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CD271 as a marker to identify mesenchymal stem cells from diverse sources before culture

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Abstract

Mesenchymal stem cells, due to their characteristics are ideal candidates for cellular therapy. Currently, in culture these cells are defined by their adherence to plastic, specific surface antigen expression and multipotent differentiation potential. However, the *in vivo* identification of mesenchymal stem cells, before culture, is not so well established. Pre-culture identification markers would ensure higher purity than that obtained with selection based on adherence to plastic. Up until now, CD271 has been described as the most specific marker for the characterization and purification of human bone marrow mesenchymal stem cells. This marker has been shown to be specifically expressed by these cells. Thus, CD271 has been proposed as a versatile marker to selectively isolated and expand multipotent mesenchymal stem cells with both immunosuppressive and lymphohematopoietic engraftment-promoting properties. This review focuses on this marker, specifically on identification of mesenchymal stem cells from different tissues. Literature revision suggests that CD271 should not be defined as a universal marker to identify mesenchymal stem cells before culture from different sources. In the case of bone marrow or adipose tissue, CD271 could be considered a quite suitable marker; however this marker seems to be inadequate for the isolation of mesenchymal stem cells from other tissues such as umbilical cord blood or wharton's jelly among others.

Key words: Mesenchymal stem cells; CD271; Low-affinity nerve growth factor receptor; p75; Bone marrow

Core tip: CD271 as a marker to identify mesenchymal stem cells from diverse sources before culture.

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INTRODUCTION

In recent decades researchers have focused on regenerative medicine as a interesting option

for the treatment of different pathologies[1,2]. Mesenchymal stem cells (MSC) are ideal candidates for cellular therapy due to their characteristics[3]. MSC are viewed as clinically promising because of their differentiation capacity and pro-regenerative features[4,5]. Moreover, MSC could be used for both autologous and heterologous transplant due to their low immunogenicity[6]. Friedenstein *et al*[7] discovered bone marrow (BM)-MSC. They demonstrated that BM contains a population of cells with a high proliferative capacity that adhere to plastic. These authors were also the first to propose the capacity of these cells to form colonies from a single cell (the fibroblast-colony forming unit F-CFU)[7-9]. Since Friedenstein *et al*[7] described MSC in the BM in the 70's several researchers have focused their attention on this type of adult stem cell. After their initial isolation from humans, MSC have since been successfully harvested from many other species including: mouse, rat, dog and horse[10]. They have also been isolated from almost every type of tissue, including: BM, adipose tissue, liver, skeletal muscle, amniotic fluid, umbilical cord blood (UCB) or dental pulp[11-14]. Due to the heterogeneity of the results obtained by many groups, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. They proposed three criteria to define MSC in culture: adherence to plastic, specific surface antigen expression and multipotent differentiation potential[15]. All these criteria perfectly define MSC in culture, however the *in vivo* identification of MSC, before culture, is not as well established. In 1999, Pittenger *et al*[16] described a small percentage of MSC present in BM (0.001%-0.01%) so this could explain the difficulty in establishing the exact phenotype of MSC before culture[16].

A suitable method of selection would allow the employment of MSC in different pathologies directly after their isolation or after their *in vitro* expansion. Pre-culture identification markers would ensure higher purity than that obtained with selection based on adherence to plastic. Many investigators direct their efforts towards find a marker or a combination of markers to ensure their selection.

Up until now, CD271 (LNGFR) has been described as one of the most specific markers for the purification of human BM-MSCs[17,18]. CD271, also known as low-affinity nerve growth factor receptor (LNGFR), nerve growth factor receptor (NGFR), or p75NTR (neurotrophin receptor), belongs to the tumor necrosis factor superfamily[19]. Taking into account that this marker has been the most commonly used in the isolation of BM-MSC this review will focus on to the identification of MSC from different tissues (Table 1).

BM

Several independent studies have confirmed the specificity of CD271 on MSC isolated from BM. At the beginning of the last decade, Quirici *et al*[18], (2002) published that the anti-CD271 antibody is specific for a population of multipotent BM cells and suggested the use of this marker as an option for the selection of MSC from BM[18]. The same year, Jones *et al*[20], conducted a study in which they carried out a cellular purification from BM with antibody-conjugated magnetic beads. They found that the D7-FIB-positive fraction contained not only all the CFU-F activity but also a unique population of $CD45^{low}/LNGFR^{+}$/ cells. These cells were adherent, proliferative, and multipotent following cell sorting and standard expansion[20]. In 2006 the same group, suggested the need to find a way to count MSC based on the use of surface markers considering that up to this point researchers were using CFU-F assays for the enumeration of MSC. Published results demonstrate the use of flow cytometry as a rapid MSC detection method and suggested *in vivo* selection of the phenotype $CD45^{low}/LNGFR^{+}/D7FIB^{+}$[21]. Following the studies started in 2002, Jones's group continued providing more data about this marker. In a comparative study, Jones *et al*[22] demonstrated that CD271 antigen (followed by CD146, CD106, D7-FIB, CD13 and CD166) remained one of the most selective markers for enriching progenitor cells from MSC of human BM. These results are supported by Kuçi *et al*[23,24], who published a study which demonstrated that the CD271 is an adequate marker for the selection of multipotent BM cells with immunosuppresive properties. Afterwards, the same group published another study in which they demonstrated that CFU-F activity was found only in the $CD271^{+}$ cell fraction, whereas no CFU-F was observed in the $CD271^{-}$ population[24]. Flores-Torales *et al*[25], (2010) proposed the use of a single marker, CD271, for the selection of MSC from BM before culture. These authors maintain that the use of this marker would reduce costs and provide a rapid and simple way of obtaining MSC. However, a high percentage of $CD271^{+}$ cells in BM and synovium co-express CD34, which disqualifies CD271 as a unique marker for the isolation of MSC. Nevertheless, these studies, among others, have confirmed the usefulness of CD271 in combination with other markers such as CD45 to isolate fresh BM-MSC. Poloni *et al*[26], (2009) carried out a selection of CD271 positive cells and cultivated them in a media supplemented with 10% allogeneic human sera, cells maintained the capacity to differentiate and no karyoptypic variations were observed. Our group utilized this marker ($CD271^{+}/CD45^{-}$) to quantify the MSC population in BM samples obtained for cell therapy using flow cytometry[27]. Recently Mabuchi *et al*[28] performed a comprehensive screening of putative surface markers to select the most useful ones for prospectively identifying a pure MSC population in human BM. They concluded that the combination marker $CD271^{+}CD90^{+}CD106^{+}$ can

Table 1 Summary of references documenting CD271 expression in different sources

Source	CD271	Ref.
Bone marrow	Positive	Alvarez-Viejo *et al*[27], 2013 Flores-Torales *et al*[25], 2010 Jones *et al*[20], 2002; Jones *et al*[21], 2006; Jones *et al*[22], 2008 Kuçi *et al*[23], 2011; Kuçi *et al*[24], 2010 Poloni *et al*[26], 2009 Quirici *et al*[18], 2002
Adipose tissue	Positive	Cuevas-Diaz Duran *et al*[31], 2013 Quirici *et al*[33], 2010
Umbilical cord blood	Negative	Attar *et al*[46], 2013 Alvarez-Viejo *et al*[44], 2013 Watson *et al*[47], 2013
Wharton's Jelly	Low/negative	Alvarez-Viejo *et al*[44], 2013 Margossian *et al*[35], 2012
Placenta	Positive/negative	Battula *et al*[50], 2008 Soncini *et al*[52], 2007
Trabecular bone cavity	Positive	Jones *et al*[56], 2010
Dermis	Positive	Vaculik *et al*[57], 2012
Peripheral blood	Negative	Attar *et al*[55], 2011

be used selectively to isolate the most potent and genetically stable MSC[28]. Therefore, CD271 may be considered a suitable marker for determining MSC in BM.

It has been demonstrated that as the age of donors increases the number and potential for differentiation of BM MSC diminish[29]. Taking into consideration that it is not easy to obtain such blood donors other sources for the obtention of MSC are required.

Adipose tissue is an interesting option for this end. MSCs can be isolated from fat tissue easily obtained by liposuction. It has been demonstrated that these cells are easily cultivated and have the capacity to differentiate into various cell lines[30].

ADIPOSE TISSUE

In 2002 a type of stem cell from adipose tissue was isolated for the first time: adipose-derived stem cell (ADSC)[31]. Since then, it has been demonstrated that adipose tissue provides an abundant source of MSC with similar yields to those obtained from BM. Furthermore, ADSC have a similar differentiation capability, morphology, and phenotype to MSC collected from BM[31]. It has been shown in mice that p75NRT seems to be a useful marker for ADSC isolation[32]. After this, Quirici *et al*[33] selected $CD271^+$ cells immune-magnetically from ADCS in humans in a similar way as has been done from BM by others authors. $CD271^+$ cells showed higher clonogenic and differentiation potential compared to plastic adherent ADSC. Thus these authors demonstrated the utility of this marker in the selection of ADSC cells[33]. Cuevas-Diaz Duran *et al*[31] used this marker to analyze if there was a relationship between donor´s age and $CD271^+$ cell yield in freshly isolated ADSC. They suggested that the proportion of $CD271^+$ ADSC decreased with age; however, positive cells were present in all age groups and their frequency was higher than what has been found in BM. Therefore, $CD271^+$ cells from adipose tissue were proposed as the primary choice for tissue regeneration and autologous stem cell therapies in older subjects[31].

The umbilical cord, placenta and placental membrane have been presented as cheap and attractive alternatives for the obtention of MSC[34].

UMBILICAL CORD

The UC is the structure that connects the foetus to the placenta. The UC has two arteries and a vein wrapped in a gelatinous connective tissue called Wharton's jelly (WJ). The UC has characteristics ideal for procuring of MSC, because its use would not generate ethical conflicts, it is easily obtained and it is an abundant resource which is discarded[35]. As described below, MSCs have been described from UCB and from WJ.

UCB

UCB was found to contain different populations of stem cells, a unique feature not shared with peripheral blood. Some authors propose that UCB derived MSC show high morphological and molecular similarities to BM derived MSC including the lack of hematopoietic surface antigens[36]. The presence or absence of MSC in UCB has been quite controversial. A few researchers have denied the presence of MSC in UBC such as Yu *et al*[37], (2004) whose results suggest that early fetal blood is rich in MSC however, the term UCB is not used. On the other hand, some authors propose the difficulty in obtaining MSC from UCB to be due to their low frequency, inferior to that in BM. Up until now, the literature does not

show interesting results regarding the obtention, isolation and purification of UCB MSCs. Some publications show that the MSC can be obtained in a low percentage of UCB units (20%-63%)[38,39]. These data bring into question the possibility of considering the UCB as a resource for acquiring MSC[40]. However, several authors have differentiated UCB-MSC *in vitro* to osteogenic, chondrogenic, neural and hepatic lineages successfully[41-43]. Our group has not been able to identify MSC from UCB using conventional methods of adherence to plastic. We observed a heterogeneous population of adherent cells, with a rounded and spindle-shaped appearance[44]. Our results are in concordance with Perdikogianni *et al*[45], (2008). We also used CD271 for the presence of cells from UCB expressing this marker. Our results showed no validity for this marker in this tissue and these data are in agreement with two studies published in parallel. Attar and coworkers showed that CD271 did not contribute to isolation of MSC from UCB[46]. Watson *et al*[47], (2013) published that, CD271 is an efficient marker for MSC isolation from BM but failed to isolate MSC from UCB. Therefore, our experience and results from the literature suggest that CD271 is not a suitable marker for the identification of MSC from UCB without culture.

WJ

Thomas Wharton was the first to describe WJ in 1656. WJ is a gelatinous substance composed of various isoforms of collagen and proteoglycans. The principal function of WJ is to protect the arteries and veins from the compression and torsion that they can be subjected to. These provide a bidirectional flow, providing oxygen and nutrients that contribute to the adequate development of the foetus and eliminating waste and carbon dioxide[48]. It has been demonstrated that human WJ-MSC present an elevated capacity for autoregeneration and have been compared with those obtained from BM[39]. MSC derived from the discarded UC, more precisely WJ, offer a low-cost and pain-free collection method of MSC that may be cryogenically stored, and are considered extremely favorable for tissue engineering purposes[35]. Data published by our group highlight the ineffectiveness of CD271 as a marker for the isolation of MSC from WJ before culture[44]. These results are supported by data published by Margossian *et al*[35]. They studied the expression of CD271 *in situ* on fresh fragments of WJ and they observed it to be weakly expressed[35]. Although few studies have focused on determining the effectiveness of this marker in WJ, those that have been carried out suggest that CD271 is not a suitable marker to identify MSC from WJ before culture.

PLACENTA

Human placenta, plays a fundamental and essential role in fetal development, nutrition, and tolerance[49]. Various reports have demonstrated that human term placenta is a plentiful source of MSC[50,51]. Considering the complexity of the structure of the placenta, Parolini *et al*[49] published a paper in order to define, as clearly as possible, the region of origin and methods of isolation of cells derived from this tissue. This work arose out the first international Workshop on Placenta Derived Stem Cells, (March 2007). One of the main characteristics is the existence of four regions in the placenta: Amniotic epithelial, amniotic mesenchymal, chorionic mesenchymal and chorionic throphoblastic. From these regions, the following cell populations are isolated: amniotic epithelial cells, amniotic mesenchymal stromal cells, chorionic mesenchymal stromal cells, chorionic trophoblastic stromal cells[49].

In 2007, Soncini *et al*[52], achieved the isolation of amnion mesenchymal cells (AMSC) and chorion mesenchymal cells (CMSC). These MSCs were isolated by a mechanical separation followed by enzymatic digestion. AMSC and CMSC show MSC characteristics, such as adherence to plastic, fibroblastic morphology and the capacity to form colonies. Both types of cells when analysed by flow cytometry show phenotypes similar to BM-MSC. Also, AMSC and CMSC demonstrate high plasticity when cultivated in adequate differentiating media, showing that these can differentiate into fat, bone and cartilage.

In order to isolate cells with MSC characteristics from human fetal membranes, AMSC and CMSC expressing CD271 were enriched by immunomagnetic isolation. $CD271^+$ cells were demonstrated to possess higher clonogenic and osteogenic differentiation potentials than CD271-depleted fractions. Based on these findings, these authors suggest that amnion and chorion can be considered as a novel and convenient sources of adult MSC[52]. Another study published in 2007 by Battula *et al*[50] confirmed the possibility of obtaining MSC from chorion; however, their results showed that CD271 is not an adequate marker for the identification of MSC. They demonstrated that CD271 is expressed only at negligible levels on naive placenta MSC. Nevertheless, they identified FZD9 (frizzled-9) as a novel marker for isolation of MSC from chorionic placenta and showed that cells with CFU-F capacity reside exclusively in the $FZD9^+$ population[50]. FZD proteins comprise a family (FZD1-10) of seven transmembrane-spanning receptors[53]. Based on the literature, it is still not possible to confirm CD271 as a suitable marker for the isolation of MSC from placenta.

Because of their attractiveness, researchers have attempted to isolate MSC from many tissues and their existence has been documented in several of them. Here, we discuss some studies which have referred to the use of CD271 for the identification of MSC from tissues other than those discussed above.

OTHER SOURCES

Chong *et al*[54] in 2012 demonstrated that MSC from peripheral blood maintain similar characteristics and have similar chondrogenic differentiation potential to those derived from BM. Based on the literature, we have found just one reference which documents that CD271 positive selection can not help isolation of MSC from granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood[55]. Another source is published by Jones *et al*[56], (2010). They showed that $CD45^{low}CD271^{+}$ MSC are abundant in the trabecular bone cavity and indistinguishable from aspirated $CD45^{low}CD271^{+}$ MSC. Moreover, MSC are found in human dermis. Vaculik *et al*[57] distinguished dermal MSC from differentiated fibroblasts. For this they selected $CD271^{+}$ and SSEA-4^{+} cells from adherent dermal cells and checked their differentiation capacity. They observed that a $CD271^{+}$ dermal population presented a greater potential for adipogenic, osteogenic and chondrogenic differentiation.

Besides CD271, several markers have been used in order to identify MSC before culture. Possibly one of the most commonly used markers for this purpose is Stro-1. After a complete review, Lv *et al*[58], concluded that Stro-1 expression appears not be a universal marker for MSC from different tissues. Another marker used to identify MSC is SSEA-4. It is an embryonic stem cell marker and it was documented in the isolation of genuine MSC from BM[59]. Conversely, other authors reported no detection of SSEA-4 expressing cells in unsorted BM[60,61].

Taken together, the data discussed in this review suggest that CD271 would not be considered as a universal marker to identify MSC before culture. In the case of BM or adipose tissue, CD271 could be considered a quite suitable marker for the isolation of MSC. As described in this review, several independent studies confirm the specificity of this marker in different tissues. However, CD271 is not adequate in the isolation of MSC from other tissues such as UC or UCB. Moreover, in the case of placenta contradictory results have been obtained by different groups. These contradictory results could be due to variations in the methodologies used by different laboratories. Hines *et al*[62] published an interesting study in which they demonstrated that membrane markers are notoriously dynamic and their expression can often be dependent on minor technical issues. Technical issues, indicating that the reproduction of results is the corner-stone of science[62].

Due to the interest in MSC for their potential applications in the clinic, it is necessary to continue research in this field, in order to find a marker or markers for optimal selection and identification of MSCs without culture. This would allow the generation of purer cultures than those obtained by adherence to plastic alone and possibly direct application avoiding cultivation costs, time and risk of contamination.

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