$ -cells (PICs) reduces the ability of the patient to regulate the rate of blood glucose, resulting in a high frequency of vascular complications that compromise quality and expectancy of life (135). Transplantation of pancreatic islet cells 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 differentiation capacity of WJ-MSCs into mature islet-like cell clusters. These islet-like cell clusters were 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 showed the enhancement of insulin and other pancreatic  $\beta$ -cell related genes, such as *pdx1* (pancreatic and duodenal homeobox 1), *hlxb9* (homeobox HB9 ou MNX1), *nkx2.2* (NK2 homeobox 2), *nkx6.1* (NK6 homeobox 1), and *glut-2* ( glucose transporter 2) in these cells (136). Various publications confirmed the pancreatic islet-like cell differentiation potential of WJ-MSCs (29, 30, 137). Kim *et al.*, comparing the PICs differentiation capacity of MSCs from many sources (WJ, BM, AT and periosteum), have confirmed that all cell lines were well differentiated



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3 with an increased insulin mRNA expression, but only PICs derived from periosteum progenitor  
4 cells showed insulin secretion to a high glucose concentration (137). More recently Hu *et al.*,  
5 studied the therapeutic potential of WJ-MSCs in patients with type 1 diabetes and they  
6 evaluated the effects of these cells for a longer treatment time. They followed two groups of  
7 patients: the first group received a basic treatment combined with WJ-MSCs implantation, while  
8 the second group also received a basic treatment with a normal saline therapy. Patients were  
9 followed-up for two years after the operations. During the follow-up period, patients treated with  
10 WJ-MSCs showed better Hba1 and C-peptide expression levels than patients of the second  
11 group. Although precise mechanisms are unknown, WJ-MSCs therapy showed a promising  
12 effect on type 1 diabetes patients and seems to be a good strategy for treatment of this disease  
13 (135).  
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32 *b. Type 2 diabetes*

33 Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and it's  
34 characterized by insulin resistance and pancreatic  $\beta$ -cell dysfunction. Hu *et al* studied the effect  
35 of intravenous infusion of human WJ-MSCs therapy and combined it with administration of  
36 sitagliptin (a dipeptidyl-peptidase IV inhibitor shown to increase insulin release and decrease  
37 glucagon levels by impacting on the  $\alpha$  and  $\beta$  cells in pancreatic islet) in a T2DM rat model. They  
38 obtained, compared to rats controls (those in diabetic control and sitagliptin group), an  
39 increased number of  $\beta$ -cells in rats treated with only WJ-MSCs and those treated with combined  
40 WJ-MSCs and sitagliptin. They also observed the decrease of glucagon level in sitagliptin rats  
41 group and (WJ-MSCs + sitagliptin) rats group compared to those in WJ-MSCs group and  
42 diabetic control group. These results suggest the therapeutic potential of WJ-MSCs in  $\beta$ -cells  
43 regeneration (138).  
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3       c. *Crohn's disease*  
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5       Crohn's disease (CD) is an inflammatory chronic disease characterized by idiopathic  
6 inflammation of the gastrointestinal tract. It can affect any part of the intestine, from mouth to  
7 anus, but preferentially involves the ileum and colon. CD's signs are skip lesions, inflamed parts  
8 of the intestine interspersed by apparent normal healthy tissue. CD's diagnosis is established by  
9 the clinical features confirmed by endoscopy. Frequent complications in CD are abscess and  
10 stricture formation, intestinal obstruction, and fistulas (abnormal connective passages from the  
11 epithelial lining of the intestines to another organ or to the skin caused by inflammation).  
12 Approximately 39% of the patients with CD will even require repeated surgery. An anti-TNF- $\alpha$   
13 therapy is the first choice in the treatment of patients with perianal fistulas. However, even with  
14 treatment, perianal fistulas often lead to physical and emotional distress and only in 46% of the  
15 cases perianal fistulas heal completely (139).  
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29       Therapeutic effects of AT-MSCs and BM-MSCs on Crohn's disease have already been proven  
30 (140, 141). Recently, studies have shown that MSCs with increased CD73 expression could be  
31 the future candidate for therapeutic intervention in Crohn's. These results suggest that WJ-  
32 MSCs in terms of their phenotypic profile could have real potential for therapeutic applications in  
33 Crohn's disease (142).  
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42       d. *Systemic lupus erythematosus*  
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44       WJ-MSCs were studied for their therapeutic potential in others autoimmune disease  
45 such as the systemic lupus erythematosus (SLE), an autoimmune inflammatory disease with  
46 multiorgan involvement including the kidney, brain, lung and hematopoietic systems. Lupus  
47 nephritis (LN) is a common major organ manifestation and is a significant cause of morbidity  
48 and mortality (143-145). Interestingly, in recent study dental pulp-MSCs were also suggested to  
49 be a promising MSC source for cell-based therapy for immune diseases such as SLE (53).  
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58       **2. Treatment of neurodegenerative diseases**  
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3 Neurodegenerative diseases are chronic and progressive disorders of the central  
4 nervous system, characterized by a steady loss of neurons in the region of the brain and spinal  
5 cord that affects the mental and motor abilities of affected people. According to the World Health  
6 Organization, Alzheimer and Parkinson are devastating diseases that will represent in 2040 the  
7 second cause of death. Multiple sclerosis is also one of these neurodegenerative diseases.  
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16 a. *Parkinson's disease*

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18 Parkinson's disease (PD) is a neurodegenerative disorder more common in the elderly.  
19 Its symptoms (tremor, rigidity, bradykinesia and postural instability) are caused primarily by the  
20 degeneration of dopamine (DA) neurons in the substantia nigra (146). Current therapies mostly  
21 relieve symptoms but do not restore the lesion side function and the loss of efficacy due to  
22 disease progression. Embryonic stem cells have been investigated as a renewable source of  
23 dopamine-producing. However, technical and ethical difficulties limited the application of this  
24 therapy. Recently more research is turning to the use of MSCs (147).  
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33 Weiss *et al.* characterized the WJ-MSCs and compared them to MSCs derived from  
34 other sources. In their study, they tested the therapeutic effect of WJ-MSCs in parkinsonian rats.  
35 Their first results showed that WJ-MSCs express growth factors and angiogenic factors,  
36 suggesting that they may be used to treat neurodegenerative disease. Indeed, WJ-MSCs  
37 characterization showed that they produced glial cell line-derived neurotrophic factor (GDNF)  
38 located in the cytoplasm. WJ-MSCs express nestin, a marker of primitive neural stem cells (3).  
39 After neural induction of WJ-MSCs, the expression of nestin was lower in differentiated cells  
40 than in undifferentiated cells, whereas the expression of tyrosine hydroxylase (a mature neural  
41 marker of catecholaminergic neurons), was greater in differentiated cells. Moreover, it has been  
42 shown that WJ-MSCs express some genes encoding for proteins with a neurotrophic effect:  
43 CNTF (ciliary neurotrophic factor), VEGF, FGF20, and TRKC (neurotrophic tyrosine kinase). In  
44 addition, when they are transplanted into parkinsonian rats, WJ-MSCs could partially reverse  
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3 the parkinsonian behavioral phenotype (18). Yan *et al.*, have managed to differentiate WJ-  
4 MSCs into neural-like cells *in vitro* and then have tested the therapeutic potential of  
5 differentiated cells by implanting them into the striatum and substantia nigra of methyl-4-phenyl-  
6 1, 2, 3, 6-tetrahydropyridine (MPTP) lesioned hemi-parkinsonian rhesus monkeys. PD monkeys,  
7 transplanted with the induced cells, showed an improvement of the behavioral measures.  
8 Furthermore, pathological and immunohistochemistry data indicated the presence of neuronal-  
9 like cells in the right brain hemisphere of PD monkeys suggesting that they may be  
10 dopaminergic neurons (147). Almost same results were obtained when WJ-MSCs are replaced  
11 by AT-MSCs (148). Also, a benefic effect of BM-MSCs on parkinsonian rat model was shown by  
12 Ye *et al.* (149).

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14 All results and observations confirm the hypothesis of Weiss *et al.* who speculated that  
15 these stem cells may be useful for treating neurodegenerative diseases. Several groups were  
16 interested in determining the effect of MSCs on central nervous system. Ribeiro *et al.* have  
17 shown that AT-MSCs and WJ-MSCs could release trophic/neuroregulatory factors that improve  
18 the metabolic viability of hippocampal neurons cultures. However, these two types of MSCs  
19 don't function similarly because their secretome act differently on the cell viability and on the  
20 densities of hippocampal neurons. In fact, while AT-MSCs need exogenous factors, such as  
21 bFGF, in culture to influence the metabolic viability and neuronal cell densities in primary  
22 cultures of hippocampal neurons, WJ-MSCs secretome is able to promote neuronal survival in  
23 the absence of exogenous factors (150).

#### 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 *b. Multiple sclerosis*

50 Multiple sclerosis (MS) is a progressive neurodegenerative disorder of the central  
51 nervous system characterized by chronic inflammation, demyelination and neuronal damage.  
52 Currently, there is no medical cure for MS, mainly owing to an incomplete understanding of its  
53 pathophysiology (151).  
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3 Recently, Payne *et al.* assessed the therapeutic efficiency of BM-MSCs, AT-MSCs and WJ-  
4 MSCs, using recombinant myelin oligodendrocyte glycoprotein (rMOG)-induced experimental  
5 autoimmune encephalomyelitis (EAE), a model of MS in which both T- and B-cells contribute to  
6 the disease pathogenesis. They have demonstrated that BM-MSCs exerted more potent  
7 immunomodulatory effects *in vitro* compared to AT-MSCs and WJ-MSCs. Unexpectedly, BM-  
8 MSCs did not impact the disease course, however transplantation of AT-MSC ameliorated  
9 clinical signs in two animal models of EAE. Furthermore, only AT-MSCs and WJ-MSCs  
10 expressed integrin- $\alpha 4$  (CD49d) but not BM-MSCs which may not be able to adhere to VCAM-1,  
11 a critical step involved in extravasation of cells into the central nervous system during EAE  
12 (152). In a recent work, Liu *et al.* showed that WJ-MSCs could have a potential therapy for MS  
13 and could be an alternative source relative to BM-MSCs extensively studied for the treatment of  
14 MS (153-155). Thus, it is believed that these cells could restore behavioral functions and  
15 attenuate the histopathological deficits of experimental autoimmune encephalomyelitis mice  
16 over the long term (50 days) (156). These results were confirmed by Liang *et al.*, who  
17 transplanted WJ-MSCs to a patient with refractory progressive MS and subsequently observed  
18 stabilization of the disease (157).  
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### 40 c. *Alzheimer's disease*

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42 Unlike WJ-MSCs, BM-MSCs and AT-MSCs have revealed *in vivo* a therapeutic potential for  
43 the treatment of another devastating disease: Alzheimer's disease (158-160).  
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46 Thus, very recently Liang *et al.*, have tried to differentiate WJ-MSCs into cholinergic-like  
47 neurons. Cholinergic neurons are neurons of the autonomic nervous system and are one of the  
48 causes of cognitive disorders. To induced differentiation, they used a neural stem cell  
49 conditioned medium supplemented with Bone Morphogenetic protein (BMP4) and fibroblast  
50 growth factors 8 (FGF8). First, they observed morphological changes of WJ-MSCs after  
51 conditioned culturing. These cells which in normal conditions have a bipolar spindle-like  
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3 morphology, exhibited changed into a bulbous shape with the thin extensions touching each  
4 other to a certain extent after 16 days of differentiation induction. These observations could  
5 already suggest a structural organization in axons. Moreover, they confirmed their hypothesis by  
6 showing an expression of cholinergic neuron markers including CHAT (choline  
7 acetyltransferase) and NF by immunofluorescence and RTPCR after 20 days of induction of  
8 WJ-MSCs.  
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These results demonstrated that WJ-MSCs could be induced into cholinergic-like neurons suggesting that WJ-MSCs may be very good candidate for the treatment of Alzheimer's disease (161).

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## VI. Conclusion

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Since now, Bone Marrow MSC have been considered as the gold standard for a use in therapeutic MSC based therapies. In search for an alternative source to overcome BM-MSC loss of proliferation and senescence, different sources of MSC have been explored such as adipose tissue, dental pulp or amniotic fluid showing variable proliferation potential or multilineage differentiation. Among all these different sources, WJ-MSC offer a high proliferation potential, a high differentiation capacity and because of their immaturity, very interesting immunomodulatory properties. These properties may allow them to overcome host immune system rejection, even when they are used after differentiation. The studies presented in this review, taken together, suggest that WJ-MSCs emerge as promising candidates for cellular therapy in the fields of regenerative medicine, or autoimmune disorders.

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Disclosure statements

No competing financial interests exist.

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## Figure Legends

### **Figure 1: Differentiation potential of WJ -MSCs compared to other sources.**

This figure shows the difference in differentiation potential between MSCs from various sources. The difference of differentiation potential between the sources is presented by the thickness of the arrows (going from thick arrows to dashed ones).

### **Figure 2: Mechanisms of immunosuppression by MSCs.**

MSCs mediate their immunosuppressive process by cell-cell contacts mechanisms or by secreting soluble factors which modulate the function of the immune cells (NO: nitric oxide, IDO: indoleamine 2,3-dioxygenase, IL-10: interleukin 10, TGF- $\beta$ : transforming growth factor  $\beta$ , HGF: hepatocyte growth factor, IL-6: interleukin 6, LIF: leukemia inhibitor factor).

### **Figure 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 DCs 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 Ig (Ig : immunoglobulin).

### **Figure 4: Proposed mechanisms involved in the homing of WJ-MSCs to sites of tissue injury.**

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. Expression of a set of MMPs by WJ-MSCs contributes to the ECM degradation which allows them to cross the ECM and reach the site of injury.

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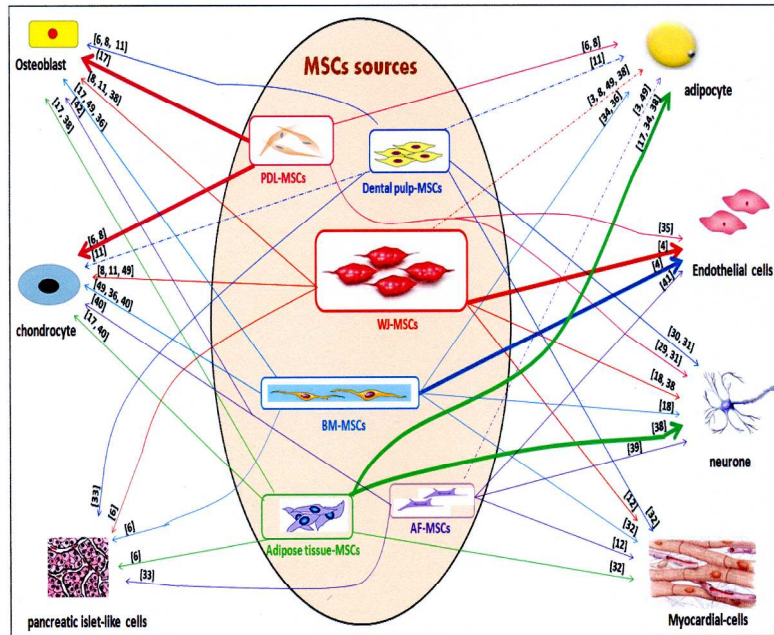
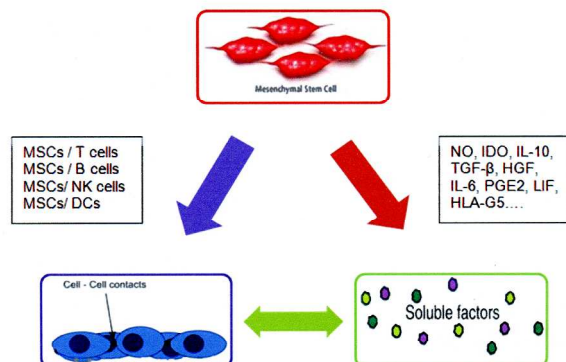


Figure 1 Differentiation potential of WJ-MSCs compared to other sources. This figure shows the difference in differentiation potential between MSCs from various sources. The highest differentiation potential is presented by a thick arrow, the weaker by a thin one and the weakest by a dashed arrow.

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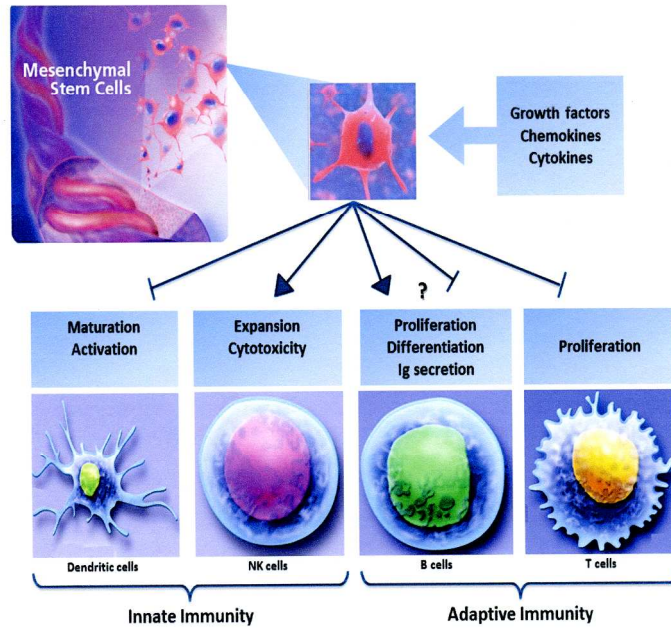
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**Figure 2.** Mechanisms of immunosuppression by MSCs. MSCs mediate their immunosuppressive process by cell-cell contact mechanisms or by secreting soluble factors which modulate the function of the immune cells (NO: nitric oxide, IDO: indoleamine2,3-dioxygenase, IL-10: interleukin 10, TGF- $\beta$ : transforming growth factor  $\beta$ , HGF: hepatocyte growth factor, IL-6: interleukin 6, LIF: leukemia inhibitor factor).

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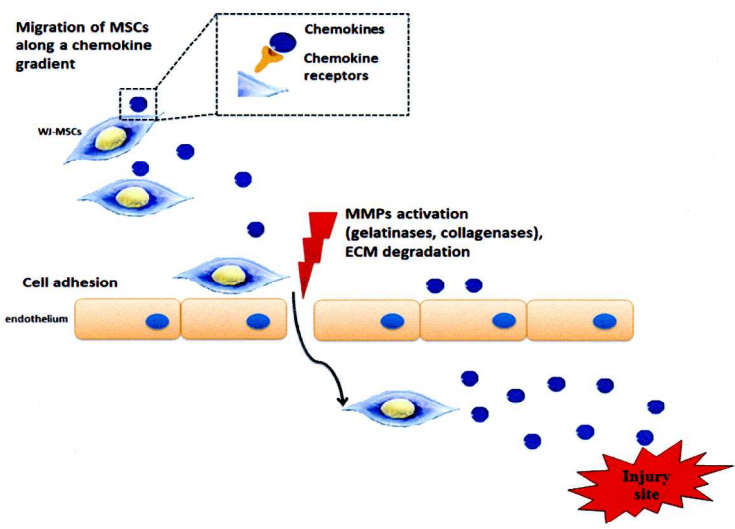


**Figure 3.** Immunomodulatory effects of the WJ-MSCs on innate and adaptive immunity which can be summarized as follows: inhibition of the maturation and activation of DCs as well as the proliferation of T cells. activation of the expansion and cytotoxicity of NK cells. Effects of WJ-MSCs on B cells are still contradictory; they can stimulate or block the proliferation and differentiation of B cells and the secretion of Ig (Ig: immunoglobulin)

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**Figure 4.** Proposed mechanisms involved in the homing of WJ-MSCs to sites of injury. MSCs home to sites 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. Expression of a set of MMPs by WJ-MSCs induces the ECM degradation which allows them to cross the ECM and reach the site of injury.

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**Table 1.** Phenotypic profile of WJ-MSCs compared to MSCs from other sources.

WJ-MSCs MARKERS	COMPARED TO [ ]	REFERENCES
<b>Positive:</b> CD29, CD105, HLA-ABC, Oct-4, Gata-4, Cx43, $\alpha$ -actin, cTnt <b>Negative:</b> CD34, HLA-DR	<b>[AF-MSCs]</b> Similar markers expression except Oct-4 : ~25% for WJ-MSC vs ~51% for AF MSC	(12)
<b>Positive:</b> CD44, CD13, CD56, CD61, CD73, CD105, CD90, CD166, CD29, HLA-ABC, CD59 <b>Negative:</b> HLA-DR	<b>[dental pulp of milk and adult wisdom teeth derived MSCs]</b> Similar markers expression	(11)
<b>Positive:</b> CD73, CD105, CD90, <b>Negative:</b> CD34, HLA-DR, CD45, CD19, CD11b	<b>[PDL-MSCs]</b> Similar markers expression	(8)
<b>Positive:</b> CD68 <b>Negative:</b> CD34, CD45, CD163	<b>[promyelocytic cell line (HL-60) : known to express CD68]</b> Similar level expression	(14)
<b>Positive:</b> CD13, CD29, CD44, CD105, CD106, CD73, CD166, HLA-ABC, CD90 <b>Negative:</b> CD14, CD34, CD38, CD45 CD31, HLA-DR	<b>[Bone marrow MSCs]</b> Similar markers expression except : <ul style="list-style-type: none"> <li>• CD106 : WJ&lt;&lt;&lt;BM</li> <li>• HLA-ABC : WJ&lt;&lt;BM</li> </ul>	(7, 10, 15)
<b>Positive:</b> CD105, CD146, CD73, CD90 <b>Negative:</b> CD14, CD34, CD31, CD45, CD3	<b>[human MSCs from : tibial plateau (TP) , trabecular bone, iliac crest (IC), BM and WJ umbilical cord]</b> Similar level expression for al markers except CD46 (twice more expressed for IC than for WJ and TP)	(16)
<b>Positive:</b> CD44, CD73, CD105, CD90, CD106, CD29, vimentin, laminin, Oct-4, Nanog, <b>Negative:</b> CD34, CD14, CD45, CD31, vWF	<b>[Adult and foetal Bone marrow (aBM-MSCs and fBM-MSCs) and adipose tissue derived-MSC (AT-MSCs)]</b> Similar markers expression except Oct-4 and Nanog expressed only by BM-MSCs and WJ-MSCs	(17)

**Table 2. A to E:** Transcriptomic profile of WJ-MSCs compared to MSCs from other sources. The table below presents the difference of genes expression, for various markers, between the different MSCs sources. The difference of expression between the sources is presented by +++, ++, +, -, and ND corresponds to a non-determined comparison.

*PPIA* : CyclophilinA , *Homo sapiens peptidylprolylisomerase A*; *ITGB1* (*Integrin, b1 :fibronectin receptor, b polypeptide*); *WNT4* (*Wingless-type MMTV inte SHH (Sonic hedgehog homolog (Drosophila))gration site family, member 4*); *SNAI2* (*Snail homolog 2 (Drosophila)*); *SNAI2* (*Snail homolog 2 (Drosophila)*); *TGFBR1* (*Transforming growth factor,  $\beta$  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*.

#### A. Genes related to bone development and neurogenesis

Gene	Gene identification/function	WJ-MSCs	BM-MSCs	PDL-MSCs	AF-MSCs	AT-MSCs	Skin-derived MSCs	references
<b>BMP4</b>	endochondral osteogenesis	++	+	ND	ND	+	+++	(25)
<b>TGFBR1</b>	bone development	++	+	ND	ND	ND	ND	(18)
<b>OPN</b>	osteogenic marker	++	++	+++	ND	ND	ND	(8, 18)
<b>STAT1</b>	bone development	+	+	ND	ND	ND	ND	(16)
<b>BSP</b>	osteogenic marker	-	ND	++	ND	ND	ND	(8)
<b>OSX</b>	osteogenic marker	-	ND	-	ND	ND	ND	(8)
<b>CXCR4</b>	mesoderm marker	+	+	ND	ND	ND	ND	(18)
<b>BMP2</b>	bone development	+	++	ND	ND	+	+++	(25)
<b>RUNX2</b>	osteogenic marker	++	+++	ND	ND	++	++	(16)
<b>CDH2</b>	neural gene	++	+	ND	ND	+	-	(18)
<b>NES</b>	neural development	++	++	ND	ND	-	+++	(25)

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3 **B. Genes related to liver and cardiovascular systems**  
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6 Gene	7 Gene 8 identification/function	9 WJ- 10 MSCs	11 BM- 12 MSCs	13 PDL- 14 MSCs	15 AF- 16 MSCs	17 AT- 18 MSCs	19 Skin- 20 derived 21 MSCs	22 references
23 <b>GATA-4</b>	24 mature myocardial 25 gene	26 +	27 +	28 ND	29 +	30 +	31 ++	32 (12, 20, 25)
33 <b>c-TnT</b>	34 mature myocardial 35 gene 36 cardiovascular	37 +	38 ND	39 ND	40 +	41 ND	42 ND	43 (12)
44 <b>VEGF</b>	45 development	46 +	47 +	48 ND	49 ND	50 ND	51 ND	52 (18)
53 <b>Cx43</b>	54 mature myocardial 55 genes	56 +	57 ND	58 ND	59 +	60 ND	61 ND	62 (12, 14)
63 <b>VCAM1</b>	64 cardiovascular 65 development	66 ++	67 +++	68 ND	69 ND	70 -	71 -	72 (25)
73 <b>GJA1</b>	74 cardiovascular 75 development	76 ++	77 +	78 ND	79 ND	80 +	81 ++	82 (25)
83 <b>AFP</b>	84 liver development	85 +	86 -	87 ND	88 ND	89 ++	90 +	91 (25)
92 <b>DSG2</b>	93 liver development	94 ++	95 -	96 ND	97 ND	98 +	99 -	100 (25)
101 <b>ENG</b>	102 cardiovascular 103 development	104 ++	105 +++	106 ND	107 ND	108 -	109 -	110 (25)
111 <b>HAND1</b>	112 cardiovascular 113 development	114 ++	115 +	116 ND	117 ND	118 +	119 ++	120 (25)
121 <b>GATA6</b>	122 cardiovascular 123 development	124 ++	125 -	126 ND	127 ND	128 +	129 -	130 (25)
131 <b>DPP4</b>	132 liver development	133 +	134 -	135 ND	136 ND	137 ++	138 -	139 (25)
140 <b>DKK1</b>	141 liver development	142 +	143 -	144 ND	145 ND	146 ++	147 -	148 (25)

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3 **C. Genes related to stemness**  
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Gene	Gene identification/function	WJ- MSCs	BM- MSCs	PDL- MSCs	AF- MSCs	AT- MSCs	Skin- derived MSCs	references
<b>ACTG2</b>	stemness-related genes	+	++	ND	ND	ND	ND	(18)
<b>TERT</b>	stemness-related genes	++	+/-	ND	ND	ND	ND	(3, 18)
<b>ESG1</b>	stemness-related genes	++	++	ND	ND	ND	ND	(18)
<b>Oct 3/4</b>	stemness-related genes	+	++	ND	ND	ND	ND	(18)
<b>ABCG2</b>	stemness-related genes	+++	++	ND	ND	ND	ND	(18)
<b>LIFR</b>	stemness-related genes	+++	+++	ND	ND	ND	ND	(18)
<b>SOX2</b>	stemness-related genes	+	ND	+	ND	ND	ND	(8, 18)
<b>Nanog</b>	stemness-related genes	+	ND	++	ND	ND	ND	(8)
<b>Oct-4</b>	stemness-related genes	++	++++	++	ND	ND	ND	(8)
<b>THY1</b>	MSCs marker	++	+	ND	ND	+	-	(25)

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33 **D. Adipogenic and chondrogenic genes**  
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Gene	Gene identification/function	WJ- MSCs	BM- MSCs	PDL- MSCs	AF- MSCs	AT- MSCs	Skin- derived MSCs	references
<b>PPAR<math>\gamma</math></b>	adipogenic marker	+++	+++	+++	ND	ND	ND	(7, 8)
<b>FABP4</b>	adipogenic marker	+	++	ND	ND	ND	ND	(7)
<b>LPL</b>	adipogenic marker	-	++	+	ND	ND	ND	(7, 8)
<b>LEPR</b>	adipogenic marker	-	+	ND	ND	ND	ND	(7)
<b>CD36</b>	adipogenic marker	-	ND	+	ND	ND	ND	(8)
<b>CEBPA</b>	adipogenic differentiation	+	ND	++	ND	ND	ND	(8)
<b>COL2</b>	chondrogenic marker	+	+	+	ND	+	+	(8)
<b>SOX9</b>	chondrogenic marker	+		+	ND	ND	ND	(8)

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## E. Genes implicated in morphogenesis, adhesion, cell structure and other mesodermal markers

Gene	Gene identification/function	WJ- MSCs	BM- MSCs	PDL- MSCs	AF- MSCs	AT- MSCs	Skin- derived MSCs	references
<b>ACTA</b>	maintains the cytoskeleton	+	ND	ND	+	ND	ND	(12)
<b>ACTB</b>		+++	+	ND	ND	ND	ND	(18)
<b>CDH5</b>	controls the cohesion and intercellular junctions	++	+	ND	ND	+	+	(25)
<b>ITGB1</b>	adhesion molecule	++	++	ND	ND	ND	ND	(18)
<b>STAG1</b>	cohesion	+	+	ND	ND	+++	++	(25)
<b>WT1</b>	kidneys and gonads development	++	-/+	ND	ND	-/+	-/+	(25)
<b>WNT4</b>	associated with morphogenesis	+	+	ND	ND	ND	ND	(18)
<b>SNA2</b>	associated with morphogenesis	++	++	ND	ND	ND	ND	(18)
<b>SHH</b>	associated with morphogenesis	-/+	+	ND	ND	ND	ND	(18)
<b>Neuregulin 4</b>	associated with morphogenesis	++	++	ND	ND	ND	ND	(18)
<b>COL1A1</b>	mesodermal marker	+++	+++	ND	ND	-	-	(25)
<b>CXCR4</b>	mesoderm marker	+	+	ND	ND	ND	ND	(18)
<b>ICAM1</b>	mesodermal lineage specification	+++	+	ND	ND	+	+	(25)
<b>CD44</b>	mesoderm marker	++	++	ND	ND	ND	ND	(18)
<b>PECAM1</b>	cell adhesion marker	++	+/-	ND	ND	+/-	+	(25)
<b>CD9</b>	differentiation, adhesion,	+	-	ND	ND	++	++	(25)
<b>Collagen X</b>	mesoderm marker	-	+++	ND	ND	ND	ND	(18)
<b>FIK-1</b>	mesoderm marker	++	-	ND	ND	ND	ND	(18)