World Journal of Stem Cells

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DOI: 10.4252/wjsc.v6.i3.367

World J Stem Cells 2014 July 26; 6(3): 367-370
ISSN 1948-0210 (online)

MINIREVIEWS

Umbilical cord fibroblasts: Could they be considered as mesenchymal stem cells?

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Received: January 10, 2014 Revised: March 11, 2014
Accepted: May 16, 2014
Published online: July 26, 2014

Abstract

In cell therapy protocols, many tissues were proposed as a source of mesenchymal stem cells (MSC) isolation. So far, bone marrow (BM) has been presented as the main source of MSC despite the invasive isolation procedure related to this source. During the last years, the umbilical cord (UC) matrix was cited in different studies as a reliable source from which long term *ex vivo* proliferating fibroblasts were isolated but with contradictory data about their immunophenotype, gene expression profile, and differentiation potential. Hence, an interesting question emerged: Are cells isolated from cord matrix (UC-MSC) different from other MSCs? In this review, we will summarize different studies that isolated and characterized UC-MSC. Considering BM-MSC as gold standard, we will discuss if UC-MSC fulfill different criteria that define MSC, and what remain to be done in this issue.

Key words: Mesenchymal stem cells; Cord matrix; Bone marrow; Differentiation; Immunophenotype

Core tip: Umbilical cord matrix derived fibroblasts [umbilical cord-mesenchymal stem cells (UC-MSC)] have gained importance in the last years. We have studied these cells and noticed some differences when compared with bone marrow-MSC in term of differentiation and phenotype. Other studies pointed out similar differences. Recently, some studies have doubted of the MSC nature of these cells. Starting from our own results and those from literature, we summarized in this minireview different studies that isolated and characterised UC-MSC. In the discussion we confronted the contradictory data about the differentiation and immunophenotype of UC-MSC and highlight what remains to be done to answer the question: are cord matrix isolated fibroblasts stem cells or not?

Zeddou M, Relic B, Malaise MG. Umbilical cord fibroblasts: Could they be considered as mesenchymal stem cells? *World J Stem Cells* 2014; 6(3): 367-370 Available from: URL: http://www.wjgnet.com/1948-0210/full/v6/i3/367.htm DOI: http://dx.doi.org/10.4252/wjsc.v6.i3.367

INTRODUCTION

Initially, mesenchymal stem cells (MSC) were defined as a rare population of multipotent progenitors with a defined immunophenotype, having the capacity for self-renewal and differentiation into various lineages of mesenchymal tissues[1]. Based on this definition, and in order to find an alternative to the invasive bone marrow (BM) isolation procedure, fibroblasts from various adult tissues such as adipose tissue, muscle, heart and liver[2,3], or fetal tissues like umbilical cord (UC) blood[4], or umbilical cord matrix[5,6] were analyzed for stemness. Scientists focused their interest on the available tissues, easy to isolate and without serious ethical considerations. According to these criteria, many protocols which show MSC isolation from

the umbilical cord matrix were proposed. Most of the studies were based on the published minimal criteria defining multipotent mesenchymal stromal cells. First, the cells must be plastic adherent, with an important proliferation potential. Second, these proliferating fibroblasts must express CD105, CD73 and CD90, and lack the expression of CD45, CD34 and CD14. Third, they should be able to differentiate *in vitro* under specific conditions, at least in osteocytes, adipocytes and chondrocytes[1]. Fibroblasts isolated from UC (UC-MSC) presented the same aspect as MSC from BM (BM-MSC), but with a significantly higher proliferative potential[6]. However, with the apparition of more MSC exclusive immunophenotype, some differences between UC-MSC and BM-MSC were highlighted. Furthermore, different studies presented contradictory results about the capacity of UC-MSC to differentiate into defined cell lineage. Here, we underlined the most striking immunophenotype and gene expression differences between UC-MSC and BM-MSC. We also discussed the contradictory data concerning the differentiation potential of UC-MSC, in an attempt to clarify whether these cells have different stemness potential in comparison with standard BM-MSC.

COMPARATIVE IMMUNOPHENOTYPE AND GENE EXPRESSION OF UC-MSC AND BM-MSC

Until recently, immunophenotyping of mesenchymal stem cells was essentially concentrated on the determination of the expression of CD90, CD73, CD105, CD13, CD44 and the absence of CD14 and CD34[1]. However, it is now admitted that these markers are not exclusive for MSCs. Indeed, foreskin fibroblasts also show this phenotype without being ranked as MSC[7]. Using more specific markers, UC-MSCs were distinguished from MSC of other tissues. We demonstrated that UC-MSCs were totally negative for SSEA-4 and LNGFR antigens, whereas BM-MSC presented an important fraction of positive cells for these markers[6]. SSEA-4 is an early embryonic glycolipid antigen, commonly used as a marker for undifferentiated pluripotent human embryonic stem cells. On the other hand, LNGFR (CD271) was found to be involved in the development, survival and differentiation of neural cells. These two markers have been proposed to identify the adult mesenchymal stem cell population[8,9]. Other differences based on the expression of CD56 and CD146 were described between UC-MSC and BM-MSC. Indeed, immunophenotyping analysis has distinguished UC-MSC ($CD56^+$, $CD146^{++}$) from BM-MSC ($CD56^-$, $CD146^{+++}$)[7].

Proteomic is an excellent tool to study and compare expressed protein profile of MSCs. 2D gel analysis revealed that BM-MSCs highly express proteins involved in cell migration (CTSB, CTSD and PHB), which correlates with their important migration potential[10]. These migration-enhancing proteins were minimally expressed in UC-MSC, which expressed migration inhibitory proteins (PAI-1 and MnSOD).

Other studies reported further differences in UC-MSC in contrast to BM-MSC and cord blood MSCs. Indeed, UC-MSC exhibited a different expression profile for *HOX*-gene; a transcription factor implicated in embryologic development[7].

As far as we know, no study has identified MSC with a single marker in humans. In mice, Méndez-Ferrer *et al*[11], identified a population of Nestin expressing population ($Nestin^+$ MSC). In a Nestin-GFP transgenic mouse, they identified the entire mesenchymal stem cell activity of bone marrow $CD45^-$ cells within the $Nestin^+$ population. These results suppose that Nestin could be a potential single marker able to define murine MSCs. It would be interesting to check for Nestin expression in human BM-MSC and UC-MSC.

We also compared the cytokine expression profile in BM-MSC and UC-MSC. BM-MSC expressed leptin that was enhanced in the presence of glucocorticoids, wherase UC-MSCs were not able to express this adipokin[12]. We found a high constitutive pSmad2 expression in UC-MSC, while it was low and modulated in BM-MSC. Indeed, the investigation of leptin expression mechanism showed pSmad-2 as an inhibitory factor[12].

Taking into account the above listed studies, it appears that UC-MSC constitute a cell population that can be distinguished from BM-MSC in term of immunophenotype and the expression of some genes implicated in development, differentiation and migration.

The main immunophenotype differences between UC-MSC and BM-MSC are summarized in Table 1.

UC-MSCS DIFFERENTIATION POTENTIAL

Despite *in vivo* transplantation assays are the most suitable to assess the MSC differentiation potential, *in vitro* differentiation assays were performed in most of the studies. Conventional staining for adipogenic, osteogenic and chondrogenic differentiation (Oil red O, Alkaline phosphatase, Von Kossa, *etc*.) were used to establish UC-MSC differentiation potential. Compared to cells derived from other tissues, UC-MSCs were reported to fail in osteogenic differentiation[7,13]. We have also published a lack of *in vitro* osteogenic differentiation potential of UC-MSC, demonstrated by the absence of alkaline phosphatase staining, and runx-2 expression, even when cells were cultured in the presence of osteogenic mixture for more than 4 wk[12]. We demonstrated that this osteogenic inability of UC-MSC was due to their incapacity to express leptin[12]. In fact, leptin was admitted to be implicated in osteogenic differentiation[14].

Furthermore, BSP, a marker for osteoblastic differentiation was shown to be highly expressed in cell-lines with high osteogenic capacity, while non-osteogenic cell line did not. Human UC-MSC did not express BSP, which can account for their inability to differentiate into osteoblasts[7].

UC-MSCs were also shown to differentiate into adipocytes in a very limited manner[13,15]. Adipogenic potential

Table 1 Immunophenotype and differentiation potential comparison between umbilical cord matrix derived fibroblasts and bone-marrow-mesenchymal stem cells

		Immunophenotype	Gene expression	Differentiation potential
BM-MSC	Positive for:	CD90, CD73, CD105, CD13, CD44[1] SSEA-4, LNGFR[6], CD146^{+++}[7]	*CTSB*, *CTSD*, *PHB*[10], *BSP*[7], *Leptin*[12] *HOX*-gene[7], *DLK-1*[16], *pSmad2* (low and modulated)[12]	No report for osteo-, adipo or chondrogenic differentiation failure
	Negative for:	CD14, CD34[1], CD56[7]		
UC-MSC	Positive for:	CD90, CD73, CD105, CD13, CD44[1] CD56, CD146^{++}[7]	*PAI-1*, *MnSOD*[10], *HOX*-gene (different expression pattern)[7] *pSmad2* (high and constitutive)[12]	Reports for adipo-[13,15] and osteogenic[7,12] differentiation failure
	Negative for:	CD14, CD34[1], SSEA-4, LNGFR[6]	*CTSB*, *CTSD*, *PHB*[10], *BSP*[7], *Leptin*[12], *DLK-1* (or weakly)[16]	

UC-MSC: Umbilical cord-mesenchymal stem cells; BM-MSC: Bone marrow-mesenchymal stem cell.

was inversely correlated with DLK-1 expression in mesenchymal stem cells isolated from cord blood-MSC (CB-MSC). UC-MSCs do not or weakly express DLK-1; which can explain their failure to differentiate into adipocytes[16].

Bosch *et al*[7], went further by wondering if UC-MSC are true "mesenchymal stromal stem cells". In fact, UC-MSC isolated by this group failed to differentiate into adipo-, osteo- and also into chondrocytes. Indeed, UC-MSC did not express Sox9 factor after 21 d incubation in an *in vitro* pellet culture system.

The above-summarized studies clearly presented results that demonstrated failure of UC-MSC to differentiate into osteo-, adipo-, and chondrocytes, at least in these conditions. The majority of these studies also provided explanations for the described UC-MSC differentiation inability. However, one should not forget that many other studies have concluded to successful UC-MSC differentiation.

DISCUSSION

It seems confusing to see the above contradictory data about the immunophenotype and the differentiation potential of UC-MSC! So what could explain these contradictions? It could be the contamination of umbilical fibroblasts by endothelial cells of the vein and arteries embedded in jelly connective tissue. These cells could proliferate over UC-MSCs and give contradictory results, especially in terms of differentiation. To address this question, we have looked to UC-MSC isolation procedure used by different laboratories and found that even in the studies that concluded to different immunophenotype and differentiation potential between UC-MSC and BM-MSC, the removal of veins and arteries before cord matrix processing was carefully performed[7,12].

Umbilical cord blood was also proposed as a source of MSC (CB-MSC). It could also be possible that cord matrix handling method may have caused the transfer of circulating MSC from the cord blood compartment in the endothelial/subendothelial layer of the umbilical cord matrix. So the studies that concluded by identical characteristics of BM-MSC and UC-MSC may have in reality characterized umbilical CB-MSC. In fact, despite CB-MSCs are difficult to isolate, studies are unanimous about resemblance of these cells with BM-MSC in term of phenotype and differentiation potential. In addition, the use of different media and culture conditions may also have an effect on the heterogeneity of UC-MSC population isolated in different laboratories; contributing to the selection and expansion of specific cell populations, which may have not the same behavior under specific culture conditions. In this context, a strategy based on counterflow centrifugal elutriation was used to identify different subpopulations in cultured UC-MSC[17]. The authors revealed that UC-MSC cultures were composed of different sized populations. The smallest cells exhibited the highest proliferative capacity, with a reduced amount of aging cells, compared with larger diameter cells. This study is a clear proof of UC-MSC heterogeneity. It is obvious that the MSC-like characteristics fit with the small sized subpopulation, however this might be proven. Performing comparative analysis on sorted small and large diameter UC-MSCs for differentiation potential could give key information that may explain the above listed contradictions.

Another technique used to detect heterogeneity among MSCs is the single cell transcriptional profiling, where more than 48 genes could be analyzed. This study was performed on adipose-derived stromal cells (ASC). Based on transcriptional profiles, ASCs were grouped into different clusters. Statistical analysis was used to find out correlations between the expression of specific markers and an increased differentiation gene expression. One striking finding was that low expressing endoglin (CD105) ASC subpopulation showed an increased osteogenic differentiation potential[18]. Such technique would be of great interest to study UC-MSCs heterogeneity.

It clearly appears that UC-MSC is a heterogeneous population, composed of distinct subpopulations, with distinct characteristics that may account for the contradictory results presented by different laboratories. However, a question remains unanswered: Is it necessary for all mesenchymal cells to have the same differentiation characteristics as BM-MSC to be defined as stem cells, or is there different MSCs with different development stages that make them different in their behavior under specific conditions?

Finally, unless a clear explanation of the contradictions in the literature about the immunophenotype and the differentiation potential of UC-MSC is proposed, cord matrix could not be considered as a reliable source for MSC for the moment. Furthermore, the above-cited studies raise the necessity to update the criteria that de-

fine multipotent stromal cells.

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P- Reviewers: Blanco LP, Kan L **S- Editor**: Wen LL
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