Phenotype and Differentiation Potential of Stromal Populations Obtained from Various Zones of Human Umbilical Cord: An Overview

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Abstract: Fibroblast-like cells with properties similar to mesenchymal stromal cells (MSCs) are present in human umbilical cord (hUC). In accordance with the international minimal criteria for defining multipotent mesenchymal stromal cells, hUC cells are designed MSCs being plastic adherent, positive to specific non hematopoietic lineage biomarkers, able to be both *in vitro* long term cultured and differentiated into osteoblasts, chondroblasts and adipocytes. In this review, a panoramic view of phenotypic characteristics of hUC cells derived from various UC parts are described. The high heterogeneity of extraction, culture and analysis procedures hinder the ability to precisely identify UC stromal cells. As a result, different phenotypic profiles are detectable not only among the cells obtained from the various parts of cord, but also inside the same UC regions, suggesting that UC-MSCs may represent an unique cell family whose components present various degree of stemness. However, *in vitro* and *in vivo* evidence indicates Wharton's jelly as the best source of MSCs, because its cells present a wide range of potential therapeutic applications.

Keywords: Umbilical cord, phenotype, cell differentiation.

1. INTRODUCTION

Since 1991, when McElreavey *et al.,* [1] reported the isolation of fibroblast-like cells from the Wharton's jelly of human umbilical cord (hUC), a great deal of effort has been devoted to obtaining, characterizing and evaluating both *in vitro* and *in vivo* hUC cells in order to identify a new source of mesenchymal stromal cells (MSCs) for therapeutic use. The hUC cells have shown to share with MSCs the surface phenotype, plastic adherence, and multipotency. Indeed, under suitable stimuli, they differentiate *in vitro* into adipocytes, osteoblasts, chondrocytes, hepatocytes, cardiac and neural cells [2-5]. Differentiation of UC cells into germ like cells has also been achieved [6]. Other properties make these cells more attractive than other MSCs. Firstly, UC is considered as medical waste and its use is completely free from ethical concerns. Secondly, hUC cells present reduced immunogenicity: the transplantation of hUC cells into non-immune-suppressed animals does not induce acute rejection [7]. Thirdly, hUC cells may modulate immune function because they produce several cytokines and growth factors and lack costimulatory molecules [8]. Furthermore, when compared with MSCs from bone marrow (BM), they exhibit higher frequency of colony-forming-unit fibroblasts (CFU-F) and shorter population doubling time [9].

To date, hUCs possessing MSC properties can be obtained from the UC lining, the Wharton's jelly, the subendothelial layer, the perivascular zone, or whole UC. Several extraction methods and culture media have been used according to the cell source. According to the isolation method and *ex vivo* culture conditions, hUC cells show a highly heterogeneous self-renewal capacity, immunophenotype, and differentiative potential suggesting that populations with different stemness grade may be distributed within all UC compartments. In the present work, a panoramic view of the phenotypic characteristics of hUC cells derived from various UC parts are described. Furthermore, despite several contradictory findings due to the high heterogeneity in the extraction and culture procedures, comparisons between hUC cells and bone marrow (BM)-MSCs have been attempted.

2. HUMAN UMBILICAL CORD

The hUC is embryologically derived on day 26 of gestation from extraembyronic mesoderm and/or embryonic mesoderm. At birth, it weighs approximately 40 g, its length is about 30–65 cm, and its mean diameter is 1.5 cm. During pregnancy, the hUC represents a vascular link between mother and fetus, and protects the blood vessels assuring blood supply and removal of biological waste to and from fetus, respectively. Its surface is covered with a simple epithelium of amniotic derivation, which stratifies in late gestation. Transverse section reveals two umbilical arteries and a single umbilical vein surrounded by mucous connective tissue, named Wharton's jelly (Fig. **1**). The umbilical arteries lack both internal and external elastic lamina and the adventitia is replaced by mucous connective tissue. The umbilical vein is characterized by a thick muscularis layer containing variously oriented smooth muscle fibers. All vessels are normally organized as left spiral turns. No capillaries and lymphatics are present. Wharton's jelly is mainly composed of ground substance, rich in sulfated proteoglycans, and several types of collagenous fibers, but

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1875-0435/11

lacking in elastic ones. Due its resistance to twisting and compression, its main role is to protect blood vessels thus allowing an efficient blood flow between fetal and maternal circulation. The most abundant glycosaminoglycan is hyaluronic acid, whereas fibers are mainly composed of collagen types I and III. The structure of hUC extracellular matrix has been reviewed by Can and Karahuseyinoglu [10]. The stromal cells of the Wharton's jelly are fibroblast-like cells which produce the extracellular matrix. Like myofibroblasts, they express vimentin, desmin, and α-smooth muscle actin (SMA) [11]. To date, various cell populations have been identified in the defined hUC compartments: amnion-derived epithelium, subamniotic region, Wharton's jelly, perivascular stroma and vessels.

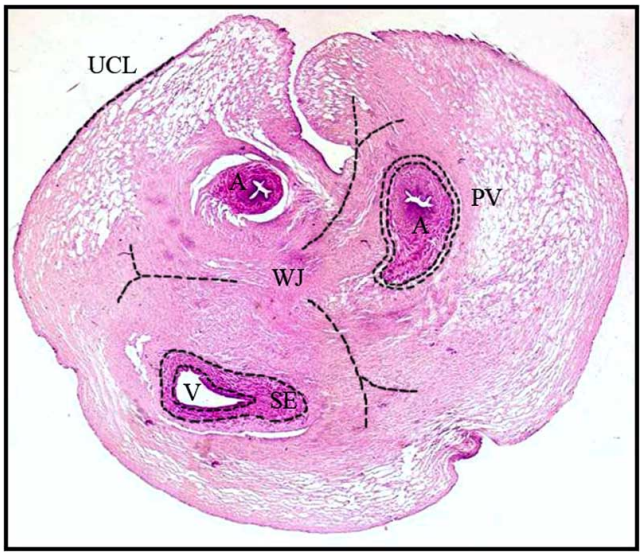

Fig. (1). Hematoxyllin-eosyn staining of hUC sections (magnification: x200). A: artery; V: vein; SE: subendothelial layer; PV: perivascular layer; WJ: Wharton's jelly; UCL: umbilical cord lining.

3. MESENCHYMAL STROMAL CELLS OF HUMAN UMBILICAL CORD

In the last few decades a growing number of researchers have focused on MSCs due to their potential use not only in cell therapy, but also in relation to development and regeneration. MSCs have been isolated from several tissues of both fetal and adult origin, including hUC [12]. Being quite numerous in fetal blood during the first trimester of pregnancy [13], it has been hypothesized that MSCs could leave the blood stream and localize into hUC during advancing gestation. MSCs are present in many parts of hUC from the vessels to the amnionic membrane. They all share a fibroblast-like morphology, a high proliferation rate, and multipotent differentiation properties. Nevertheless, it seems very difficult to compare MSCs obtained from the various parts of hUC with each other as the heterogeneity of cell isolation methods and used culture media is high. Indeed, different cell populations can be obtained by enzymatic procedures or explant cultures. Moreover, according to the various research groups, the panel of stemness markers used to characterize these cell populations varies a great deal. And furthermore, it has to be highlighted that the phenotypic characterization has been carried out at different culture passages and the percent variations in marker expression during culturing have been reported in only few papers. In this section, the phenotypic features of MSCs obtained from hUC compartments together with the corresponding extraction methods, the used culture conditions and the differentiation potential are summarized. Overall, all MSCs fit the defining criteria of Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy [14]. They share fibroblast-like morphology and a nonhematopoietic phenotype, lacking hematopoietic markers, such as CD14, CD34, CD38, CD45, and CD133. In addition, they express human leukocyte antigen (HLA) class I but not HLA class II.

3.1. MSCs from the Subendothelial Layer

To retrieve the subendothelial layer cells, the cord vein is filled with collagenase and incubated at 37°C. After washing and gently massaging the cord, cell suspension is collected and seeded. Despite this common procedure, each research group has used different types of collagenase (I or IV), enzyme concentration (0.1, 0.5, and 1%), incubation times and culture media [15-22]. In particular, a five hour incubation period compared to shorter ones (ranging from 10 to 20 min) could assure complete digestion of the subendothelial layer leading to an enrichment of cell suspension. Nevertheless, it can not be excluded that a decrease in cell viability may occur. Then, the cells are cultured in DMEM low glucose (LG), MEM or M199 media supplemented with 10% fetal bovine serum (FBS) or fetal calf serum (FCS), but without exogenous growth factors that could induce cell differentiation. Although endothelial cells are present in primary cultures, they do not spread, migrate or proliferate using the above mentioned media. As a result, the cultures contain only MSCs and are free of contaminating endothelium. However, the absence of endothelial markers, such as CD31, von Willebrand factor (vWf) and kinase insert domain receptor (KDR), has been verified in only half the papers on MSCs from the subendothelial zone [17-19, 22]. As reported in Table **1**, in addition to CD105, CD73, and CD90, MSCs express CD29, CD44, CD49e, CD54, and CD13. CD29 (integrin β-1) is usually considered as a stem cell associated marker and its expression has been reported in several studies on neural stem cells [23], adipose-derived stem cells [24], cancer stem cells [25], and MSCs [26]. The CD49e molecule, also known as integrin α5, mediates cell adhesion and participates in cell-surface mediated signalling [27]. CD44 (H-CAM), a cell membrane glycoprotein involved in cell-cell interactions, cell adhesion and migration, is expressed on stem cell populations isolated from adipose tissue [24], bone marrow [28], astrocyte precursor cells [29], and hemopoietic stem cells [30]. The CD13 protein, a zinc-metallopeptidase that cleaves single neutral amino acids, is expressed not only on the surface of early committed granulocyte and monocyte progenitors and endothelial cells, but also on BM stromal cells [31]. CD54 (ICAM-1, intercellular adhesion molecule-1) is constitutively expressed at low level in MSCs and the role it plays in stromal-induced immunosuppresion remains unknown [32].

Contradictory results have been obtained on the expression of CD106 (VCAM-1), a cell surface sialoglyco-

Table 1. Phenotype of hUC Cells Obtained from the Subendothelial Layer of the Umbilical Vein

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
0.1% collagenase at 37°C for 15 min	DMEM-LG, 20nM HEPES, 2mM L-glutamine, 1mM sodium piruvate, 10% FBS	2	ICC	CD106, SMA, fibronectin, collagen type I	CD34	Adipocyte, osteoblast	[15]
1% collagenase at 37°C for 20 min	α-MEM, 2mM L-glutamine, 20% FCS	2	FC	CD13, CD29, CD44, CD49e, CD54, CD90, HLA-I	CD14, CD45, CD51/61, CD106, CD235a, HLA-DR	Adipocyte, osteoblast	[16]
1% collagenase at 37°C for 20 min	M199, 20% FCS	3	FC	CD13, CD29, CD44, CD49e, CD54, CD90, HLA-I	CD14, CD34, CD45, CD49d, CD133, CD31, CD51/61, KDR, HLA-DR	Adipocyte, osteoblast, chondrocyte	[17]
0.1% collagenase type I at 37°C for 10 min	M199, 10%FBS	n.r.	ICC	n.r.	CD31, vWf, KDR	Adipocyte, osteoblast	[18]
0.1% collagenase type IV at 37°C for 20 min	DMEM, 10%FBS	n.r.	FC	CD29, CD44, CD54, CD73, CD105, CD166	CD31, CD34, CD45	Adipocyte, osteoblast, cardiomyocyte	[19]
0.5% collagenase at 37°C for 5 h	DMEM-LG, 10% FBS	1-4	FC RT-PCR	CD29, CD44, CD73, CD90, CD105 ABCG2, COL10A1, CXCR4, LIFR, OPN	CD3, CD34, CD45, HLA-DR	n.r.	[20]
0.5% collagenase at 37°C for 5 h	DMEM-LG, 10%FBS	n.r.	FC RT-PCR	CD29, CD44, CD73, CD105 Nanog, STAT3, AP, Oct-4, BMP4, SCF, PAX3, PAX6, ADAM12, nestin, HLA-I	CD34, CD45, HLA-DR	Neuron	[21]
0.1% collagenase at 37°C for 20 min	DMEM-LG, 10% FBS	n.r.	FC	CD29, CD73, CD90, HLA-I	CD31, CD34 CD45, CD117, HLA-DR	Adipocyte, osteoblast, skeletal muscle cell	[22]

ICC: immunocytochemistry; FC: flow cytometry; n.r.: not reported.

protein belonging to the immunoglobulin superfamily [32]: Romanov *et al*., [15], but not Covas *et al.,* [16], reported the immunoreactivity against this marker. RT-PCR revealed the expression, as mRNAs, of molecules of the undifferentiated state [Nanog, Oct-4, leukemia inhibitory factor receptor (LIFR), and ABCG2], mesoderm (CXCR4, CD44), and extracellular matrix (collagen) [20, 21]. Nanog and Oct-4 (octamer-binding transcription factor-4), also known as POU5F1 (POU domain, class 5, transcription factor 1), are involved in self-renewal of undifferentiated embryonic stem cells [33]. According to various reports, the expression of these markers are not restricted to embryonic stem cells, but also to stem cells isolated from umbilical cord, adipose tissue or induced pluripotent stem cells [33].

It has been demonstrated that MSCs from the subendothelial layer can differentiate *in vitro* into adipogenic, osteogenic, chondrogenic and myogenic (skeletal and cardiac muscle) lineages. Interestingly, nestin and PAX6, related to early differentiation of neuronal ectoderm, were found in MSCs, suggesting that these cells may be easily differentiated into neuronal lineage [21]. Nevertheless, these cells fail to differentiate *in vitro* into functional active neurons. Thus, the improvement of neurobehavorial function and the reduction of the infarct volume observed after injection of hUC-MSCs in ischemic stroke rats seemed to be related to the secretion of neutrotrophic factors rather then the integration of these cells into the host nervous system [21].

3.2. MSCs from the Perivascular Zone

UC vessels are isolated together with their surrounding matrix and then sutured to form a loop, from which MSCs are obtained by enzymatic digestion at 37°C using collagenase (0.5-1 mg/mL) [34, 35] or a cocktail composed of dispase, hyaluronidase, collagenase, and trypsin [36]. The incubation times are very variable ranging from 1 to 24 h. Except for Farias *et al*., [36] which have used a culture media supplemented with growth factors, such as dexametasone, ascorbic acid, epidermal growth factor (EGF) and insulin, that may induce cell differentiation, the other Authors have cultured MSCs, previously depleted of CD45-positive cells, with α-MEM containing 15% FBS [34, 35, 37]. The phenoypic profile of MSCs from the perivascular zone seems to resemble, at least in part, that of the subendothelial layer. Indeed, cells express, as protein, CD105, CD73, CD90, CD29, CD44, and CD54, but lack Oct-4 (Table **2**). Moreover, the presence of CD10 and CD68, not reported for cells of the subendothelial layer, has been verified. The

Table 2. Phenotype of hUC Cells Obtained from the Perivascular Zone

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
1mg/mL collagenase at 37°C for 18-24 h	α-MEM, 15% FBS	2	FC	CD44, CD54, CD73, CD90, CD105, CD117, HLA-I	CD34, CD45, CD106, CD123, CD235a, SSEA-4, HLA-II, Oct-4	Osteoblast	[37]
1mg/mL collagenase at 37°C for 4 h	α-MEM, 15% FBS, 1%, L-glutamine	2-5	FC	CD29, CD44, CD73, CD90, CD105, CD146	CD14, CD34, CD45, CD106, CD117	Adipocyte, osteoblast, chondrocyte	[34]
0.5 mg/mL collagenase I at 37°C for 18-24 hours	α-MEM, 15% FBS	2-9	FC RT-PCR	CD44, CD73, CD90, CD105, CD106 Collagen IA1, Desmin, $MyoD^{Low}$	CD34, CD45	Adipocyte, osteoblast, chondrocyte, fibroblast, skeletal muscle cell	[35]
1.38 mg/mL dispase, 0.2 mg/mL hyaluronidase, 0.8 mg/ml collagenase IV for 45 min (x2) at 37 °C and 0.25% trypsin-EDTA at 37 °C for 20 min	60% DMEM-LG, 2% FBS, 40% MCDB-201, 1x insulin transferrin selenium, 1x linoleic acid-bovine serum albumin, 10^{-9} M dexamethasone, 10^{-4} M ascorbic acid 2-phosphate, 10 ng/mL EGF, 10 ng/mL PDGF-BB	3-5, 9	ICC FC	CD10, CD29, CD44, CD54, CD68, CD73, CD90, CD105, caldesmon, desmin, SMA, CKAE1/AE3, Ki67, podoplanin, vimentin CD10, CD14, CD29, CD44, CD54, CD73, CD90, CD105, SMA	CD31, CD34, CD45 CD34, CD45	n.r.	[36]

ICC: immunocytochemistry; FC: flow cytometry; n.r.: not reported.

protein CD10 is a surface enzyme with neutral metalloendopeptidase activity and able to inactivate a variety of biologically active peptides. It is expressed not only in B-cell lymphomas, but also in other cell types, such as BM-MSCs and human fibroblasts [38]. Farias *et al*., [36] has suggested that all cells from perivascular area derive from Wharton's jelly, as they are 100% positive for CD10, an *in vivo* expressed marker of Wharton's jelly cells, but not of amnion or arterial smooth muscle layers. During culturing, it is reported that the cells acquire the expression of CD68, a myelomonocytic marker, that is present on adipose tissue-derived MSCs cultured under defined conditions [39]. The same Authors also reported that perivascular area cells resemble myofibroblasts, because of the expression of SMA and caldesmon and the capacity to contract under stimulation with trasforming growth factor (TGF)-β1 and platelet derived growth factor (PDGF). Interestingly, Sarugaser *et al*., [37] have reported a cell population negative to both HLA class I and II that increases during the first five passages and after cryopreservation. These data are particularly attractive in view of their clinical application in cell-based therapies. Some discrepancies among the various studies exist about the immuno-reactivity against CD106 and CD117 [c-kit, stem cell factor (SCF) receptor] [34, 35, 37]. Surprisingly, Sarugaser *et al*., using the same extraction method, culture conditions and method of analysis, have reported that cells were positive to CD106 in one work [35] and negative in another one [37]. MSCs from the perivascular zone were reported to differentiate *in vitro* into osteoblastic, adipogenic, chondrogenic, myogenic and fibroblastic lineages [34, 35, 37]. Furthermore, when injected into the femoral marrow cavity of NOD-SCID mice, these cells produced bone, cartilage and fibrous stroma, suggesting that they can contribute to tissue repair in response to injury [36]. No expression of cardiac markers were detected after inductive treatment with 5-azacytidine [34].

3.3. MSCs from the Wharton's Jelly

Most of researchers use Wharton's jelly as a source of MSCs [3, 8, 40-63]. After removal of vessels, the mucous tissue is cut in small pieces and incubated at 37°C with enzymatic solution containing various types of collagenase and/or hyaluronidase, trypsin, and dispase (Table **3**). Also here, incubation times greatly differ from one extraction protocol to another. Alternatively, the fragments are put in culture plates and cells are allowed to migrate from explants. Culture media are mainly composed of DMEM supplemented with 10-20% FBS or FCS, but MCDB 201, RPMI 1640, α-MEM, F12, and MesenCult have been also used. In a few works [41, 42, 57] culture media have been enriched with growth factors, such as basic fibroblast growth factor (bFGF), PDGF, dexametasone, and ascorbic acid. The explant method may present some advantages because cell damage induced by the enzymatic treatment is avoided and the growth factors contained in Wharton's jelly may support the proliferation of MSCs. Primary cultures obtained by the explant method seem to be heterogeneous. Indeed, Majore *et al*., [47] have isolated by means of Counterflow Centrifugal Elutriation (CCE) a small-size (diameter about 11.1 μm) cell population that exhibited higher proliferative rates and expression of MSC markers in respect to large-sized (diameter about 19 μm) cells. These data have suggested that small cells may represent precursors of the larger ones. Overall, cells from Wharton's jelly fit with the minimal criteria for MSCs. Nevertheless, Kadam *et al*., [52] have not found any expression of CD105, while Bakshi *et al*., [46]

have observed that this marker is missing in cultures of earlier passages but is present at the 5th passage. The mesenchymal features of Wharton's jelly cells have been confirmed by the expression of specific lineage cytoskeletal markers, such as SMA [3, 43, 52] and vimentin [3, 52, 61, 62]. Furthermore, embryonic stem cell markers, such as Oct-3/4, SSEA4, nucleostemin, SOX-2, and Nanog, have also been revealed [41, 56, 58, 60-63]. Other detected cell surface molecules, whose expression has not been verified on MSCs from UC subendothelial layer and perivascular zone, are CD59 [41] and CD146 [34, 48, 62]. CD59 is involved in the complement system regulation: it inhibits the formation of pore forming membrane attack complex thus preventing cell lysis. CD146, also known as cell surface glycoprotein MUC18, is a cell adhesion molecule expressed not only on endothelial cells but also on MSCs. As reported in Table **1**, the protein CD51 or vitronectin receptor, member of the integrin family, was not found on MSCs from the subendothelial layer [16, 17]. It is unlikely that MSCs from Wharton's jelly demonstrated to be positive to this marker [40, 49].

Regarding to the immunomodulatory properties of MSCs from Wharton's jelly, it has been demonstrated that cells, such as plancental ones, express the HLA-G6 isoform [8]. HLA-G seems to play a role in the immune tollerance during pregnancy avoiding a maternal immune response against the

Table 3. Phenotype of hUC Cells Obtained from Wharton's Jelly

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
2mg/mL collagenase for 16 h, 2.5% trypsin for 30 min at 37°C	DMEM-HG, 10%FBS	1	FC	CD29, CD44, CD51, CD73, CD105	CD34, CD45	Adipocyte, osteoblast, chondrocyte, cardiomiocyte	[40]
Explants	DMEM, 10% FBS, 5ng/mL bFGF, 5 ng/mL EGF	3-8	FC	CD29, CD44, CD59	CD14, CD33, CD34, CD38, CD40, CD40L, CD45, CD80, CD86, CD117	Adipocyte, osteoblast, neuron	[41]
Hyaluronidase, trypsin, and collagenase at 37°C for 45-60 min	56% DMEM-LG, 37% MCBD 201, 2% FBS, 1x insulin-transferrin-selenium A, 10 nM dexametasone, 50 μM ascorbic acid, 10 ng/mL PDGF-BB	4-8	FC gene array	CD10, CD13, CD29, CD44, CD49e, CD90, CD105, HLA-I *ESG1, SOX-2, TERT, SHH*, neuregulin-1 and 4, Patched, *SNA2, WNT4,* N-cadherin, V-cadherin, R-cadherin, integrin-β_1, integrin-α, VCAM-1, CD49b, integrin-α_V, integrin-β_5, integrin-α_4, era, *CNTF, VEGF, FGF20, TRKC*, ACTG2, ACTA2, BMP1, PDGFB, CK 8, insulin	CD14, CD31, CD34, CD45, CD56, CD133, HLA-DR	Neuron	[42]
Explants and immuno-separated CD105+/ CD31-/KDR-cells	DMEM-LG, 20% FBS	CD105+ CD31- KDR-	ICC	CD90, HLA-I, SMA	CD34, CD38, CD45, CD54, SREBP, MYF5	Adipocyte, osteoblast, skeletal muscle cells	[43]
Collagenase type I at 37°C for 14-18 h	DMEM, 10% FCS	4-8	FC	CD13, CD29, CD44, CD49b, CD73, CD90, CD105, CD166, HLA-I	CD1q, CD3, CD10, CD14, CD31, CD34, CD45, CD49a, CD56, CD117, HLA-DR	Adipocyte, osteoblast, neuron	[44]
Explants	RPMI 1640, 20% FBS	2	FC	CD29, CD44, CD49b, CD58, CD 73, CD105, CD166, HLA-I	CD3, CD7, CD14, CD19, CD33, CD34, CD40, CD45, CD49d, CD80, CD86, CD117, CD133, HLA-DR, 3G5, STRO-1	n.r	[45]
1mg/mL collagenase type I at 37°C for 18-24 h	DMEM-LG, 10% FBS	4	ICC, FC, RT-PCR	CD13, CD29, CD29b, CD44, CD49e, CD54, CD73, CD90, CD105, $CD117^{low}$, CD166, HLA-I, SMA, vimentin, fibronectin	CD14, CD34, CD45, CD140a, HLA-II	Adipocyte, osteoblast, hepatocyte	[3]
Hyaluronidase, trypsin, and collagenase at 37°C for 45-60 min		4	FC RT-PCR	HLA-G6	CD40, CD80, CD86 HLA-G5	n.r.	[8]

(Table 3) Contd…..

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
1mg/mL collagenase type I at room temperature for 18-24 h	DMEM-LG, 2 mM glutamine, 10% FBS	3-13	FC	CD29, CD44, CD73, CD90, CD105, CD166, HLA-I	CD34, CD38, CD45, CD117, HLA-DR	n.r.	[46]
Explants	α-MEM, 15% FBS, 1% L-glutamine	2	FC	CD29, CD44, CD73, CD90, CD105, CD146	CD14, CD34, CD45, CD106, CD117	Adipocyte, osteoblast, chondrocyte	[34]
Explants + CCE	MEM, 15% of human serum	n.r.	FC	CD44, CD73, CD90, CD105	CD34, CD45	n.r.	[47]
Explants	DMEM, 20% FCS	1-10	FC	CD44, CD73, CD90, CD105, CD144^{low}, CD146, HLA-I	CD34, CD45	Osteoblast	[48]
Explants	DMEM, 10% FBS	8-10	FC	CD29, CD44, CD51, CD73, CD105	n.r.	n.r.	[49]
0.2 mg/mL collagenase type I for 24 h and 0.2% trypsin for 30 min at 37°C	DMEM-HG, 10% FBS	n.r.	ICC	CD44, CD73, CD90, CD105, CD106, CD144	CD45	n.r.	[50]
Explants	DMEM-LG, 10% FBS	3-4	FC	CD73, CD90, CD105	CD14, CD45	n.r.	[51]
Collagenase type IV and dispase II for 1h and trypsin plus EDTA for 30 min at 37°C	DMEM/F12 (1:1), 10% UC blood serum	1	FC ICC	CD44, CD73, CD90, CD117 SMA, vimentin	CD33, CD34 CD45, CD105	Adipocyte, osteoblast, chondrocyte, neuron	[52]
2 mg/mL collagenase at 37°C for 16 h, 2.5% trypsin for 30 min at 37°C	HMSCGM, growth supplements	2, 4, 8, 12	FC	CD13, CD29, CD73, CD90, CD105, CD117, CD166	CD14, CD34, CD45	Adipocyte, osteoblast, chondrocyte	[53]
Collagenase (300 U/mL) /hyaluronidase (1 mg/mL) for 1 h, 0.1% trypsin-EDTA solution for 30 min	Medium 1: Growth Medium #1 (GM#1) containing FBS; Medium 2: StemPro MSC SFM with 2% GMP-certified human serum (SP+2%HS); Medium 3: MesenCult ACF (MC) xeno- and serum-free	n.r.	FC	CD73, CD90, CD105	CD34, CD45, CD80, CD86, HLA-DR	Adipocyte, osteoblast, endothelial cell	[54]
Explants	αMEM, 15% human serum	3-7	FC	CD44, CD73, CD90, CD105	CD34, CD45	n.r.	[55]
1) Explant 2) 0.4% collagenase type II, 0.01% DNAse I at 37 °C for 30 min	DMEM/F12 (1:1), GLUTAMAX –I, 10% FBS, 40ng/mL bFGF	from 3	FC RT-PCR	CD29, CD73, CD90, CD105, HLA-I Nanog, REX2, SOX-2, Oct-4	CD34, CD45, CD80, CD86, HLA-DR	Adipocyte, osteoblast	[56]
Collagenase type I, collagenase type IV and 100 IU hyaluronidase at 37 °C for 45 min	DMEM-HG, 20% FBS, 16 ng/mL bFGF, 1 mM L-glutamine, 1:200 dilution of insulin-transferrin-selenium	3 to 15	RT-PCR, microarray	CD10, CD13, CD29, CD44, CD73, CD90, CD105, CD166, HLA-I	CD14, CD11b, CD18, CD31, CD33, CD34, CD45, CD115, vWf, CDH5, ICAM-2, KDR, FLT1, HLA-DR	n.r.	[57]
1 μg/mL collagenase type B at 37°C for 4 h	DMEM/ F12 (1:1), 10% FBS	0-7	FC, RT-PCR	CD29, CD73, CD90, CD105, CD54, Nanog, SOX-2, nucleostemin	CD31, CD34, CD45, CD133	Adipocyte	[58]
Explants	DMEM-LG, 10% FBS	2	ICC, FC	CD29, CD44, CD90, CD105	CD34, CD45	Adipocyte, osteoblast, Schwann-like cells	[59]
500 mg/mL collagenase for 20 h	KO-DMEM, 10% FBS, 2 mM L-glutamine	3-8	FC	CD54, CD73, CD90, CD95, CD105, CD166, SSEA4, HLA-I	CD19, CD34, CD45, CD50, CD80, CD86, HLA-DR	Adipocyte, osteoblast, chondrocyte	[60]

(Table 3) Contd…..

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
Collagenase Type IV: Dispase (7:1, v/v) for 30 min at 37 °C, 0.05%, trypsin and EDTA (0.02%) for 15–20 min at 37 °C	DMEM: HF12 (1:1), 10% human UC blood serum	4-6	FC ICC	CD29, CD44, CD73, CD90, CD117, Oct4 nestin, vimentin, desmin, SMA, Ki67, SSEA4	CD10, CD33, CD34, CD45, CD105, CD166, HLA-DR	Adipocyte, osteoblast, chondrocyte, neuron, islet-like cluster	[61]
Explants	DMEM-LG, 10% FBS	2-5	ICC	CD29, CD44, CD105, CD146, STRO-1, vimentin	nestin	Adipocyte, osteoblast, chondrocyte	[62]
Explants after 1.2U/mL dispase and 112U/mL type I collagenase (for three 5-min periods)	DMEM, 10% FBS	n.r.	FC RT-PCR	CD29, CD44, CD73, CD90 Nanog, Oct-3/4, SOX-2, SOX-9	CD34, CD45	Adipocyte, osteoblast, chondrocyte, neuron, skeletal muscle cell	[63]

ICC: immunocytochemistry; FC: flow cytometry; n.r.: not reported.

fetus. Compelling evidence has shown that the low rate of rejection seems to be associated to the expression of these antigens in blood and heart and liver/kidney grafts [64]. Furthermore, MSCs lack both CD80 and CD86 proteins [8, 41, 45, 54, 56, 60]. CD80, present on activated B cells and monocytes, and CD86, found on antigen-presenting cells, are costimulatory molecules inducing T cell activation and survival. The simultaneous presence of HLA-G and the lack of CD80 and CD86 suggest that Wharton's jelly-derived MSCs are particularly suitable for cell-based therapy, in accordance with the *in vivo* studies where the transplantation of heterologous MSCs did not elicit acute rejection [7]. Finally, contradictory findings have been obtained on the expression of CD106 [34, 50], CD117 [3, 34, 41, 44-46, 53, 61], and nestin [61, 62].

MSCs from Wharton's jelly seem to possess a greater differentiation potential than ones obtained from the subendothelial layer, the perivascular zone, and the umbilical cord lining (see above). Indeed, they can differentiate into not only mesodermic but also ecto- and endodermic lineages. Weiss *et al*., [42] have shown that, after neural induction, MSCs express tyrosine hydroxylase, a marker for catecholaminergic neurons. The transplantation of undifferentiated hMSCs into the brain of hemiparkinsonian rats induced a behavioral recovery of the animals, suggesting the potential use of these cells for the treatment of central nervous system diseases. Furthermore, they may also contribute to nerve repair in clinical applications because they can differentiate *in vitro* into Schwann-cell lineage [59]. In a rat brain ischemia/riperfusion model, the transplantation of MSCs improved the neurological function and the cortical neuronal activity [44]. These effects were related not only to the differentiation of the transplanted cells into glial and neuronal cells, but also into endothelial cells. This latter differentiation potential may have implications for the therapy of vascular diseases. Indeed, when differentiated MSCs were transplated into wire-injured femoral arteries in mice, a quick re-endothelization and inhibition of neointimal hyperplasia were observed [50]. These effects were probably mediated by the pigment epithelium-derived growth factor (PEDF) that inhibits smooth muscle cell proliferation and possesses antioxidant properties. It has been demonstrated that MSCs from Wharton's jelly can also form mature islets, responsive to glucose challenge and expressing all the islet hormones [61]. When immunoisolatory biocompatible capsules containing differentiated MSCs were transplanted in experimental diabetic mice, normoglycemia was restored indicating the functionality of these cells. Campard *et al*., [3] have shown that MSCs from Wharton's jelly constitutively express some markers of hepatic lineage, such as albumin, α-fetoprotein, and cytokeratin-19. Although the production of urea and storing of glycogen were detected under differentiative medium, the cells fail to form *in vitro* mature hepatocytes. Nevertheless, undifferentiated MSCs injected into the spleen of partially hepatectomized SCID mice engrafted into the liver and express albumin and α-fetoprotein.

Apart from their wide differentiation potential, MSCs from Wharton's jelly present some interesting characteristics not related to the replacement of injured cells. Indeed, they support the growth and the maintenance of hematopoietic stem cells from UC blood (UCB) through the production of hematopoietic growth factors [45, 46]. Consistent with their immunomodulatory properties [5], they also enhance human hematopoietic engraftment in SCID mice when co-transplanted with hematopoietic stem cells [45]. MSCs from Wharton's jelly possess also antifibrotic properties. The administration of MSCs in a bleomycin-induced mouse model of lung injury reduced the inflammation and collagen concentration in the lung [48]. Interestingly, injected MSCs were detected only in areas of inflammation and fibrosis but not in healthy lung tissue. Similar results were obtained in a rat model of carbon tetrachloride–induced liver fibrosis [49]. Although the transplanted MSCs did not differentiate into hepatocytes, they secreted bioactive cytokines promoting liver cell repair through the reduction of hepatic inflammation.

3.4. MSCs from the Umbilical Cord Lining

Cells from the umbilical cord lining (UCL) have also been isolated by means of explant method after separation and dissection of the UCL membrane [65, 66]. Different cell populations have been obtained depending on the culture medium (Table **4**). Gonzales *et al*., [65], using DMEM supplemented with 15% FBS, have characterized MSCs that expressed pluripotent markers, such as Nanog, Oct-4, and SSEA-4 and were able to differentiate into osteogenic, adipogenic, chondrogenic, cardiogenic, and neurogenic linea-

Table 4. Phenotype of hUC Cells Obtained from the Umbilical Cord Lining

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
Explants	DMEM, 15 % FBS, Glutamax, MEM-non essential aminoacids, MEM vitamins	from 1	ICC, FC, RT-PCR	CD14, CD44, CD73, CD90, CD105, CD106, CD166, STRO-1, SSEA-4, HLA-I, LIN, Nanog, Oct-4	CD19, CD34, CD45, CD117, CD133, HLA-DR, SOX-2	Adipocyte, osteoblast, chondrocyte, cardiomiocyte, and neuron	[65]
Explants	PTTe-1 medium, 2.5% FBS, 50 µg/mL insulin-like growth factor-1, 50 µg/mL PDGF-BB, 5 µg/mL TGF-β1, 5 µg/mL insulin	up to 20	FC ICC, RT-PCR	CD34, CD45, CD73, CD105, CD151, CD166, HLA-I, CD227 vimentin, E-cadherin, CK7, CK14, CK19, Oct-4, Nanog, SSEA-4, SOX-2, REX1, p63	CD31, HLA-II TERT	Epithelial cells	[66]

ICC: immunocytochemistry; FC: flow cytometry

ges. Interestingly, these cells also expressed CD14, that is a component of the innate immune system and they are present on macrophages, neutrophil granulocytes, and dendritic cells [67]. On the other hand, Reza *et al*., [66] have grown UCL cells in PTTe-1 medium containing components that preferentially support epithelial cell growth. As a result, a cell population possessing properties of both epithelial cells and embryonic stem cells has been obtained. Indeed, the cells highly expressed MUCIN1, whose function is to protect the epithelium by binding to pathogens [68], CD151, involved in epithelial cell-to-cell adhesion [69], p63, an adult epithelial stem cell marker [70], and CK7, CK14 and CK19 in varying degrees. Furthermore, cells were positive to several embryonic stem cell markers, such as Oct-4, Nanog, ring-exported protein-1 (REX-1), SOX-2, telomerase reverse transcriptase (TERT), and SSEA-4.

3.5. MSCs from whole Umbilical Cord

Starting from the presence of MSCs in many parts of hUC, several research groups have used whole hUC in the attempt to increase the yield of MSCs (Table **5**) [71]. After mechanical chopping, explant culture of the cord is carried out or UC fragments are incubated at 37°C with various types of collagenase (0.1% or 0.075%, w/v) and trypsin (0.25% or 0.125%, w/v) [2, 4, 72-80]. Also in this case, the incubation times greatly differ from one laboratory to another, ranging from 10 min to 3 h. The most frequently used culture medium is DMEM-LG supplemented with 10% FBS or FCS. However, growth factors, such as vascular endothelial growth factor (VEGF), EGF, and bFGF, have been also added [72, 76, 79]. A comparison between the explant method and enzymatic digestion was carried out by Schugar *et al*., [81], which demonstrated that the isolation method affects population phenotype. Indeed, collagenase digestion lead to a cell population characterized by higher levels of MSC surface markers compared to that detected in cultures obtained by means of explant method or dispase digestion.

Although there is a wide heterogeneity of markers used to characterize the cells, MSCs from whole hUC express most of CD proteins detected in hUC cells from the subendothelial layer, perivascular area, umbilical cord lining, and Wharton's jelly (Table **5**). Jo *et al*., [76] have shown that pluripotent stem cell markers, such as Oct-4, SSEA-3, SSEA-4, Tra-1–60, and Tra-1–81, are expressed, as both mRNA and protein, by MSCs from the whole cord and their expression levels are maintained up to the 9th passage. Furthermore, Qiao *et al*., [75] have detected nucleostemin, a p52 binding protein localized in nucleoli of stem cells [82], and BMI-1 involved in self-renewal [83], whereas La Rocca *et al*., [77] have demonstrated, for the first time, the presence of both isoforms of Oct-4 (A and B). The same Authors have extensively studied this cell population, verifying the expression of several markers related to the tumor cells, the immune recognition of MSCs by the immune system, neuroectodermal and endodermal differentiation. MSCs lack the expression of the carcino-embryonic antigen (CEA) [84] and an embryonic form of the Ras oncogene, known as Eras [85], suggesting that these cells could be used in cell therapy without any risk of tumorigenicity. As already reported for Wharton's jelly cells, the expression of HLA-G and the absence of CD86 have been detected. In addition to the presence of mesenchymal markers, such as vimentin, SMA, and prolyl-4-hydroxylase, neuroectodermal and endodermal markers have been revealed, confirming that these cells possess differentiation potential towards these lineages. Indeed, MSCs were positive to GATA-4, a transcription factor involved in cardiomyocyte differentiation [86], and connexin-43, highly expressed in cardiomyocytes [87]. When MSCs were induced to cardiomyocyte differentiation, changes in cell morphology with formation of myotube structures were observed [4].

Furthermore, about 50% of cells expressed cytokeratins (CK) 8, 18, and 19, none of which have been previously detected in MSCs. The feasibility to differentiate MSCs into neuronal lineage, already proposed for cells isolated from the subendothelial layer [21], is here supported by the expression of nestin [77], nerve growth factor receptor [73], which plays a role in survival and differentiation of neuronal cells, and CD56, the neural cell adhesion molecule involved in neurite outgrowth and synaptic plasticity [76]. Consistent with these findings, MSC cultures presented a high percentage of neuron specific enolase-positive cells after neuronal induction [72]. As already demonstrated for MSCs from Wharton's jelly, also ones from whole cord are able to differentiate *in vitro* into endothelial cells [79] and *in vivo* into hepatocyte-like cells, improving the recovery of carbon tetrachloride-injured mouse liver [73].

Other markers recognized on MSCs from whole cord are involved in cell adhesion and migration: CD9, a cell surface glycoprotein which forms a complex with integrins, CD49b,

Table 5. Phenotype of hUC Cells Obtained from whole UC

Extraction method	Culture medium	Passage	Methods of analysis	Positive markers	Negative markers	Differentiation potential	Refs.
Collagenase type II 0.075% for 30 min, 0.125% trypsin for 30 min at 37°C	DMEM-LG, 5% FBS, 10 ng/mL VEGF, 10 ng/mL EGF	2-6	FC	CD13, CD29, CD44, CD73, CD105, CD106, CD166, HLA-I	CD14, CD31, CD34, CD38, CD45, HLA-DR	Adipocyte, osteoblast, neuron	[72]
3mg/mL collagenase 1S at 37°C for 3h	αMEM/F12 (1/1), 10% FBS	n.r.	ICC, FC	CD29, CD49b, CD73, CD105	CD31, CD34, CD38	Cardiomiocyte	[4]
Explants	DMEM-LG, 10% FBS	2	FC	CD13, CD29, CD44, CD105, NGFR	CD34, CD38, CD45, CD71, HLA-DR	Adipocyte, osteoblast	[73]
Explants	DMEM-LG, 10% FBS	2	FC	CD13, CD44, CD73, CD105, HLA-I	CD14, CD31, CD45, CD144, HLA-DR	Adipocyte, osteoblast, chondrocyte	[74]
Explants	DMEM-LG, 10% FBS	2	FC RT-PCR	CD13, CD44, CD29, CD105, HLA-I nucleostemin, BMI-1	CD34, CD38, CD71, HLA-DR	Adipocyte, osteoblast	[75]
0.1% collagenase for 2 h and 0.25% trypsin for 1 h at 37°C	DMEM-LG, 10% FBS, 10 ng/mL bFGF	2,5,7,9	FC ICC, RT-PCR	CD9, CD10, CD13, CD29, CD44, CD49a, CD49b, CD54, CD56, CD62e, CD68, CD73, CD90, CD105, CD117, CD120, CD133, CD166, CD235a, HLA-I Nanog, Oct-4, SSEA-3, SSEA-4, Tra-1–60, Tra-1–81	CD11, CD14, CD31, CD34, CD40, CD45, CD66, CD71, CD106, STRO-1, HLA-DR	Adipocyte, osteoblast, chondrocyte	[76]
Explants	DMEM-LG, 10% FCS, 1x non essential aminoacids	up to 15	ICC, RT-PCR	CD10, CD13, CD29, CD44, CD73, CD80, CD90, CD105, CD106, CD117, CD133, CD166, Oct-1, Oct-4 (A and B), HLA-A, HLA-G, Nanog, GATA-4/5/6, SMA, vimentin, nestin, CKs 8/18/19, connexin-43, GFAP, NSE, HNF-4α, Prolyl-4-Hydroxylase	CD31,CD33, CD34, CD38, CD45, CD79, CD86, CEA, HLA-DR, ERas, CK7, MyoD, Myosin (smooth muscle), vWf, Oct-2	Adipocyte, osteoblast	[77]
Explants	DMEM-LG, 10% FCS, 1x non essential aminoacids	n.r.	ICC, RT-PCR	CD68	CD34, CD45, CD163	n.r.	[78]
Explants	DMEM-LG, 10% FBS, 5 ng/mL bFGF	1-18	FC	CD90, CD105, CD73	CD14, CD19, CD34, CD45, HLA-DR	Adipocyte, osteoblast, chondrocyte, endothelial cell	[79]
0.1% collagenase at 37°C for 20 min	DMEM-LG, 10% FBS	n.r.	FC	CD13, CD29, CD44, CD73, CD90, HLA-I	CD31, CD34, CD45, CD117, HLA-DR	Adipocyte, osteoblast, chondrocyte	[80]
Explants	DF-12, 10% FBS	up to 25	FC	CD90, CD105, CD73	n.r.	Adipocyte, osteoblast, chondrocyte	[2]

ICC: immunocytochemistry; FC: flow cytometry; n.r.: not reported.

and integrin α subunit, and CD62, also known as E-selectin. As reported above for MSCs obtained from the various parts of hUC, contradictory results were obtained concerning the presence of CD106 and CD117. Finally, MSCs from whole cord have been shown to express cytokines, such as IL-6, SCF, Flt-3, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), that are associated with hematopoietic stem cell proliferation, suggesting a potential hematopoiesis supportive function [72].

3.6. Comparison between MSCs from Various Parts of hUC

Table **6** summarizes the availability of some markers in MSCs obtained from the various parts of hUC. In the attempt to make a comparison among the UC cell phenotypes, only the markers studied in at least two separate cell populations have been reported. All MSCs seem to resemble each other. Indeed, they do not possess a hematopoietic profile, whereas mesenchymal and embryonic stem cell markers are exp-

Table 6. Availability of Markers on hUC Cells by Flow Cytometry, Immunocytochemistry, RT-PCR, Gene Expression Assays

Marker	SE cells	PV cells	UCL cells	WJ cells	wUC cells
CD10	n.r.	+	n.r.	+/-	+
CD13	+	n.r.	n.r.	+	+
CD14	-	+/-	+	-	-
CD19	n.r.	n.r.	-	-	-
CD29 (integrin β1)	+	+	n.r.	+	+
CD31 (PECAM)	-	-	-	-	-
CD34	-	-	+/-	-	-
CD44 (HCAM)	+	+	+	+	+
CD45	-	-	+/-	+/-	-
CD49a	n.r.	n.r.	n.r.	-	+
CD49b (integrin α2)	n.r.	n.r.	n.r.	+	+
CD49d (integrin α4)	-	n.r.	n.r.	-	n.r.
CD49e (integrin α3)	+	n.r.	n.r.	+	n.r.
CD54 (ICAM-1)	+	+	n.r.	+/-	+
CD73 (SH3)	+	+	+	+	+
CD80	n.r.	n.r.	n.r.	-	+
CD86	n.r.	n.r.	n.r.	-	-
CD90 (Thy-1)	+	+	+	+	+
CD105 (SH2)	+	+	+	+/-	+
CD106 (VCAM-1)	+/-	+/-	+	+/-	+/-
CD117 (c-kit)	-	+/-	-	+/-	+/-
CD133	-	n.r.	-	-	+
CD166 (ALCAM)	+	n.r.	+	+/-	+
CD235a (glycophorin A)	-	-	n.r.	n.r.	+
HLA-I	+	+	+	+	+
HLA-G	n.r.	n.r.	n.r.	+	+
HLA-DR	-	-	-	-	-
Nanog	+	n.r.	+	+	+
Nestin	+	n.r.	n.r.	+/-	+
nucleostemin	n.r.	n.r.	n.r.	+	+
Oct-4	+	-	+	+	+
SMA	+	+	n.r.	+	+
SOX-2	n.r.	n.r.	+/-	+	n.r.
SSEA-4	n.r.	-	+	+	+
STRO-1	n.r.	n.r.	+	+/-	-
vimentin	n.r.	+	+	+	+
vWf	-	n.r.	n.r.	-	-

SE: subendothelial layer; PV: perivascular; UCL: umbilical cord lining; WJ: Wharton's jelly; wUC: whole umbilical cord; n.r. : not reported; +/-: contradictory results.

ressed. Moreover, HLA class I, but not HLA class II, are present. Nevertheless, a different expression of some markers exists among the various hUC-MSCs. The CD133 and CD235a proteins have been revealed in cells from the whole UC, but not in those obtained from the other parts of the cord. The UCL cells and, at least in part, the perivascular cells express CD14 which has not been detected on cells isolated from the subendothelial zone, the Wharton's jelly, and whole UC. Other differences in stem cell markers, such as Oct-4, SSEA-4, and STRO-1, and integrins α are also visible. These findings suggest that the each part of hUC may contain a MSC population that differs from those of the other parts. Furthermore, different cell types could be contained within the same part of UC because of the discrepancies in marker expression. However, it seems impossible to draw a conclusion starting from the available data obtained by means of high heterogeneous protocols. To verify whether hUC-MSCs could represent an unique cell population or a family composed of cells at different stemness and/or committed degrees, a great effort must be made through the various research groups to standardize extraction methods, culture media, and conditions and analysis output. Indeed, phenotype characterization should be carried out using the same culture passage and a more homogenous panel of markers. The percentage of the expression should also be assessed. Furthermore, the *in situ* identification of stem cell niche and MSCs inside the cord could be very useful. In this context, Schugar *et al*., [81] have demonstrated that CD146-, CD144-, and CD105-positive cells are largely distributed in the vessel walls and the perivascular region of the Wharton's jelly, whereas CD105 and CD34 are strongly expressed in endothelial cells. CD73 expression has been found throughout the vessels and endothelium, absent in the perivascular region, and highest in the epithelium and sub-epithelial regions of Wharton's jelly matrix. CD90 is exp-ressed in most regions but not in the endothelial lumen lining. A high expression of vimentin, CKs (1, 4, 5, 6, 8, 10, 13, 18 and 19), desmin and SMA [88] has been detected in the subamniotic layer and perivascular region. On the contrary, the intervascular layer presents high positivity for vimentin and desmin but the expression level of CKs and SMA is low. Taken together, these findings corroborate the presence of different MSC populations inside hUC.

Overall, compelling evidence indicates Wharton's jelly as the best source of MSCs. Indeed, the findings collected by several studies suggest a wide range of therapeutic applications for these cells. The differentiation potential makes them attractive tools for the replacement of injured cells. The production of cytokines and growth factors as well as the immunomodulatory properties [89] represent the rationale for their employment in the treatment of fibrotic diseases, to support the *ex vivo* expansion of hematopoietic stem cells from UCB and improve their *in vivo* engraftment. Furthermore, Ayuzawa *et al*., [90] have suggested that MSCs from Wharton's jelly may be a useful tool for cancer cytotherapy. Indeed, they reduce MDA 231 metastatic lung tumor growth in SCID mice. This effect is related to the cell homing in on the tumour site, where MSCs probably produce cytotoxic compounds altering the cell cycle of cancer cells.

4. COMPARISON BETWEEN MSCS FROM UMBILICAL CORD AND BONE MARROW

To date, due to their application in regenerative medicine, BM-MSCs represent the "gold standard" for the evaluation of stemness properties of MSCs obtained from various sources. In this section, some research works, where the phenotype of UC-MSCs has been directly compared to that of BM-MSCs by means of flow cytometry (FC), RT-PCR, or immunocytochemistry (ICC) techniques, have been summarized. Overall, although the two cell population share several cell surface proteins, the differences seem to be mainly related to markers of committed states and molecules with immunomodulatory properties.

Comparing the gene expression profiles of BM-MSCs and subendothelial layer-derived UC-MSCs, Panepucci *et al*., [17] have shown similarities among the two lineages. Nevertheless, BM-MSCs seem to be more committed to osteogenesis, because they express genes such as biglycan, *TSC22*, CD44, and vitronectin. On the contrary, genes related to angiogenesis, such as *CXCL6, IL-8*, IL-1 receptor-like ligand*, MMP1,* have been expressed exclusively or at higher levels in the UC-MSCs. Using ICC analysis and FC, Suldal'tzeva *et al*., [91] have compared the phenotypic profile of subendothelial layer-derived UC-MSCs, BM-MSCs, and skin fibroblasts. Their results have shown that UC-MSCs, but not BM-MSCs, express nestin, collagen type 1 and 2, whereas CD106 is only present on BM-MSCs. The Authors have suggested that the phenotypic profile of UC-MSCs is intermediate between those of BM-MSCs and skin fibroblasts, because UC-MSCs expressed markers of both stem cells and differentiated cells. On the other hand, Covas *et al*. [92] have reported that MSCs from the subendothelial layer present a gene expression profile very similar to that of BM-MSCs and related to pericytes. Indeed, although at various degrees, they have found a common expression of genes, such as CD146, NG2, CD271, and CD140B. These findings indicate that the vascular wall contains a MSC compartment composed of fibroblasts, pericytes, and UC-MSCs that may derive from a common stem cell. Furthermore, it has been proposed that fibroblast-specific protein-1 (FSP-1) and CD146 may be used to monitor the differentiation pathways because the percentage expression of these markers are different between the two cell populations: in fibroblasts the expression levels of FSP-1 and CD146 are higher and lower, respectively, than those determined in MSCs.

Although MSCs derived from Wharton's jelly also present a phenotypic profile resembling, at least in part, that of BM-MSCs, some differences exist between the two cell populations. Friedman *et al*., [45] have reported that CD49b is expressed by UC-MSCs, whereas BM-MSCs lack this marker. The expression levels of HLA-I and CD73 are lower in UC-MSCs than those detected in BM-MSCs. The presence of 11 cell surface markers have been verified by Martin-Rendon *et al*., [34] in MSC cultures derived from BM, Wharton's jelly, and the perivascular zone of hUC. The three cell populations were phenotypically similar, except for CD106 that was weakly expressed in BM-MSCs and absent in UC-MSCs. Yoo *et al*., [51] have determined not only cell surface stem cell markers but also cytokines, chemokines, and growth factors that are secreted by BM-MSCs and Wharton's jelly-derived MSCs. In addition to IL-6, IL-8, MCP-1 that were produced by both cell types, IL-12, IL-15, RANTES and PDGF-AA were secreted only by UC-MSCs, while VEGF was expressed by BM-MSCs, but not by UC-MSCs. Furthermore, the Authors have demonstrated that UC-MSCs, like BM-MSCs, were able to suppress mitogen-induced T-cell proliferation, suggesting that they can be used, instead of BM-MSCs, to enhance the engraftment of allogeneic hematopoietic stem cells and to reduce the incidence of graft *versus* host disease.

This latter observation agrees with the results obtained by Lu *et al*., [72], which have compared BM-MSCs with MSCs derived from whole UC. Indeed, they have reported that UC-MSCs expressed HLA-I at lower levels than BM-MSCs and produced G-CSF and GM-CSF, cytokines inducing hematopoietic stem cell proliferation, that was not revealed in BM-MSCs. Taken together, these findings support the use of UC-MSCs for allogeneic cell therapy. Moreover, the same Authors have demonstrated that the CFU-F frequency was higher in UC-MSCs than BM-MSCs. Higher proliferation rates in MSCs cultures derived from Wharton's jelly in comparison with BM-MSC ones were also reported by Chen *et al*., [79]. As already observed for subendothelial layer-derived MSCs [17], Wharton's jelly-derived MSCs seem to possess a higher endothelial differentiation potential than BM-MSCs. Indeed, after endothelial induction, UC-MSCs expressed higher levels of endothelial markers, such as KDR, vWf, and VE-cadherin, than BM-MSCs.

CONCLUSIONS AND PERSPECTIVES

A great deal of evidence obtained both *in vitro* and *in vivo* have demonstrated that UC-MSCs isolated from the various parts of the cord are very promising tools for clinical applications. However, MSCs from Wharton's jelly seem to possess greater therapeutic potential than the ones obtained from the other UC zones. Indeed, they possess a wide differentiation potential suggesting their use for tissue-engineered constructs [93] and cell therapy [61] to replace injured tissues and cells. They have been successfully used in animal models for increasing angiogenesis in brain ischemia [44], inducing liver regeneration after hepatectomia [3], and replacing neuronal elements in Parkinson's models [42]. Moreover, MSCs from Wharton's jelly, like BM-MSCs, are able to support hematopoietic stem cell proliferation [46] and increase the engrafment of these cells in NOD-SCID mice [45]. Recently, their potential use for cancer cytotherapy has been suggested [90]. All these effects seem to be related to the production of a wide panel of factors. One of the main issues to be addressed is the identification of these factors to fully understand the exact mechanisms responsible for the properties of MSCs. In this context, a detailed report on transcriptome profile of MSCs from Wharton's jelly has been provided by Fong *et al*., [94] through Affymetrix DNA microarray analysis. The results have shown that the upregulation of genes associated with immune system processes related to antigen processing and presentation, immune system development and activation can be responsible for the immomodulatory properties. On the other hand, the homing in on tumour cells can be due to semaphorin E, promoting cell migration and possessing a high affinity for receptors over-expressed in tumor cells.

Furthermore, the expression of several tumour suppressor genes may account for the lack of *in vivo* teratoma induction.

When compared with BM-MSCs, MSCs from Wharton's jelly present several advantages related to easier availability, higher proliferation rates, lower expression of HLA-I coupled to the presence of HLA-G and better immunomodulatory properties. Furthermore, they maintain their multipotent characteristics for longer periods *in vitro* than BM-MSCs [94].

Despite these compelling evidence, the high heterogeneity in extraction, culture and analysis procedures hinder precisely identifying these cells. As a result, different phenotypic profiles are detectable not only among the cells obtained from the various parts of UC, but also inside the same UC regions [95-97]. The available data suggest the UC-MSCs may represent a unique cell family whose components present various degrees of stemness. However, standardized and shared procedures should be useful not only to verify this hypothesis and define the identity of each cell subset, but also to identify the most primitive cell population possessing the highest potentialities for clinical applications.

Finally, since the amount of MSCs isolated from Wharton's jelly (about 4 x 10^6cells/cm^2) is not sufficient for transplantation therapy [98], the optimization of protocols for the expansion and storage of these cells are needed to assure their efficient use for regenerative medical purposes. Regarding these issues, it has been highlighted that complex media, containing growth supplements, such as insulin-transferrin-selenium G and FGF, improve cell proliferation without affecting stemness properties [98]. On the other hand, the pretreatment of MSCs with 10μM Y-27632, a ROCK inhibitor, for 24h before freezing has been shown to increase cell survival and maintain stemness and differentiation potential after thawing [99].

Undoubtedly, MSCs from Wharton's jelly represent a great promise for human health. Although their versatility makes them particularly attractive, several questions must be answered and several problems must be solved before their entry in clinical settings. What is the most primitive MSC population inside the cord? By which mechanisms MSCs modulate the immune system and exert other functions not strictly related to cell replacement? What are the most suitable protocols to enhance their *in vitro* expansion and their storage? In summary, to successfully use MSCs in cell-based therapy, it seems noteworthy to further investigate the mechanisms underlying the functions of these cells and standardize protocols for their isolation, expansion, and characterisation.

ABBREVIATIONS

ACTG2 = Gene (protein-coding), actin, gamma-enteric smooth muscle

ACTA2 = Gene (protein-coding), actin, alpha 2, smooth muscle, aorta

AP = Alkaline phosphatase

bFGF = Basic fibroblast growth factor

BM = Bone marrow

CCE = Counterflow centrifugal elutriation

CEA = Carcino-embryonic antigen

CFU-F = Colony-forming-unit fibroblasts

CK = Cytokeratin

EGF = Epidermal growth factor

Esg1 = Embryonal stem cell-specific gene 1

FBS = Fetal bovine serum

FCS = Fetal calf serum

FC = Flow cytometry

FSP-1 = Fibroblast-specific protein-1

G-CSF = Granulocyte colony-stimulating factor

GM-CSF = Granulocyte-macrophage colony-stimulating factor

GFAP = Glial fibrillary acidic protein

HG = High glucose

HLA = Human leukocyte antigen

HNF4 = Hepatocyte nuclear factor 4

hUC = Human umbilical cord

KDR(Flk-1) = Kinase insert domain receptor

ICAM = Intercellular adhesion molecule

ICC = Immunocytochemistry

LIFR = Leukemia inhibitory factor receptor

LG = Low glucose

MMPs = Matrix metalloproteinases

MSCs = Mesenchymal stromal cells

MYF5 = Myogenic factor 5

NSE = Neuron-specific enolase

Oct = Octamer-binding transcription factor

OPN = Osteopontin

PDGF = Platelet-derived growth factor

PEDF = Pigment epithelium-derived growth factor

REX = Ring-exported protein

SCF = Stem cell factor

SHH = Sonic hedgehog homolog

SMA = α-smooth muscle actin

SREBP = Sterol regulatory element-binding proteins

TERT = Telomerase reverse transcriptase.

TGF = Trasforming growth factor

UCB = Umbilical cord blood

UCL = Umbilical cord lining

VEGF = Vascular endothelial growth factor

vWf = Von Willebrand factor

ACKNOWLEDGEMENT

None declared.

CONFLICT OF INTEREST

None declared.

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Received: March 14, 2011 Revised: May 20, 2011 Accepted: June 24, 2011