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Review paper

A Review on Umbilical Cord Stem Cells

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Abstract

Background and aim: There are different source of mesenchymal stem cells (MSCs) in the various compartments of Human umbilical cord (UC), such as Wharton's jelly, vein, arteries, UC lining and subamnion and perivascular regions.

Methods: In this review, we present current evidence on the properties of human umbilical cord-derived stem cells, including origin, proliferative potential, plasticity, stability of karyotype and phenotype, transcriptome, secretome, and immunomodulatory activity. Also we give a brief introduction to various compartments of UC as a source of MSCs and emphasize the potential clinical utility of UC-MSCs for regenerative medicine and immunotherapy.

Results: UC is a promising source of MSCs. Apart from their prominent advantages, such as a painless collection procedure and faster self-renewal, UC-MSCs have shown the ability to differentiate into three germ layers**,** to accumulate in damaged tissue or inflamed regions**,** to promote tissue repair, and to modulate immune response.

Conclusion: Umbilical cord Stem Cells ability to accumulate in damaged tissue and can modulate immune response.

Keywords: *Umbilical cord, Stem cells, Differentiation, Mesenchymal stem cells*

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Introduction

- *What are Stem Cells?*

Stem cells (SCs) are specified shortly as cells have three basic characteristics including:

1- Renewal potential which means cell division in order to produce identical daughter cells and maintain the stem cell population.

2- The differentiation capacity to become specialized progeny cells [1]. Asymmetric division may occur during stem cells differentiation, which leads to an identical cell and a daughter cell production. The last cell acquires characteristics of a specific cell type, for example, particular morphology, phenotype, and physiological properties that categorize it belonging to a specific tissue [2].

Pluripotent Stem cells are those group of SCs that may differentiate into tissues derived from all three germ layers, ectoderm, endoderm, and mesoderm. Embryonic stem cells (ESCs) are the best example of pluripotent SCs which are derived from the inner cell mass of early embryos. In contrast, most SCs that have been ever characterized are multipotent, because they may differentiate to derivatives of 2 of the 3 germ layers.

3- Tissue renewal capacity. The SCs may renew the tissues in which they live. All tissue compartments contain cells lines that satisfy the description of "stem cells" [3]. For example, blood-forming SCs, gut epithelium SCs, and skin-forming SCs must be constantly replaced for normal health. In contrast, the nervous system SCs that replace neurons are relatively quiescent and do not participate in tissue renewal process. Then lost or injured neurons will not be replaced [4].

In the body, SCs live in specialized microenvironments called "niches", included SCs support cells and special extracellular matrix. The niche microenvironment plays a fundamental role in the maintenance of SCs characteristics including pluripotency and self-renewal, also regulates the growth and differentiation of SCs [5]. Understanding the role of the various "support" cells and the niche environmental factor, is essential for in vitro manipulation of SC populations. As an example, normal atmospheric oxygen concentration of 21% is relatively toxic to SCs, and "hypoxic" conditions of 2–3% oxygen concentration is preferred. Extracellular matrix and growth and angiogenic factors as other components of the niche, play fundamental roles in SC regulation. Understanding the SC microenviornment is rapidly processing and is an important topic which, however, is beyond the scope of this article [4], [6].

Stem Cells Sources in Therapeutic Approach

Next to hematopoeitic stem cells (HSCs), the most widely studied bone marrow SCs are marrow-derived MSCs, also known as marrow stromal cells [7]. Mesenchymal stem cells (MSCs) transplantation or MSCT consider to be the most effective cell therapy tool due to its capacity in the paracrine, trophic, immunomodulatory, and differentiation activation simultaneously which affect all stages of the regeneration of damaged tissues [8]. MSCs in the adult persons are found in the most concentration in the marrow cavity. MSCs also are found at lower density in umbilical cord and peripheral blood, umbilical cord tissue, adipose tissue, amniotic fluid, skin, dental pulp, synovium, placental complex, endometrium, and others. Moreover MSC-like cells can be isolated from umbilical cord blood, placenta, perivascular areas, amniotic fluid, and from the tissue surrounding the umbilical cord vessels, i.e., Wharton's jelly [9]. Bone marrow-derived MSCs (BM-MSCs) are historically accepted as "gold standard" of MSCs and are most extensively characterized. Nevertheless, researcher have been able to extract these cells from other sources, recently. In fact, evidence has suggested that MSCs may be present naturaly in any vascularized

tissue throughout the whole body. Our review focuses on umbilical cord-derived MSCs (UC-MSCs). These cells have an unique combination of prenatal and postnatal SC properties [8].
The collection of MSC-like cells from tissues that are discarded at birth is easier and less expensive than collecting MSCs from a bone marrow aspirate. During the collection of these tissues, there is no health impact on either the mother or the newborn. At least in theory, these cells may be stored frozen and then thawed to provide stem cells for therapeutic use decades after cryogenic storage [10], [11].
In the last 10 year, umbilical cord blood has been shown to be therapeutically useful for rescuing patients with bone marrow-related deficits and inborn errors of metabolism. Umbilical cord blood offers advantages over bone marrow because cord blood does not require perfect human leukocyte antigen (HLA) tissue matching, has less incidence of graft vs host disease, and may be used allogenically. In addition, cord blood may be banked, and thus is available for use "off-the-shelf [12].

History of UC-MSCs

During pregnancy, the fetus and mother connection is supplied by UC. UC prevents umbilical vessels from compression, torsion and bending, while providing good blood circulation. McElreavey et al. [4] for the first time reported isolation of fibroblast-like cells from Wharton's Jelly of human UC in 1991. The UC-derived cells have the similar surface phenotype, plastic adherence and multipotency as those of MSCs derived from other sources. It was 3 years earlier that the first cord blood (CB) transplantation was performed in France in 1988 [13]. After that, together with the development of CB banking, CB transplantation has become the alternative source of hematopoietic stem cells. Although CB-derived MSCs cannot be consistently isolated [14], [15], [16], [17], MSCs were considered to circulate in the blood of preterm fetuses and able to be successfully isolated and expanded [3]. Where these cells home at the end of gestation is not clear [13]. Thus, UC has inevitably become a focus of attention as a source of MSCs because it contains CB [17]. One key study appeared concerning CB-derived MSCs appeared around 2003 [18]. Mitchell et al. [19] successfully isolated matrix cells from UC WJ using explant culture and Romanov et al. [18]. Isolated MSCs-like cells from the subendothelial layer of UCV.

Characterization of Umbilical Cord-Derived MSCs

Recently the minimal defining characteristics of MSCs was the subject of a blue ribbon panel of scientists [13]. This panel ascribed three defining characteristics to MSCs:

1- MSCs are plastic-adherent when maintained in standard culture conditions.
2- MSCs express the cell surface markers CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR.
3- MSCs differentiate to osteoblasts, adipocytes, and chondroblasts in vitro [14].

Mesenchymal-like cells collected from the umbilical cord, placenta, and from umbilical cord blood, perivascular space, and placenta all share a relatively consistent set of surface markers, which is apparently consistent with the hypothesis that they are MSC-like. In a study which focused on human umbilical cord matrix (UCM) cells, cells isolated in large numbers from the Wharton's jelly of human cords [15]. Two other research labs have published on the isolation and characterization of cells from the Wharton's jelly: Dr. Davies' lab at the University of Toronto [16] and Dr. Y. S. Fu at the National Yang-Ming University, Taipei [17], [18]. All three groups reported that UCM cells are MSC-like cells and are robust. These cells can be isolated easily, frozen/thawed, clonally expanded, engineered to express exogenous proteins, and extensively

expanded in culture. Human UCM cells express a marker of neural precursors, nestin, without exposure to differentiation signals [3], [19], [20]. In response to differentiation signals, human UCM cells can differentiate to catecholaminergic neurons, expressing tyrosine hydroxylase TH [18], [21]. Human UCM cells meet the basic criteria established for MSCs described previously. Similarly, MSC-like cells are derived from other umbilical cord tissues, e.g., umbilical vein subendothelium, umbilical cord blood, amnion, placenta, and amniotic fluid [3].

It is currently not clear whether UCM cells are MSC-like or fit into a unique niche. For instance, in using the vital stain Hoechst 33342 in the dye exclusion test, about 20% of the UCM cells excluded dye [3] about 85% expressed CD 44, the hyaluronate receptor marker found on several stem cell populations, and about 85% of the cells expressed ABCG2, the receptor believed to mediate dye exclusion. Enrichment of the Hoechst-dim cells was successful to a certain extent, with maximal enrichment at about 32%. Culture conditions are assumed to limit further enrichment of what is assumed to be the most primitive populations [22], [23].

A review of the literature revealed a question about the stability of umbilical cord cells in culture. Two groups discovered that the cell surface marker expression shifted over passage [3], [20]. Sarugaser found that HLA-1 was lost as a consequence of cryopreservation. While umbilical cord perivascular cells failed to maintain cell surface staining for HLA-1 with freeze– thaw, HLA-1 surface staining was consistent out to passage 5 for cells maintained in culture [3]. In contrast, Weiss et al. [20] reported a decline in the percentage of cells that expressed CD49e and CD105 when human UCM cells were maintained in culture for passage 4–8 and no significant changes were made in HLA-1 expression. This question about the stability of surface marker expression may signify the effect of that epigenetic phenomena associated with cell culture on the cord MSC-like cells. There is a need for further characterization of the cord MSC-like cells to understand the mechanisms involved in these changes [3], [24].

The gene expression analysis of MSCs obtained from the umbilical cord and their reverse-transcription polymerase chain reaction (RT-PCR) was reported by one lab using the National Institutes on Aging (NIA) human 15k gene array [25]. The results showed that human UCM cells express genes in cells that are derived from all three germ layers to some extent. It was reported in at least one study that UCM cells express the pluripotency gene markers Oct-4, nanog, and Sox-2 at low levels relative to ESCs [26]. These findings support the interpretation that cord matrix stem cells are pleiotropic and express a relatively large number of genes in relatively low abundance. It may also provide evidence for the existence of a subset of primitive stem cells in the cord matrix cell population. Because gene array is not a sensitive method for examining low copy number message, massively parallel signature sequencing (MPSS) is a better method for the assessment of matrix cell gene expression. For characterizing cord matrix stem cells, RT-PCR alone is not useful: there is a need for quantitative RT-PCR to be able to make meaningful statements about gene expression and to compare gene expression in different experimental conditions [27], [28].

Properties of Umbilical Cord Matrix Stem Cells

MSC-like cells have been isolated from the umbilical cord tissues or blood in several studies. Based on the results, those cells may express neural markers when differentiated [29], and differentiate into neural cells when transplanted into rat brain. This is natural, because the adult bone marrow-derived MSCs injected into fetal rat brain engrafted, differentiated along neural-like lineages, and survived into the postnatal period [30]. In the same line, Jiang et al. [31] provided convincing evidence that bone marrow-derived MAPCs might be differentiated in vitro to change into cells with electrophysiological properties of neurons. What is interesting is that reports

indicate that bone marrow-derived cells may differentiate, initially to neurospheres and subsequently to neurons with proper neuronal electrophysiological characteristics [32].

In one study in 2003, the investigators discovered that UCM cells can be stimulated in vitro to turn into cells with morphological and biochemical characteristics of neurons [19]. These findings have been confirmed and extended by others, for example, neurons cardiac muscle, bone, and cartilage [33], [34]. Wang et al. [35] used two in vitro differentiation methods to show that umbilical cord matrix stem (UCMS) cells could be induced to exhibit cardiomyocyte morphology and synthesize cardiac muscle proteins such as N-cadherin and cardiac troponin I. The cells responded to five azacytidine or culture in cardiomyocyte-conditioned media [36], [37]. Fu et al. [3], [20] induced human UCMS cells using media conditioned by primary rat brain neurons to synthesize NeuN neurofilament. In addition, they were able to invoke an inward current in UCM cells with glutamate. They reported that exposure to neural-conditioned media also raised the proportion of cells that synthesized the astroglial protein glial fibrillary acidic protein (GFAP) from 94% initially to 5% after 9 d, although the percentage had been reduced to almost 2% by the 12^{th} day. The multilineage potential of UCMS cells was further supported and confirmed by Wang et al. [35]. They found that UCMS cells could be induced in vitro into chondrogenic, osteogenic, and adipogenic lineages.

Nonetheless, a subpopulation of MSC-like cells obtained from Wharton's jelly adjacent to umbilical vessels (which are referred to as human umbilical cord perivascular cells) cultured in nonosteogenic media demonstrated a functional osteogenic phenotype with the elaboration of bone nodules [38]; adding osteogenic supplements further enhanced this population. These findings indicate that cord matrix stem cells such as bmMSCs are multipotent meaning that they are capable of making ectoderm- and mesoderm-derived cells [39], [40].

Some studies showed that porcine UCM stem cells can be xeno-transplanted into nonimmune suppressed rats, where they engrafted, proliferated in a controlled fashion, and exhibited TH expression in some cells [41]. Most recently, some studies showed that UCM cells ameliorate behavioral deficits in the hemi-parkinsonian rat, and UCM cell transplantation led to significantly more dopaminergic neurons in the substantia nigra compared with lesioned, nontransplanted rats that responded to the transplant [42], [43].

These findings point to the suggestion that UCM cells have merits over stem cells as a source of therapeutic cells. First, UCM cells are obtained from a noncontroversial, inexhaustible source, and can be harvested noninvasively at low cost. Second, as against the human ESCs, UCM cells did not induce teratomas or death after transplanting 1 × 106 to 6 × 106 human UCM cells either intravenously or subcutaneously into severe combined immune deficient beige mice. Third, UCM cells are easy to start and do not require feeder layers or medium with high serum concentrations to be maintained. Fourth, when transplanted as xenografts in nonimmune-suppressed rats, they are not acutely rejected. For example, in one study it was shown that pig UCM cells undergo a moderated expansion after being transplanted into rat brain without obvious untoward behavioral effects or host immune response [12], [44].

Homing

Besides their immune-suppressive properties, MSCs seem to display a tropism for damaged or rapidly growing tissues. For example, after being injected into the brain, MSCs travel along known pathways when injected into the corpus striatum [45]. MSCs migrate throughout forebrain and cerebellum, integrate into the central nervous system cytoarchitecture, and express markers that are typical of mature astrocytes and neurons after being injected into the lateral ventricle of

neonatal mice [46], [47]. MSCs injected into the injured spinal cord formed guiding "cord," ushering in regenerating fibers [45]. MSCs may be useful for regeneration in stroke or myocardial ischemia by releasing trophic factors like brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, or angiogenic factors. Because UCM stem cells are strongly related to MSCs, not surprisingly they also will home to tumors [48]. The signals recruiting transplanted or endogenous cells to inflammation or neoplasia regions are still unknown. However, stromal cell-derived factor-1α has a considerable role in bone marrow-derived cells' recruitment to the heart after myocardial infarction. Matrigel invasion assays have implicated such molecules as platelet-derived growth factor-BB, epidermal growth factor, and stromal cell-derived factor-1α as chemokines for MSCs; however, both the basic FGF (bFGF) and vascular endothelial growth factor (VEGF) had no effect. In any event, the directed migration of umbilical and other mesenchymal stem cells to tumors is promising in that they may be a platform for targeted delivery of high local levels of protein.These proteins often have a short half-life and/or result in major side effects when administered systematically [49], [50].

The Origin and Morphology of the Human Umbilical Cord

The umbilical cord develops from the yolk sac and allantois and becomes a conduit between the developing embryo or fetus and the placenta. The umbilical cord stroma contains Wharton's jelly, a gelatinous substance named after Thomas Wharton (1614–1673), an English physician and anatomist [8]. Wharton's jelly keeps the blood vessels (two umbilical arteries and one umbilical vein) from clumping and makes the cord flexible. This substance is mainly made from glycosaminoglycans, especially hyaluronic acid and chondroitin sulfate. Collagen fibers are the main fibrillary component, while elastic fibers are not present. The cell component is presented by mesenchyme-derived cells (fibroblasts, myofibroblasts, smooth muscle cells, and mesenchymal stem cells) [51]. In contrast to most tissues in the body, no capillaries exist in Wharton's jelly: there is an active process of hematopoiesis and capillaries formation in umbilical cord stroma in the sixth week of development; however, within 7–9 weeks, hematopoiesis stops and capillaries regress [52].

The Umbilical Cord as a Source of Mesenchymal Stem Cells

In 1974, umbilical cord blood was certified to be the source of hematopoietic stem and progenitor cells [53], and the umbilical cord tissue remaining was believed to be medical waste without any scientific value. This belief was completely modified in 1991 following McElreavey et al.'s isolation of fibroblast-like cells from Wharton's jelly and characterizing them [54]. Then, in 2004, these fibroblast-like cells were certified to be MSCs since they expressed CD29, CD44, CD51, CD73, and CD105, did not express CD34 and CD45, and could differentiate into cells of the adipogenic and osteogenic lineages [35]. Currently, the umbilical cord MSCs include cells derived from the total umbilical cord or its different sections (perivascular, intervascular, and subamnion zones of Wharton's jelly and subendothelial layer but not from umbilical cord lining or inner blood vessel walls) [51]. Some main points: (1) MSCs must be plastic-adherent when maintained in standard culture conditions; (2) MSCs must express CD105, CD73, and CD90 but not CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLADRsurfacemolecules; (3) MSCs must differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [55], [56].

The Origin of Wharton's Jelly MSCs

Wang et al. (2008) assumed that early in embryogenesis, hematopoietic cells and MSCs migrate

from the yolk sac and aorta-gonad-mesonephros to the placenta and then go back to the fetal liver and bone marrow through the umbilical cord. During these two migration phases, some cells become trapped in Wharton's jelly and are retained therein throughout the whole period of gestation. The new microenvironment changes the features of the migrating cells, which may account for their differences from BM-MSCs [57].

Isolation of Primary Cell Culture from Wharton's Jelly Most protocols for primary cell culture isolation from Wharton's jelly involves three steps: (1) Removing the epithelial, vascular, and perivascular tissues; (2) Mechanical grinding and enzymatic digestion using trypsin, collagenases I, II, or IV, dispase, protease, and hyaluronidase; (3) Transfer into the culture medium (standard culture media with human or fetal calf serum which may be supplemented with growth factors FGFb, EGF, PDGF, and VEGF) [51], [58], [59]. An explant culture method can be also used; it prevents the damaging effects enzymes have on cells and minimizes the processing time of the biomaterial ("plate and wait" procedure) [8]. In the common explant method of isolating UC-MSCs, the umbilical cords are minced into small fragments, which are then attached to a culture dish bottom from which the cells migrate. One of the disadvantages associated with this method is that the cell recovery rate is reduced by the fragments frequently floating up from the bottom of the dish. In some protocols, a stainless steel mesh is used to prevent the tissue from floating [60]. According to some reports, the explant method enables the selection of a cell fraction with a higher proliferative potential [61], [62], but a remarkable variation of cell phenotype expressions was distinguished compared to enzymatic digestion [63], [64]. In a recent study, a comparison was made between three explant culture methods and three enzymatic methods. The advantages of MSC isolation using the 10mm size tissue explant method included shorter primary culture time, larger numbers of isolated cells, and higher proliferation rates compared with other isolation methods. There was no significant difference among the six groups in their immune phenotype and multilineage differentiation capacity [64]. Furthermore, UC-MSCs that were isolated using the explant technique always reached proliferation arrest earlier, regardless of the initial population doubling times; however, the mechanism accounting for this effect is still obscure [65]. On the contrary, further studies showed that the cells derived from explants demonstrated similar characteristics (morphology, population doubling time, post thaw survival, differentiation capacity, and phenotype) to those from enzymatic protocols [66]. According to published data [67], [68] the efficiency of isolation of primary cell culture from Wharton's jelly is 100%. Comparatively, the efficacy of MSC isolation from umbilical cord blood does not exceed 60%; amniotic fluid is 90%; placenta ranges from 62.5% to 100% [68]. Wharton's jelly tissue bears the highest concentration of allogeneic mesenchymal stem cells: yields for bone marrow ranged from 1 to 317,400 cells/mL; yields for adipose tissue varied from 4,737 to 1,550,000 cells/mL of tissue; and yields for umbilical cord tissue ranged from10,000 to 4,700,000 cells/cm of umbilical cord [69]. It is worth noting that umbilical cords obtained after Caesarean sections are used in almost all culture laboratories; the reason is that is the increased risk of contamination of primary biological material by vaginal delivery. Some researchers believe that viable MSCs can be isolated solely from fresh umbilical cord tissue and not from frozen tissue fragments [70]. According to another report, MSCs obtained from frozen cord tissue displayed reduced plating efficiency and increased doubling times but near equivalent maximum cell expansion in comparison to fresh cord tissue [71].

The Proliferative Potential and the Karyotype Stability of UC-MSCs have higher proliferative potential than BM-MSCs (the "gold standard" for MSCs comparison) or MSCs from other postnatal (adipose tissue) and neonatal sources (placenta and amniotic membrane) [72], [73]. The

mean of CFU-F (colony-forming unit-fibroblast) colonies per 1 × 10 6 nucleated cells was significantly higher inUC-MSCs (800, range 300–2000) than in BM-MSCs (36, range 16–64) as determined by the CFU-F assay based on Castro-Malaspina's method [73].
CFU-F frequency, which is determined by limiting dilution assay also showed a higher frequency for CFU-F in UC-MSCs compared to BM-MSCs [73]. Another report indicated that typical CFU-F efficiency (the ratio of number of cells that form colonies under clonal conditions and the number of cells seeded directly following isolation) for BM-MSCs was between 0.001% and 0.01%; however, for UCMSCs, it reached 0.2–1.8% [74]. It has been found that cell doubling time for UC-MSCs is approximately 21 h, 24h, 40h, and 45h [8]. More importantly, based on the recent data, each individual UCMSCs sample showed different population doubling rates and reached senescence at different passages depending on its unique genetic and epigenetic profiles, regardless of the isolation protocol [65]. A sufficient amount of the starting biomaterial (umbilical cord weight is nearly 40 g) and high telomerase activity of UC-MSCs make it possible to obtain 109 cells from one cord while their normal karyotype are maintained for 6 passages [75], [76]. Since passage 7, the telomerase activity of UC-MSCs reduce to a considerable extent, but cell karyotype is stable for a minimum of 25 passages [77], [78].

UC-MSCs Phenotype

The expression profile of surface markers and pluripotency markers of UC-MSCs has been extensively examined. Particular attention has been paid by researchers to CD105 (endoglin, a part of the TGF beta receptor complex). According to ISCT decision, CD105 is an essential marker for the verification of MSCs [55]. However, the existing data are contradictory. Most of the studies indicate that CD105 presents on the surface of UC-MSCs [58], [79], and maintains its expression during long-term cultivation (at least 16 passages) [80]. However, there are some studies showing that UC-MSCs do not actually express CD105 at all [81] or until passage 5 [82]. Reduced expression of mesenchymal marker (CD73, CD90, and CD105) on UC-MSCs may occur under ischemic conditions mainly under the influence of hypoxia [83]. Based on laboratory data, CD105 is expressed by more than 98% of the UC-MSCs on passages 2–5, as measured by flow cytometry. The data related to the expression of pluripotent specific markers on UC-MSCs also contradict each other. Different reports showed the expression of these markers only under certain conditions, for example only on the early passages [84], or when grown with human embryonic stem cells medium on mouse feeder cells, or after O2 concentration was lowered from 21% to 5% level [85], or after CD105+ cells were selected and were subsequently cultivated under the suspension culture condition [86]. Based on flow cytometric analysis, neural ganglioside GD2(+)-sorted UC-MSCs showed increased expression of SSEA-4, OCT4, SOX2, and NANOG compared to unsorted or GD2-negative cells [87].

Transcriptomic Profile of UC-MSCs

De Kock et al. (2012) examined the global gene expression profiles of four human mesoderm-derived stem cell populations. Human UC-MSCs showed significant enrichment in functional gene classes that are involved in the development and functioning of the liver and cardiovascular system in comparison to MSCs obtained from adipose tissue, bone marrow, and skin [88]. Hsieh et al. (2010) published interesting data comparing the gene expression profiles of BM-MSCs and UC-MSCs. They found, no common genes among the top 50 known genes most strongly expressed in the two MSC types. Top 10 genes for UC-MSCs included those encoding somatostatin receptor 1, member 4 of immunoglobulin superfamily, gamma 2 smoothmuscle actin, reticulon 1, natriuretic

peptide precursor B, keratin 8, desmoglein 2, oxytocin receptor, desmocollin 3, and myocardin. The study also showed that the genes associated with cell proliferation (*EGF*), PI3K-NFkB signaling pathway (*TEK*), and neurogenesis (*RTN1*, *NPPB*, and *NRP2*) were up regulated in UC-MSCs rather than BMMSCs [89]. The UC-MSCs and BM-MSCs were also screened based on surface expression of HLA antigens, costimulatory factors, and immune tolerance molecules [72], [79]; the expression of MHCII molecules (HLA-DMA, -DRA, and -DPB1) in the BM-MSCs was 16-fold, 36-fold, and 4- fold higher, respectively, than the UC-MSCs. The levels of expression of immune-related genes *TLR4*, *TLR3*, *JAG1*, *NOTCH2*, and *NOTCH3* in the BM-MSCs were also 38-fold, 4-fold, 5-fold, 3-fold, and 4-fold higher, respectively, than the UC-MSCs [72]. These results show that allogeneic UC-MSCs can be successfully used for clinical trials in the future. In a review study, El Omar et al. [79] presented a more detailed comparison of the UC-MSCs transcriptome.

Secretome of UC-MSCs

MSCs produce different varieties of bioactive compounds that provide a paracrine mechanism for their therapeutic activity. However, UC-MSCs' secretome is significantly different from MSCs obtained from other sources such as bone marrow and adipose tissue. The most clear difference is the lack of synthesis of the main proangiogenic factor VEGF-A: the secretion level is 102 lower than AT-MSCs and 103 lower than BMMSCs [74], [90], [91], where transcription level of VEGF gene expression can be detected and, according to some reports, is has a great similarity to that of BM-MSC [92]. There is also a reduced production of some proangiogenic factors (including angiogenin and PLGF) by UC-MSCs, and also some antiangiogenic factors (including thrombospondin-2 and endostatin) compared with BM-MSCs and AT-MSCs [90], [91]. Conversely, UC-MSCs showed higher expression levels of angiogenic chemokines such as CXCL1, CXCL, CXCL5, CXCL6, and CXCL8 and angiogenic growth factors like HGF, bFGF, VEGF-D, PDGF-AA, TGF-β2, G-CSF, and TGF-β2 [90], [93], [94]. As a result, UC-MSCs' proangiogenic capacity is realized by a VEGF-A-independent pathway [91]. UC-MSCs have been also reported to exhibit increased secretion of neurotrophic factors such as bFGF, nerve growth factor (NGF), neurotrophin 3 (NT3), neurotrophin 4 (NT4), and glial-derived neurotrophic factor (GDNF) in comparison to BM-MSCs and AT-MSCs [95]. Based on these and published data, we believe that UC-MSCs are possibly pre-committed to an ectodermal fate. Furthermore, higher amounts of several important cytokines and hematopoietic growth factors, including G-CSF, GM-CSF, LIF, IL-1α, IL-6, IL-8, and IL-11 are secreted by UC-MSCs compared to BM-MSCs; thus, they seem to be a better candidate for the expansion of hematopoietic stem cells [96].

The Multilineage Differentiation Potential of UC-MSCs

Very high differentiation capacity was demonstrated by in vitro UC-MSCs: they were able to differentiate into chondrocytes, adipocytes, osteoblasts, odontoblast-like cells, dermal fibroblasts, smooth muscle cells, skeletal muscle cells, cardiomyocytes, hepatocyte-like cells, insulin-producing cells, glucagon-producing cells, and somatostatin-producing cells, sweat gland cells, endothelial cells, neuroglia cells (oligodendrocytes), and dopaminergic neurons [59], [97], [98]. In 2014, research findings showed that UC-MSCs expressed markers of male germ-like cells and primordiallike germ cells under specific conditions; this possibility had been found to be limited to embryonic stem cells (ESCs) or induced pluripotent stem cells [99], [100]. Numerous studies have examined differentiation potential of UC-MSCs and MSCs from other sources (bone marrow and adipose tissue); some have demonstrated the possibility of UC-MSCs' differentiation after

genetic modification (transduction or transfection). UC-MSCs overexpressing hepatocyte growth factor (HGF) were able to differentiate into dopaminergic neuron-like cells secreting dopamine, tyrosine hydroxylase, and dopamine transporter [101]. They promoted nerve fiber remyelination and axonal regeneration one week after being transplanted in rats with collagenase-induced intracerebral hemorrhage [33]. After being infected with adenovirus containing SF-1 cDNA, UC-MSCs showed increased expression of all steroidogenic mRNAs (including P450 sidechain cleavage enzyme, 3β-HSD, 17β-HSD type 3, LH-R, ACTH-R, P450c21, and CYP17), caused higher secretion of steroidogenic hormones (including testosterone and cortisol), and led to significantly higher cell viability compared to differentiated BM-MSCs [102].

Interestingly, the UC-MSCs plasticity may be dependent on the pregnancy conditions. UC-MSCs of preeclamptic patients were more committed to neuroglial differentiation: a significant increase was observed in the protein expressions of neuronal (MAP-2) and oligodendrocytic (MBP) markers in cells from preeclampsia versus gestational age-matched controls [103]. At the same time, preterm birth did not impact neuronal differentiation of UC-MSCs in comparison to term delivery [104]; however, it decreased the osteogenic potential [103]. UC-MSCs from gestational diabetes mellitus patients were similar to UC-MSCs from normal pregnant women in expression levels of CD105, CD90, and CD73 to but displayed reduced cell growth and earlier cellular senescence with accumulation of p16 and p53, showed significantly lower potentials of osteogenic and adipogenic differentiation. They also exhibited low mitochondrial activity as well as reduced expression of the mitochondrial function regulatory genes *ND2*, *ND9*, *COX1*, *PGC-1*α, and TFAM [105]. Thus, impaired metabolism of the maternal organism during pregnancy significantly affects neonatal MSCs biological properties. This should be considered when selecting a source of cells for clinical use [8].

The Immunomodulatory Properties of UC-MSCs

Weiss et al. (2008) were the first to delve into the immunomodulatory properties of UC-MSCs. In vitro study pointed to five main conclusions: (1) UC-MSCs caused suppression of the proliferation of Con-Astimulated rat splenocytes (xenograft model) or activation of human peripheral blood mononuclear cells (allogeneic model) (2). They did not stimulate the proliferation of allogeneic or xenogeneic immune cells (3). They produced an immunosuppressive isoform of human leukocyte antigen HLA-G6 inhibiting the cytolytic activity of NK cells (4). Immune response-related surface antigens CD40, CD80, and CD86, which participated in T lymphocytes activation, were not expressed by UC MSCs. And, finally, UC-MSCs produced anti-inflammatory cytokines that provided their immunomodulatory properties [106].

The immunomodulatory activity of UC-MSCs is currently believed to be provided by the paracrine mechanism. For example, UC-MSCs produce IL-6, which instructs the dendritic cells to acquire tolerogenic phenotypes [107], prostaglandin E2 (PGE2), which suppresses NK cells cytotoxicity [108] and CD4+ and CD8+ T-cell proliferation [109], and indoleamine 2,3-dioxygenase (IDO), which inhibits the differentiation of circulating T follicular helper cells. UC-MSCs secrete anti-inflammatory cytokine IFN-α in contrast to BMMSCs and AT-MSCs [110]. After being exposed to proinflammatory cytokine IL-1β for 48 hours, UC-MSCs displayed a relatively higher expression of immunomodulatory molecules TGFβ1, IDO, TNF-stimulated gene 6 protein (TSG-6), and PGE2, compared to MSCs from bone marrow or placenta [78]. The activated MSCs secrete PGE2, which drives resident macrophages with M1 proinflammatory phenotype toward M2 anti-inflammatory phenotype and TSG-6's interaction with CD44 on resident macrophages decreases TLR2/NFκ-B signaling and, thereby decreases the secretion of proinflammatory mediators of

inflammation. These findings confirm the role of MSCs (and especially UC-MSCs due to their secretome) as the early regulators of inflammation [111]. Interestingly, culture conditions are likely to influence the immunomodulatory properties of UCMSCs: in an allogeneic mixed lymphocyte reaction, UC-MSCs-mediated suppression of T-cell proliferation is more effective in xeno-free (containing GMP-certified human serum) and serum-free media than in standard fetal bovine serum-containing cultures. Therefore, the xenogeneic components of the culture medium are to be removed for future clinical study design in regenerative and transplant medicine [112].

Tumorigenic Potential of UC-MSCs

Perinatal stem cells have the characteristics of both embryonic stem cells and adult stem cells due to their pluripotency properties, as well as multipotent tissue maintenance; they act as a bridge between embryonic and adult stem cells [113]. Expression of markers of pluripotency is higher in the UCMSCs compared to BM-MSCs [114], [115] but lower than in ESCs [116]. This might be the reason for the crucial difference between UC-MSCs and ESCs: as against ESCs, UC-MSCs do not induce tumorigenesis. In one of the initial studies on the subject, using the immunodeficient mouse model the tumor-producing capabilities associated with UC-MSCs were compared with human ESCs. The animals receiving human ESCs developed teratomas in six weeks (s.c. 85%; i.m. 75%; i.p. 100%), which contained ectoderm, mesoderm, and endoderm tissues. None of the animals receiving human UC-MSCs developed tumors or inflammatory reactions at the injection sites when maintained for a prolonged period (20 weeks) [56], [117]. Moreover, it was discovered that UC-MSCs could be immortalized via transduction with a lentiviral vector that carries hTERT (human telomerase reverse transcriptase) catalytic subunit gene, but even then, the transfected UC-MSCs did not transform into tumors in nude mice [118]. In vitro model of cell culture transformation, in which the cells were grown in the presence of breast and ovarian cancer cell conditioned medium for 30 days, led to no changes in UC-MSCs' morphology, proliferation rates, or transcriptome compared to BM-MSCs that changed into tumor-associated fibroblasts [119]. Therefore, human UC-MSCs, as they are nontumorigenic, can be used for safe cell-based therapies [8].

Preclinical Studies on the Use of UC-MSCs

Promising results have been found in recent preclinical studies on the use of UC-MSCs for the purpose of treating different diseases using animal models. Reports on the early period of MSC-based cell therapy for tissue repair revealed that the injected MSCs may survive, engraft, and differentiate into specific cell types and help to repair the injured tissues. However, further studies supported the fact that the UC-MSCs engraftment level in the host organs of the recipient animals was low after systemic administration but rather high after local administration [120]. There is not much evidence for the differentiation of UC-MSCs into relevant cells; it may be attributed to the xenogeneic transplantation used in most of these studies. Currently, the proposed mechanisms related to UC-MSCs therapeutic activity include trophic and paracrine effects on the immune system cells, remodeling of the extracellular matrix, angiogenesis, apoptosis, and stimulation of the migration and proliferation of resident progenitor cells [78], [121], [122], [123], [124]. All the studies point to appropriateness of UC-MSCs for clinical use [8].

The UC-MSCs' Ability to Differentiate into Adipogenic, Chondrogenic and Osteogenic Lineages

UC-MSCs originating from the extraembryonic mesoderm and their capacity for differentiation

into adipogenic, chondrogenic and osteogenic lineages have been extensively studied [125], [126]. Concerning the osteogenic differentiation ability, Hsieh et al. [89] showed that the gene profiles of UC-MSCs are similar to ESCs; they display delayed and insufficient differentiation into osteocytes. On the other hand, BM-MSCs have already expressed an osteogenic gene profile and can differentiate into osteocytes quite easily. Among the three compartments, UCWJ, UCV and UCAs, UCWJ-MSCs have a clearly defective ability in differentiating into osteocytes, even though osteocyte-related genes expression is detected by reverse-transcriptase PCR, at similar levels to the other two tissues/compartments [127]. Mennan et al. [128] compared cord regions in six samples in terms of osteogenic differentiation finding that UCWJ-MSCs and whole UC-derived MSCs have the best differentiation, rather than UCA-, UCV- and UC lining MSCs [129].

With regard to adipocytic differentiation, according to Mennan et al. [128], UC-MSCs produce small lipid vacuoles while BM-MSCs produce more mature adipocytes (unilocular lipid vacuoles). UC-MSCs could keep their multipotency for a longer time than BM-MSCs can [130].

As to chondrogenic differentiation, UCMSCs revealed no obvious differences between different cord regions (sources) [128]. In addition, comparing BM-MSCs and UCMSCs in their chondrogenic potential showed that UC-MSCs produce collagen three times more than BM-MSCs indicating that UC-MSCs may be a better choice for fibrocartilage tissue engineering [131].

With respect to other differentiation abilities, among various UC compartments, UCWJMSCs have been mostly investigated and many articles have been published in this regard [132], [133]. UCWJ-MSCs differentiate not only into osteocytes, chondrocytes and adipocytes, but also into cardiomyocytes (with the gene expression of N-cadherin and cardiac troponin), neurons and glia [19], oligodendrocytes [134] and hepatocytes [135]. Recently, UC-MSCs have been used for neurogenic disorders (spinocerebellar ataxia and multiple system atrophy of the cerebellar type) and liver disorders in clinical trials [136], [137].

UC-MSCs Biomarkers

Flow cytometry is used to analyze the immunoprofile of UC-MSCs, based on the standard definitions provided for MSCs described by the position paper of the International Society for Cellular Therapy [55]. There are no single specific markers to be used for detecting multipotent MSCs. MSCs are positive for such adhesion markers as CD29 and CD44; mesenchymal markers such as CD90, CD73 and CD105; and human leukocyte antigen class I (HLAABC), but negative for endothelial cell marker CD31; hematopoietic cell markers such as CD34, CD45 and CD117; and human leukocyte differentiation antigen class II (HLA-DR) [138]. Among the different UC compartments, UCWJ-, UCV- and UCA-derived MSCs show a similar fibroblast-like spindle shape and the MSCs from these three types of tissues display no significant differences in the immunoprofile. These cells are positive for CD13, CD29 (integrin β1), CD73 (SH3), CD90 (Thy-1), CD105 (SH2; endogrin) and HLA-ABC at the cellular frequency higher than 90% but negative for CD34, CD45, CD133 and HLA-DR, with the cellular frequency lower than 1% [127], [139]. Mennan et al. [40] also discovered that MSC immunophenotypes are not significantly different among the different sources: BM, umbilical cord arteries, vein, WJ and UC lining membrane. Karahuseyinoglu et al. [128] found that CD73 is expressed throughout the vessels and endothelium and is not present in the perivascular region, but the strongest expression is seen in the epithelial and subepithelial regions of WJ. CD90 is positive in majority of the compartments and negative in the endothelial lumen lining. A high expression of vimentin, CKs [68], [127], [140], [141], desmin and SMA was demonstrated in the subamniotic layer and the perivascular region. Schugar et al. [142] discovered that CD146 (endothelial progenitor marker) is expressed in the walls of the

vessels (100%) and the perivascular region of UC (62%); however, is not expressed in UCWJMSCs any longer [51], [143]. These markers might aid in determining the multipotency of the isolated cell population. Phenotypic characterization of UC-MSCs is probably affected by the culture passage number, culture medium and culture method [129].

Additionally, such ESC markers as Oct4, Nanog, Sox-2 and KLF4 are expressed only at low levels in UCMSCs [144]. This confirms the role of MSCs as primitive stem cells, somewhere between ESCs and mature adult stem cells. Nevertheless, pluripotent MSCs need to be precisely isolated using specific markers. The role of SSEA3 and SSEA4 in MSCs is still a controversial issue. As reported by Gang et al. [145], [146], SSEA4+ cells proliferate predominantly when the culture originates from primary BM cells in the medium supplemented with special cocktails of cytokines. In contrast, the authors were not able to recreate the same phenomena in UCWJ-MSCs in the medium consisting of α-MEM and 10% FBS. Besides, SSEA4 expression in UCWJ-MSCs has a significant correlation with the concentration of FBS in the culture medium, but an inverse correlation with SSEA3 expression. The authors concluded that SSEA4 in UCWJ-MSCs is neither a marker of either proliferation capacity nor that of multipotency [147]. Schrobback et al. [148] assessed SSEA4 expression in human articular chondrocytes, osteoblasts and BM-derived MSCs and portrayed their differentiation potential showing that SSEA4 levels in these cells have no relationship with the capacity for chondrogenic and osteogenic differentiation and the proliferation potential *in vitro* [148].

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Conflict of interests

The authors declare that they have no conflict of interest.

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