STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Review: The Surface Markers and Identity of Human Mesenchymal Stem Cells

FENG-JUAN LV,[a,b,c] ROCKY S. TUAN,[d] KENNETH M.C. CHEUNG,[a,b,c] VICTOR Y.L. LEUNG[a,b,c]

Key Words. Mesenchymal stem cells • Surface epitopes • CD271 • CD146 • Markers • Pericytes • Niche • Regenerative medicine

[a]Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, People's Republic of China; [b]Stem Cell & Regenerative Medicine Consortium and [c]Center for Reproduction, Development and Growth, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, People's Republic of China; [d]Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennyslvania, USA

Correspondence: Victor Y.L. Leung, Ph.D., 9/F, Lab Block, Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Hong Kong SAR, People's Republic of China. Telephone: +852-2819-9589; Fax: +852-2818-5210; e-mail: vicleung@h-ku.hk or Kenneth M.C. Cheung, M.D., Department of Orthopaedics and Traumatology, The University of Hong Kong Medical Centre, Queen Mary Hospital, Pokfulam Road, Pokfulam, Hong Kong SAR, People's Republic of China. Telephone: +852-2255-4254; Fax: +852-2817-4392; e-mail: cheungmc@hku.hk

Received September 16, 2013; accepted for publication February 9, 2014; first published online in STEM CELLS *EXPRESS* February 28, 2014.

1066-5099/2014/$30.00/0

http://dx.doi.org/10.1002/stem.1681

ABSTRACT

The concept of mesenchymal stem cells (MSCs) is becoming increasingly obscure due to the recent findings of heterogeneous populations with different levels of stemness within MSCs isolated by traditional plastic adherence. MSCs were originally identified in bone marrow and later detected in many other tissues. Currently, no cloning based on single surface marker is capable of isolating cells that satisfy the minimal criteria of MSCs from various tissue environments. Markers that associate with the stemness of MSCs await to be elucidated. A number of candidate MSC surface markers or markers possibly related to their stemness have been brought forward so far, including Stro-1, SSEA-4, CD271, and CD146, yet there is a large difference in their expression in various sources of MSCs. The exact identity of MSCs in vivo is not yet clear, although reports have suggested they may have a fibroblastic or pericytic origin. In this review, we revisit the reported expression of surface molecules in MSCs from various sources, aiming to assess their potential as MSC markers and define the critical panel for future investigation. We also discuss the relationship of MSCs to fibroblasts and pericytes in an attempt to shed light on their identity in vivo. STEM CELLS *2014;32:1408–1419*

INTRODUCTION

Mesenchymal stem cells (MSCs) were first identified from bone marrow mononuclear cells (BM-MNCs) as fibroblastic colony-forming units (CFU-Fs). Due to their multipotency and paracrine effect [1, 2], MSCs are ideal candidates for regenerative medicine [3, 4]. Currently, there is no consensus on a single surface molecule to identify MSCs from various sources. The minimum criteria of MSCs [5] include: (a) remain plastic-adherent under standard culture conditions; (b) express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR; (c) differentiate into osteoblasts, adipocytes, and chondrocytes in vitro. Other surface antigens generally expressed by MSCs include CD13, CD29, CD44, and CD10 [6, 7]. Although bone marrow (BM) is the most widely recognized source of MSCs, recent research has identified alternative sources of MSC-like cells, including adipose tissue (AT) [8], placenta [9], dental pulp [10], synovial membrane [11], peripheral blood [12], periodontal ligament [13], endometrium [14], umbilical cord (UC) [15], and umbilical cord blood (UCB) [16, 17]. In fact, evidence has suggested that MSCs may be present virtually in any vascularized tissues throughout the whole body [18].

Genuine MSCs are expected to possess both clonogenicity and tripotency. However, only a fraction of CFU-Fs from plastic adherence isolated MSCs (PA-MSCs) exhibited multipotency [19], indicating that PA-MSCs comprised a heterogeneous population of cells with different lineage commitment [19], which may relate to their in vivo environment. This is reflected in the differences in the protein expression profile, cytokine profile, or differentiation potency of various sources of MSCs (reviewed in [20]). For example, the percentage of BM CFU-Fs with osteogenic potency was higher than that with adipogenic potency [21]. Similarly, ectopic transplantation of BM-MSCs resulted in heterotopic bone tissue formation, while dental pulp-derived MSCs generated reparative dentin-like tissue [22]. It is now widely accepted that in the MSCs population, only a proportion of cells satisfy the "MSCs" criteria at single cell level, while the other cells are more committed. There is also a more immature population in MSCs which are embryonic stem cell (ESC)-like and express Oct-4 and Sox2 [23].

So far, the markers proposed for MSCs fall into two categories: sole markers and stemness markers. A sole marker is an alternative MSC selection tool to plastic adherence, which alone is sufficient to identify or purify MSC-

like cells from their in vivo environment [5]. A "stemness" marker is able to identify a subset of MSCs with high CFU-Fs and trilineage potential or even identify ESC-like population. Ideally, such stemness marker may facilitate selection and therefore enrichment of subpopulation that exhibit superior CFU-Fs and multipotency. Based on the nature of the two different types of markers, the sole markers are normally highly expressed, while the stemness markers may be moderately detected.

The majority of MSC markers are identified for BM-MSCs. To date, however, whether these markers can be applied to other sources of MSCs is not very clear. Moreover, the exact in situ identity of MSCs is not entirely clear, although reports have suggested they may have a fibroblastic or pericytic origin. This review attempt to address these issues through a comprehensive analysis of the current findings on MSC isolated from various tissues via the use of single surface markers. Moreover, the capacity of stemness markers representing the subset of more primitive MSCs is revisited and the origin of MSCs is discussed.

Sole and Stemness Markers of MSCs

A number of molecules have been suggested as MSC markers, as shown in Table 1. Among them, Stro-1, CD271, stage-specific embryonic antigen-4 (SSEA-4), and CD146 are the ones that have received the most attention and adopted in studies as markers to sort MSCs. The expression of these four molecules in various sources of MSCs is listed in Table 2.

Stro-1

Stro-1 is one of the most well-known markers for MSCs. Stro-1 is a cell membrane single pass type I protein that translocates from the endoplasmic reticulum to the cell membrane in response to the depletion of intracellular calcium [69]. By combining negative selection against glycophorin-A, Stro-1 enriched CFU-Fs from BM with multipotency [24]. However, it did not enrich CFU-Fs from human endometrial stroma [14]. The degree of homogeneity of the Stro-1-selected MSCs was further enhanced 1000-fold by positive selection for CD106 compared to PA-MSCs [25]. Injection of human Stro-1(+) but not Stro-1(−) BM-MNCs into rat myocardium led to arteriogenesis and functional cardiac recovery [70]. Further in vivo research demonstrated that Stro-1(−) MSCs supported higher hematopoietic stem cells (HSC) engraftment in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice while no support was detected by Stro-1(+) MSCs. However, Stro-1(+) MSCs exhibited greater capability in homing to spleen, BM, and kidney [26]. Conditioned medium from Stro-1(+) MSCs could induce a greater degree of cardiac vascular repair than PA-MSCs [25]. These suggest that Stro-1 may be involved in clonogenicity and play a role in homing and angiogenesis of MSCs.

However, Stro-1 is not universally expressed in all reported types of MSCs. Stro-1 is expressed in dental- [10], synovial membrane- [11], decidua parietalis-derived MSCs [39] and multipotent dermal fibroblasts [28]. AT- [54], UCB- [57], UC- [16], peripheral blood-derived MSCs [59] are negative/low for Stro-1 expression. It is reported that placenta-derived MSCs gradually lose Stro-1 expression in culture [62]. In contrast, however, the expression of Stro-1 in BM-MSCs increases with culture time [36].

The potential of Stro-1 as an MSC marker is limited in several ways. It is unclear whether Stro-1 expression correlates with multipotency. Stro-1 is also unsuitable as a sole marker to separate MSCs from its harboring tissue, at least not from BM, as greater than 95% of Stro-1(+) cells in the human BM were glycophorin A expressing nucleated erythroid cells [24]. Moreover, Stro-1 expression appears not universal for various MSC types.

CD271

CD271 (also named as low-affinity nerve growth factor receptor) is a receptor for neurotrophins, which stimulate neuronal cells to survive and differentiate. CD271 has been used to select CFU-Fs from BM-MNCs. The percentage of CD90(+)CD105(+)CD45(−)CD34(−)CD79(−) cells in BM-MNCs coincided with the amount of CD271(+) cell subset (0.54%) [71]. CFU-Fs could only be generated from CD271(+) subsets of CD45(−)glycophorin-A(−) human BM-MNCs while the CD271(−) fraction showed no residual CFU-F activity [7, 29]. CD271(+) BM MSCs were shown to have enhanced capability in promoting HSC engraftment compared to PA-MSCs [72] and also induced superior chondral repair than the CD271(−) BM MSCs [73]. These together suggest a role of CD271 in maintaining clonogenicity and function of MSCs. However, the majority of the CD271(+) cells were found not to coexpress CD90 and CD73, the two general markers of MSCs. The percentage of CD90 and CD73 positive cells was found to be very low (<10%) in CD271(+) cells from BM [29] and from AT (10%–20%) [72]. Moreover, nearly 50%–99% of the CD271(+) cells in BM [29] and synovium [47] coexpressed CD34, which disqualifies CD271 as a sole marker to isolate MSCs from various tissues [5].

Similar to Stro-1, CD271 is not universally expressed in various MSCs. CD271 shows high levels of expression in BM and AT MSCs [6, 55] and is also expressed in periodontal ligament MSCs [13]. However, it is expressed at low levels in placenta-derived MSCs [31, 63] and not expressed in synovial membrane- [47, 65, 66], peripheral blood- [60], UC-, and UCB-MSCs [48, 49]. Although Watson et al. [58] reported detection of CD271 in UCB-MSCs, CD271 failed to enrich CFU-Fs and multipotency. The potency of CD271(+) cells as a stemness marker was further challenged by the finding of lower trilineage differentiation potential in CD105(+)/CD271(+) expanded BM-MNCs subsets compared to unsorted BM-MNCs [30]. Therefore, CD271 may not be considered as a MSC stemness marker.

SSEA-4

SSEA-4 is an embryonic stem cell marker. It has been documented to isolate genuine MSCs from BM [32]. SSEA-4(+) BM cells can expand extensively, while SSEA-4(−) subsets fail to grow. SSEA-4(+) cells also show tripotency [57]. SSEA-4 expression gradually increases in BM culture over time [57]. Besides in BM-MSCs, SSEA-4 expression was also detected in placenta- [64], periodontal ligament- [74], dental pulp- [61], and synovial membrane [65]-derived MSCs. On the contrary, AT-, UC-, or UCB-derived MSCs [48, 51] do not express SSEA-4. A more important question that whether the clonogenicity and multipotency of SSEA-4(+) cells is superior to the

Table 1. Potential MSC sole markers and their expression in unsorted MSC population

Markers	Marker potential	% positive	MSC source	References
Stro-1	Enrich CFU-Fs from whole BM	10	Human BM-MNCs	[24]
		11.2	Human BM-MNCs	[25]
		6	Human BM-MSCs after 1 week in culture	[26]
		2.1	Human endometrial stroma cells	[14]
		1.29	Human amnion MSCs at passage 0–2	[27]
		8	Human dermal fibroblasts at passage 3	[28]
		28.96	Inflamed periodontal ligament MSCs	[13]
		37.84	Healthy periodontal ligament MSCs	
CD271	Enrich CFU-Fs from CD45/α-glycophorin A depleted BM-MNCs	2.3	Human BM-MNCs	[7, 29]
	Higher differentiation-related gene expression after induction compared to MNC-derived MSCs			[30]
		Negligible	Human placenta MSCs	[31]
SSEA-4	Enrich CFU-Fs from whole BM	1–2	Mouse BM-MSCs at day 2 in culture	[32]
		71	Mouse BM-MSCs after 100 days in culture	
		2–4	Human BM cells	
		37.82	Human amnion MSC at passage 0–2	[27]
CD146	Enrich CFU-Fs from BM-MSCs	9.4	Human endometrial stroma	[14]
	Enrich cells with multipotency from BM-MSCs	1.5 CD146(+)PDGF-Rβ(+)	Human endometrial stroma	[33]
	Downregulated in differentiated cells	∼0.1	Human BM-MSCs	[34, 35]
		∼1.2	Human CD45 depleted BM-MNCs	[35]
		42.7	Human BM-MSCs	[17]
		17.2–37.9	Human UC-MSCs	
		11.55	Inflamed periodontal ligament MSCs	[13]
		21.85	Healthy periodontal ligament MSCs	
		9.4	Fresh isolated human endometrial stromal cells	[14]
CD49f	Enrich clonogenicity and differentiation potency	88.1	human BM-MSCs at passage 1	[36]
	Knocking down results in differentiation of HSCs	∼15	Human BM-MSCs	[37]
		∼55	Human UCB-MSCs	[37]
CD349	Enrich CFU-Fs from whole placenta cells	0.2	Total human plancenta cells	[31]
GD2	High specificity for isolating MSCs from BM	95	Human CD45(−)CD105(+)CD73(+) BM-MNCs	[38]
		∼65	Human BM-MSCs	[37]
		∼3	Human UCB-MSCs	[37]
3G5	Enrich CFU-Fs		Decidua parietalis	[39]
		63	Dental pulp CFU-Fs	[40]
		14	BM CFU-Fs	
SSEA-3	Enrich cells with clonogenicity and ectodermal, endodermal, and mesodermal differentiation potency	∼1	BM-MSCs	[23]
SUSD2	Enrich CFU-Fs and tri-potency	4.2	Human endometrial stromal cells	[41]
Stro-4	Enrich CFU-Fs	<5	Human and ovine BM-MSCs	[42]
MSCA-1	MSCA-1 positivity enrich CFU-Fs by 90-fold from BM-MNCs; MSCA-1 and CD56 double positivity enrich CFU-Fs by 180-fold from BM-MNCs; MSCA-1(+)CD56(−) selects for better adipogenesis in BM-MSCs; MSCA-1(+)CD56(+) selects for better chondrogenesis in BM-MSCs	0.5–5.5 coexpressed	$CD271^{bright}$ BM cells	[43]
CD200	Enrich CFU-Fs from BM-MNCs; downregulated in differentiated cells	0.15	Human BM-MNCs	[44]
PODXL	PODXL decreases in high-density cultures	∼90	Human BM-MSCs at passage 2	[36]
		∼13	Human BM-MSCs	[37]
		∼25	Human UCB-MSCs	[37]
Sox11	Downregulated during culture. Knockdown affects proliferation and osteogenesis potential	Not tested	Human BM-MSCs	[45]
TM4SF1	Enriched in MSCs compared with their source tissue or fibroblasts	Not specified	BM, AT, and UCB	[46]

AT, adipose tissue; BM, bone marrow. BM-MNCs: bone marrow mononuclear cells; CFU-Fs, fibroblastic colony-forming units; MSC, mesenchymal stem cell; UCB, umbilical cord blood.

Table 2. Detection of Stro-1, CD271, SSEA-4 and CD146 in various MSC sources

	Stro-1		CD271		SSEA-4		CD146	
Sources of MSCs	**Presence**	**References**	**Presence**	**References**	**Presence**	**References**	**Presence**	**References**
Bone marrow	+	[24]	+	[6, 7, 29, 47, 48]	+	[32, 43, 48]	+	[17, 19, 34, 35, 49, 50]
			−	[49]	−	[51–53]		
Adipose tissue	−	[54]	+	[55]	−	[51]	+	[56]
							−	[49]
Umbilical cord	−	[16]	−	[48]	−	[16, 48]	+	[17]
Umbilical cord blood	−	[57]	−	[48, 49]	−∼−/+	[48, 51]	+	[17, 49]
			+	[58]				
Peripheral blood	−	[59]	−	[60]				
Dental pulp	+	[10, 40]	−/low	[31]	+	[61]	+	[40]
Placenta	−	[62]	−/low	[63]	+	[64]	+	[63]
Synovial membrane	+	[11]	−	[47, 65, 66]	+	[66]	+	[47]
			+	[67]				
Periodontal ligament	+	[13]			+	[64]	+	[13]
Dermis	+	[28]	+	[68]	+	[68]	+	[68]
Endometrium	+	[14]					+	[14, 33]
Decidua parietalis	+	[39]					+	[39]

MSCs, mesenchymal stem cells; +, expression detected; −, expression not detected; −/low, expression detected but very low.

traditional PA-MSCs or MSCs sorted by other molecules is unanswered. Notably, the expression of SSEA-4 in UCB-HSCs was suggested to be an artificial induction in the in vitro culture as fetal calf serum (FCS) contained globoseries glycosphingolipids which can be recognized by a SSEA-4 antibody, and in vitro FCS exposure may induce SSEA-4 expression [75]. In fact, some other studies reported no detection of SSEA-4 expressing cells in unsorted BM [51, 52, 76]. These findings raise the issue of the physiological relevance and reliability of SSEA-4 as the marker for MSCs.

CD146

CD146 is a key cell adhesion protein in vascular endothelial cell activity and angiogenesis. Notably, CD146 emerged as an attractive candidate for identifying genuine MSCs. In human endometrial stroma population, CD146(+)PDGF-Rβ(+) cells show higher CFU-F enrichment compared with CD146(−) PDGF-Rβ(−) cells ($7.7 \pm 1.7\%$ vs. $0.7 \pm 0.2\%$) [33]. CD146 has a greater CFU-Fs enrichment capacity than CD90, Stro-1, or CD133 [14]. CD146 expression also defines MSCs with higher multipotency. Russell's group reported that the expression level of CD146 in the tripotent clones is twofold of that in the unipotent clones [19]. Additionally, CD146 also identifies MSCs with higher supporting capacity for hematopoiesis, as in vitro, CD146(+) MSCs show more than 100-fold increase in the long-term culture colony output by 8 weeks compared to unsorted BM-MNCs [34], and in vivo, when transplanted into mice, CD146(+) BM stroma subendothelial cells exhibit the capacity to reorganize the hematopoietic microenvironment to heterotopic sites [35].

Importantly, the expression of CD146 was found not only in BM-MSCs [50] but also in almost all the other sources of MSCs, including MSCs derived from AT [56], UC [15, 17], synovial membrane [47], UCB [49], placenta [63], dermis [68], periodontal ligament [13], and intervertebral disc [77]. In fact, CD146-expressing MSC clones from multiple organs were found to exhibit trilineage potency [18].

CD49f

CD49f (α6-integrin) regulates signaling pathways in a variety of cellular activities. Oct-4 and Sox-2 directly regulates the expression of CD49f, and that the knockdown of CD49f in ESCs results in differentiation into three germ layers, indicating CD49f is involved in the maintenance of pluripotency and is an ESC marker [78]. CD49f has also been identified as a specific HSC marker and shown to enrich cells capable of generating long-term multilineage grafts [79]. To date, CD49f expression has been detected in BM-MSCs [36], fetal urinary bladder-derived MSCs [80], and UCB-MSCs [37]. It is possible that the expression of CD49f may implicate the stemness of MSC culture. In fact, study has shown that CD49f is associated with high clonogenicity and multipotency in less confluent MSC culture [36]. Condition that induces MSC sphere formation can enrich CD49f(+) population compared with MSCs in monolayer [78]. Moreover, higher expression level of CD49f, such as in UCB-MSCs, is functionally linked with a higher lung clearance rate in systemic infusion [37]. Nonetheless, CD49f may not necessarily be of value as a single specific marker of MSCs since it is also widely expressed in epithelial cells as well as endothelial cells, monocytes, platelets, and thymocytes [79].

CD349

CD349 (frizzled-9) is a transmembrane-spanning receptor that is activated by Wnt ligands. It has been proposed to enrich CFU-Fs from placenta cells [31]. Additionally, CD349 expression has been reported in periodontal ligament-derived MSCs [74]. However, whether CD349 being essential to the enrichment of clonogenicity has been questioned by other reports. For example, while CFU-F could be enriched by 60-fold in the CD349(+)CD10(+)CD26(+) fraction, the CD349(+)CD10(−) CD26(−) subsets did not show CFU-F capacity, implying that CD349 alone is not sufficient for CFU-F enrichment. In fact, CD349(−) subset has been shown to proliferate at a higher rate than CD349(+) subset in periodontal ligament MSCs [74]. Moreover, CD349(−), rather than CD349(+) placenta MSCs, show a function in recovering blood flow following vascular occlusion [81]. These suggest CD349 might not be a critical MSC marker or essential in enriching MSC function.

GD2

GD2, the neural ganglioside, was found by Martinez et al. [38] as a single surface marker sufficient to isolate MSCs from BM

as GD2 is highly expressed in CD45(−)CD73(+) MSCs (>90%) but not in CD45(−) BM cells. It is also expressed in AT-MSCs and UC-MSCs [82], but not on foreskin fibroblasts. However, a portion of CD34(+) or CD19(+) BM cells also express GD2 [38], suggesting GD2 expression is not limited within BM-MSCs.

3G5

3G5 is a pericyte marker. Khan et al. [83] reported that plastic-adherence isolated BM-MSCs were negative for CD271, CD56, and Stro-1 but positive for 3G5. To date, 3G5 expression is detected on BM-MSCs, dental pulp- [40], and decidua parietalis-derived MSCs [39]. Shi and Gronthos [40] showed that a minor population of BM-MSCs positive for Stro-1 expression is also positive for 3G5. 3G5 positivity accounts for 14% of BM CFU-Fs and 63% of dental pulp CFU-Fs [40]. However, a large proportion (54%) of hematopoietic BM cells express 3G5, eliminating its potential as a sole marker to isolate MSCs from human bone marrow [40].

SSEA-3

Stage-specific embryonic antigen-3 (SSEA-3) is a pluripotent stem cell marker. Recently, evidence showed that a minor subset of SSEA-3(+)CD105(+) cells in MSCs, namely multilineage-differentiating stress enduring (MUSE) cells, are able to differentiate into ectodermal, endodermal, and mesodermal lineage cells in vivo [23]. Induced pluripotent stem cells (iPSCs) were only found to be derived from the MUSE cell subset in fibroblasts but not the non-MUSE subset [84], suggesting that SSEA-3 and CD105 expressing MSCs (≈1%) as progenitor cells reminiscent of, but not identical to, pluripotent-like ESCs. MUSE cells coexpressed some other pluripotency markers including Nanog, Oct3/4, PAR-4, Sox2 [23]. Since MSCs are strongly positive for CD105, MUSE cells can be represented as the SSEA-3(+) subset of MSCs. MUSE cells are not tumorigenic and can differentiate in vivo without prior genetic manipulation or growth receptor induction [23], hence they may have practical advantages for regenerative medicine.

SUSD2 (W5C5)

Type 1 integral membrane protein Sushi domain containing 2 (SUSD2) has been recently reported to enrich for CFU-Fs and tripotency from endometrium- [41], and BM-derived MSCs [85]. SUSD2 can be detected by the W5C5 antibody [85]. SUSD2 is not expressed in hematopoietic cells. In the endometrium, it is predominantly expressed in perivascular regions. W5C5(+) cells are also capable of producing endometrial stromal-like tissue in vivo [41]. Whether SUSD2 being a common MSC marker remains to be consolidated.

Others

There are also some other MSC sole or stemness markers proposed by some researchers which are well-investigated. Stro-4, MSCA-1, CD56, CD200, and PODXL have been proposed as MSC markers by their CFU-Fs enrichment capacity. The antibody Stro-4 identified the beta isoform of heat shock protein-90. It was found expressed in BM-, dental pulp-, periodontal ligament-, and AT-derived MSCs, and enriched CFU-Fs from both human and ovine BM by 16- and 8-fold compared to BM-MNCs [42]. MSCA-1 (mesenchymal stem cell antigen-1) is identical to tissue nonspecific alkaline phosphatase [52]. Compared to unsorted BM-MNCs, MSCA-1 selection resulted in a 90-fold increase in enrichment of CFU-Fs and a 180-fold increase when coselected for CD56 [43]. Another surface molecule CD200 was reported [44] to enrich CFU-Fs from BM-MNCs to 333-fold. PODXL, a sialomucin in the CD34 family, was also reported to decrease in high-density cultures which have lower clonogenicity and differentiation potency compared to less confluent cultures [36].

Unlike the above molecules, neuron-glial antigen 2 (NG2), Sox11, and TM4SF1 were proposed largely based on their expression. NG2 is first observed on the surface of neural progenitors and is a pericyte marker whose expression is also shared by BM-MSCs [18, 86, 87]. Sox11, a transcription factor previously identified in neural progenitor cells, was found to significantly decrease during MSC passages and knockdown of Sox11 with siRNA decreased the proliferation and osteogenic differentiation potential of MSCs [45]. TM4SF1 is another surface protein highly expressed in BM-, UCB-, and AT-MSCs which is not detected in mononuclear cells and fibroblasts, suggesting it may be a potential marker for MSC selection [46].

Comparison of MSCs Isolated by Different Markers

With the array of potential markers identified in MSCs, it is still unclear whether these markers define different or overlapping subpopulations of MSCs. One of the reasons is that the phenotypic or functional differences among the MSC subpopulations selected by different markers are still poorly understood. Here we aim to review studies that were designed to compare various MSC populations sorted in parallel and directly from single sources with respect to their coexpression of MSC markers, CFU enrichment capacity or differentiation potential.

CFU-Fs Enrichment Capacity

Delorme et al. [44] reported that, among nine molecules, CD73, CD130, CD146, CD200, and integrin $\alpha V/\beta 5$ were able to enrich CFU-Fs from CD235a(−)/CD45(−)/CD11b(−) BM-MNCs, while CD49b, CD90, and CD105 showed less enrichment. Among various methods of MSC isolation from BM-MNCs, including plastic adherence, RosetteSep-isolation, and CD105(+) and CD271(+) selection [30], CD271(+) fraction showed the highest number of CFU-Fs colonies. Double selection for CD56 or MSCA-1 enriched CFU-Fs to 3- or 2-fold, respectively, in CD271 (bright) BM-MNCs [43]. Schwab et al. [14] found that CD146 but not Stro-1 or CD133 selection enriched CFU-Fs from human endometrial stromal cells. Sorting of CD34(−)CD45(−) [88] or CD45(−) [76] human BM-MNCs with CD271 and CD146 revealed that CFU-Fs units remained exclusively in CD271(+) population regardless of CD146 expression, with a tendency toward more CFU-Fs in CD271(+)CD146(+) cells relative to CD271(+)CD146(−) cells. CD146(+) subsets accounted for 96% of CFU-Fs in unfractionated human dental pulp cells, while Stro-1(+) and 3G5(+) subsets accounted for around 80% and 60%, respectively [40]. In addition, the cloning efficiency of W5C5(+)CD146(+) cells was found significantly higher than CD140b(+)CD146(+) cells. $W5C5^{hi}$ cells had a high clonal capacity equivalent to

Table 3. Comparison of MSC sorting protocols for CFU-Fs enrichment

Cell subsets analyzed	Whole cell population	Result	References
Stro-1(+), CD133(+), CD90(+), CD146(+)	Human endometrial stromal cells	Only CD146 showed CFU-Fs enrichment	[14]
CD49b(+), CD90(+), CD105(+), CD73(+), CD130(+), CD146(+), CD200(+), $\alpha V/\beta 5$(+)	Human BM-MNCs	CD49b, CD105, and CD90 showed low CFU-Fs enrichment. CD73, CD130, CD146, CD200, and integrin $\alpha V/ß5$ showed higher CFU-Fs enrichment	[44]
MSCA-1(+), CD271(+), CD56(+)	Human BM-MNCs	CD271(+)CD56(+) fraction enriched CFU-Fs to threefold compared to CD271(+)CD56(−) fraction. MSCA-1(+)CD56(+) fraction gave rise to two fold higher CFU-Fs than MSCA-1(+)CD56(–) cells.	[43]
PA-MSCs, RosetteSep-, CD105(+) or CD271(+) sorted	Human BM-MNCs	CFU-Fs was most enriched in CD271(+) fraction.	[30]
CD271(+),CD146(+)	CD34(−)CD45(−) human BM-MNCs	CFU-Fs remained exclusively in CD271(+) population; CD271(+)CD146(+) cells had more CFU-Fs relative to CD271(+)CD146(−) cells	[87]
CD271(+),CD146(+)	Human BM-MNCs	CFU-Fs was not observed in CD271(-) cell fraction. CD146 positivity further enhanced CFU-Fs to 2.1 times in CD271(+)CD45(−) fraction.	[76]
Stro-1(+), CD146(+), 3G5(+)	Human dental pulp cells	No colony formation could be detected in STRO-1^{bright}/CD146(−) human bone marrow. CD146(+) subsets accounted for 96% of CFU-Fs in unfractured dental pulp cells, while Stro-1(+) and 3G5(+) subsets accounted for around 80% and 60%, respectively.	[40]

BM-MNCs, bone marrow mononuclear cells; CFU-Fs, fibroblastic colony-forming units; MSC, mesenchymal stem cell; PA-MSCs, plastic adherence isolated MSCs.

W5C5(+)CD146(+) cells [41]. Taken together, these findings imply that CD146 and CD271 positivity indicates superior CFU-F capacity in MSCs. These findings are summarized in Table 3.

Differentiation Potential

Battula et al. [43] reported that chondrocytes and pancreatic-like islets are predominantly induced from MSCA-1(+)CD56(+) BM-MNCs whereas adipocytes emerge exclusively from MSCA-1(+)CD56(−) subsets, indicating that CD56 is involved in differentiation tendency. Jarocha et al. [30] reported that CD271(+) or CD105(+) MSCs have lower lineage marker expression than PA-MSCs after osteogenic, chondrogenic, and adipogenic induction. Arufe et al. [67] reported that when comparing CD73, CD106, or CD271 positive human synovial membrane cells, CD271(+) cells are highly chondrogenic, whereas the CD73(+) cells are less chondrogenic and the CD106(+) cells mostly undifferentiated after induction. Vaculik et al. [68] reported that CD271(+) but not SSEA-4(+) dermal cells exhibit osteogenic and chondrogenic differentiation potential. Dermal SSEA-4(+) cells, in contrast, are only responsive to adipogenic induction. In adult human BM-MNCs, CD271(+)CD146(−/low) and CD271(+)CD146(+) subsets [76] show a similar capacity to differentiate and to support hematopoiesis, but the two subsets have been found at different sites; CD271(+)CD146(−/low) cells are bone-lining, while CD271(+)CD146(+) cells have a perivascular localization, suggesting that the two subsets play different roles in HSC niche. The function of surface markers in multipotency enrichment is summarized in Table 4.

Surface Marker Coexpression

Relevant studies on the degree of coexpression of surface markers on MSCs is summarized in Table 5. The expression of CD106 and CD146 was found to be restricted to the MSCA-1(+)CD56(−) MSCs and CD166 to MSCA-1(+)CD56(+/−) MSCs [43]. Vaculik et al. [68] reported that in human dermis, the expression pattern of SSEA-4 is almost analogous to CD271. Both were found only weakly expressed and coexpressed with CD45. Van Landuyt and Quirici also reported the detection of CD34 expression on CD271(+) subpopulation of human synovial and BM-MSCs [29, 47]. In human dermis, CD73 and CD105 are coexpressed [68]. A minor population of the human dermis CD73(+) cells is CD90(−). Dermis CD271(+) cells were CD73(+) and CD105(+), whereas the majority of CD271(+) cells are CD90(−) [68]. Similarly, in two other reports, only a minor subset of the CD271(+) cells express CD90, CD73 (<10% in cultured CD271(+) cells from BM [29], 10%–20% in freshly purified CD271(+) cells from adipose tissue [72]). Maijenburg further reported that the distribution of CD271(+)CD146(−) and CD271(+)CD146(+) subsets correlates with donor age. The main subset in pediatric and fetal BM was reported to be CD271(+)CD146(+), whereas CD271(+)CD146(−) population was dominant in adult marrow [88]. In endometrial MSCs, 28% of W5C5(+) cells are CD146(+), while 60% of W5C5(+) cells are Stro-1(+). A small population of W5C5(+) cells also express other

Table 4. Comparison of multipotency of sorted MSCs

Cell subsets analyzed	Whole cell population	Capacity compared	Result	References
CD73(+), CD106(+), CD271(+)	Human synovial membrane cells	Chondrogenesis	Chondrogenic potential: CD271 (+) > CD73(+) > CD106 (+)	[67]
MSCA-1(+), CD56(+)	Human BM-MNCs	Chondrogenesis, adipogenesis	MSCA-1(+)CD56(+): Chondrogenic and pancreatic differentiation potential, no adipogenic potential.	[43]
		Pancreatic differentiation	MSCA-1(+)CD56(−): Adipogenic potential, no chondrogenic and pancreatic differentiation potential.	
CD271(+), SSEA-4(+), CD73(+), CD90(+)	Human dermis MSCs	Chondrogenesis, adipogenesis, osteogenesis	CD271(+) cells had tri-lineage potential. Dermal SSEA-4(+) cells could only go for adipogenesis. CD73(+) cells showed a significantly higher adipogenic differentiation capacity than CD90(+) cells.	[68]
PA-MSCs, RosetteSep-, CD105(+) or CD271(+) sorted	Human BM-MNCs	Osteogenesis, chondrogenesis, adipogenesis	CD271(+) or CD105(+) MSCs showed lower differentiation related marker expression than PA-MSCs after osteogenic, chondrogenic and adipogenic induction.	[30]
CD271(+), CD146(+)	Human BM-MNCs	Osteogenesis, chondrogenesis, adipogenesis	CD271(+)CD146(−/low) and CD271(+)CD146(+) subsets showed a similar differentiation potential	[76]

BM-MNCs, bone marrow mononuclear cells; MSCs, mesenchymal stem cells; SSEA-4, surface-specific embryonic antigen.

Table 5. Comparison of coexpressed markers in sorted MSCs

Cell subsets analyzed	Whole cell population	Result	References
MSCA-1(+), CD271(+), CD56(+)	Human BM-MNCs	CD271 (+)CD56(−) cells expressed CD106 and CD146. CD271(+)CD56(+) cells exclusively expressed CD166. CD271(+)CD56(+) double positivity enriched SSEA-4 expression. CD271(+)CD56(+) double positivity enriched MSCA-1 expression.	[43]
CD271(+), SSEA-4(+)	Human dermis cells	Expression pattern of SSEA-4 in dermis was analogous to CD271. CD271 and SSEA-4 both coexpressed with CD45(+) cells. CD73 and CD105 were coexpressed. A minor population of the CD73(+) cells was CD90(−). CD271(+) cells were CD73(+) and CD105(+), whereas the majority of CD271(+) cells were CD90(−).	[68]
W5C5(+)	Human endometrial cells	W5C5(+) cells were 28% CD146(+), 60% Stro-1(+), 11.6% CD24(+), 5.3% CD31(+), 4.7% CD45(+).	[41]

BM-MNCs, bone marrow mononuclear cells; MSCs, mesenchymal stem cells; SSEA, surface specific embryonic antigen.

lineage markers, like CD24 (11.6%), CD31 (5 %), CD45 (4.7 %), and epithelial cell adhesion molecule [41].

MSC Identity In Vivo: Fibroblasts or Perivascular Cells?

Adult stem cells are found in specialized niches that store and maintain stem cells and mediate the balanced response of stem cells to the needs of organisms. The definition of MSCs has been based on their ability to self-renew and to differentiate into certain mature cell types in vitro. Their identity in vivo, however, remains unclear. Unlike the well-established niche of BM for HSCs [89], the true identity of MSCs and their niche in vivo is still under debate. Currently, it has been raised that MSCs may derive from fibroblasts or pericytes.

Fibroblasts are a type of cells synthesizing collagen, the major structural framework for animal tissues, and in the human body they are found in virtually every organ and tissue. MSCs have a close resemblance to fibroblasts [90]. Fibroblasts and MSCs are both plastic adherent and share similar cell morphology. Human dermal fibroblasts express many cell

Table 6. Physical expression of MSC markers in vivo

Markers	Expression site	References
CD73, CD90, and CD105	Dermis: Vascular and perivascular expression	[68]
	Skeletal muscle, placenta, and white adipose tissue: Perivascular expression	[18]
CD90	Endometrium: Expressed on all the stroma of the human endometrium, including the fibroblasts, perivascular and endothelial cells	[14]
Stro-1	BM: Expressed on blood vessel walls	[40]
	Dental pulp: Expressed on blood vessels and around perineurium surrounding nerve bundles	[40]
	Endometrium: On endothelial cells and on the stroma around blood vessels	[14]
	Placenta: Expressed around the vessels	[105]
NG2	Skeletal muscle, pancreas, placenta, white adipose tissue, fetal heart, fetal skin, lung, brain, eye, gut, bone marrow, and umbilical cord: Only expressed in periphery of capillaries and microvessels in almost all tissues	[18]
CD146	Skeletal muscle, pancreas, placenta, white adipose tissue, fetal heart, fetal skin, lung, brain, eye, gut, bone marrow, and umbilical cord: Expressed on perivascular cells surrounding capillaries, arterioles and venules, and on endothelium in capillaries, but not on microvessel endothelial cells	[18]
	BM and dental pulp: Blood vessel wall expression	[40]
	Endometrium: Expressed on perivascular and endothelial cells	[14]
	Placenta: expressed around the vessels	[105]
3G5	Placenta: expressed on scattered cells around the vessels.	[105]
CD271	Dermis: presented on cutaneous nerve fibers, Schwann cells, dermal single cells, and, faintly, on clusters of basal keratinocytes	[68]
	BM: CD271(+)CD146(−/low) cells were bone-lining, while CD271(+)CD146(+) had a perivascular localization	[76]
SSEA-4	Dermis: Presented on cutaneous nerve fibers, Schwann cells, dermal single cells, and, faintly, on clusters of basal keratinocytes	[68]
SUSD2/W5C5	Endometrial tissue: Perivascular location.	[41]

BM, bone marrow; MSC, mesenchymal stem cells; SSEA, surface specific embryonic antigen.

surface proteins similar to MSCs, including the general markers used for MSC characterization [91]. Human dermal fibroblasts also have tripotency [91, 92], although contradictory finding has been reported which suggests a lack of multipotency [51]. In addition, human dermal fibroblasts show immunoregulatory functions similar to MSCs [93, 94].

Another hypothesis is that MSCs reside throughout the body as pericytes or perivascular cells and that the perivascular zone is the in vivo niche of MSCs [95]. Pericytes are a relatively elusive cell type recognized by virtue of their anatomical location of their residence, that is on the abluminal surface of endothelial cells in the microvasculature, rather than by a precisely defined phenotype. As pericytes, MSCs may be readily released from their niche and secrete immunoregulatory and trophic bioactive factors upon tissue damage. As such, MSCs may function as a source of stem cells for physiological turnover.

A perivascular niche of MSCs is supported by the observation that in the majority of solid tissues where MSCs have been found, blood vessels may be the only common anatomical structure. Consistent with the observation, the mesenchyme acts as a "space filler" before the development of a vascular system in early embryonic limb development [96]. Similar to MSCs, pericytes or perivascular cells are able to differentiate into osteoblasts, chondrocytes, adipocytes, fibroblasts, myofibroblasts, and smooth muscle cells in vitro [97]. In fact, CD146(+) perivascular cells from multiple organs expressed general MSC surface antigens [18], as well as 3G5 [98] and NG2 [87]. Observations in vivo also support the association of pericytes with MSCs. For instance, multipotential stem cells were identified in the mural cell population of the vasculature [99]. In rat malignant glioma, intratumoral injection of MSCs [100] resulted in the engraftment of MSCs into tumor vessel walls and the expression of several pericyte markers.

Several studies have further compared the associations of MSCs with fibroblasts and pericytes. Blasi et al. [101] reported that AT-MSCs cannot be distinguished from human dermal fibroblasts in vitro by phenotype or multipotency. However, AT-MSCs, but not dermal fibroblasts, displayed strong angiogenic and anti-inflammatory activity. Sacchetti et al. [35] found that only CD146(+) MSCs, but not muscle or skin fibroblasts, are capable of reconstructing BM and conferring a hematopoietic microenvironment in immunocompromized mice. Additionally, several transcripts were found differentially expressed between HS68 fibroblasts and MSCs, whereas several inhibitors of the Wnt pathway (DKK1, DKK3, SFRP1), an important pathway in regulation of MSCs, were highly expressed in fibroblasts, suggesting that MSCs and fibroblasts have distinct gene expression profiles. Gene and microRNA expression comparison of human MSCs and dermal fibroblasts revealed a panel of MSC-specific molecular signature, which mainly encode transmembrane proteins or associate with tumors [102]. In a comprehensive study by Covas et al. [86], the cell morphology and the phenotypes were found to be comparable among 12 types of MSCs, 2 origins of pericytes, and 4 sources of fibroblasts. However, different from MSCs and pericytes, fibroblasts were reported to be weak for CD146 expression and high for the expression of fibroblast-specific protein-1 (FSP-1, also named as S100A4), a specific fibroblast marker. Furthermore, serial analysis of gene expression revealed a consistent grouping of MSCs with pericytes and hepatic stellate cells, while fibroblasts differentially clustered with smooth muscle cells and myofibroblasts rather than MSCs [86].

The close relationship of MSCs with perivascular cells is also reflected by the physical distribution of the MSC specific markers in vivo. As summarized in Table 6, the general MSC antigens, such as CD73, CD90, and CD105 have a vascular and perivascular expression pattern [18, 68], although their

Table 7. Effect of in vitro or in vivo conditions on MSC phenotype

Regulatory factors	Markers investigated	Findings	References
Inflammation	Stro-1	There was no significant difference in proliferation, differentiation or Stro-1 positivity between MSCs isolated from normal and inflamed dental pulps.	[106]
	Stro-1, CD90, CD105, CD146	Inflammed dental pulps expressed higher levels of MSC markers STRO-1, CD90, CD105, and CD146 compared with normal dental pulps.	[107]
	Stro-1, SSEA-4	More Stro-1 and SSEA-4 positive cells were found in healthy than in inflammed gingival tissues.	[108]
Culture confluency	CD49d, CD49f, CD200, CD106, PODXL	Culture confluency was shown positively correlated with the expression of CD49d, CD200 and CD106, and negatively correlated with CD49f and PODXL.	[36]
Serum	SSEA-4	FCS contained globoseries glycolipids which could be recognized by a SSEA-4 antibody, and exposure to FCS induced the cell-surface expression of SSEA-3 in cord-blood-derived HSCs	[75]
Interferon	HLA class II	HLA class II expression in MSCs was induced by IFN-γ.	[109, 110]
	HLA-DR	HLA–DR positivity upon addition of IFN remained unchanged.	[111]
	NG2	Addition of IFN-γ repressed the transcription of NG2 in MSCs after neural induction procedures.	[112]
Growth factors	CD105, CD73, CD90, CD29, CD44, CD146	FGF induced expression of HLA-DR, and lowered the expression of CD146 and CD49a, as well as the expression of CD49c. Expression of the MSC surface antigens HLA–A/B/C, CD105, CD73, CD90, CD29, and CD44 was not affected.	[111]
	Stemness markers (Oct4A, Notch 1, Hes5), neural markers (Nestin, Pax6, Ngn2)	EGF + bFGF pretreatment downregulated the expression of stemness markers Oct4A, Notch1 and Hes5, whereas neural/neuronal molecules Nestin, Pax6, Ngn2 and the neurotrophin receptor tyrosine kinase 1 and 3 were upregulated.	[113]
	CD44, CD90, CD146, CD105	FGF-2 resulted in reduced expression of CD146 and alkaline phosphatase, which was partially reversed upon removal of the supplement. There was no alteration in CD44 and CD90 with culture conditions, whereas the CD146, CD105, and ALP expression profile was regulated by supplementation with FGF-2, EGF, and PDGF-BB.	[114]
AA	CD44, CD90, CD146, CD105	There was no alteration in CD44 and CD90 with culture conditions, whereas the CD146, CD105, and ALP expression profile was regulated by supplementation with AA.	[114]

AA, ascorbic acid; ALP, alkaline phosphatase; EGF, epidermal growth factor; FCS, fetal calf serum; FGF, fibroblast growth factor; HLA-DR, human leukocyte antigen DR; HSCs, hematopoietic stem cells; IFN, interferon; MSCs, mesenchymal stem cells; PDGF-BB, platelet-derived growth factor BB.

expression can also be found in fibroblasts [14]. For the MSC specific markers, Stro-1, NG2, CD146, and 3G5 expression was mainly found in perivascular area of capillaries, microvessels, and/or venules in many tissues [18, 40], despite additional expression was found in certain endothelial cells for CD146 [14] and in endothelial cells and stroma for Stro-1 [14]. This further supports the identity of MSCs as perivascular cells in vivo, and that MSCs may bear stronger resemblance to pericytes and perivascular cells rather than to fibroblasts. However, this "perivascular niche" theory cannot explain why MSC-like cells are also detected in avascular tissues, such as in articular cartilage [103] and nucleus pulposus [77]. A further postulation is that MSCs may have more than one origin than the perivascular niche, as disclosed in the dual origin of odontoblasts in the teeth by genetic lineage tracing [104]. This postulation of a nonpericytic origin of MSCs is also supported by the fact that MUSE cells, a subset of MSCs with higher stemness, do not express CD146 [84].

Conclusions and Perspectives

A large number of markers have been brought forward to facilitate the isolation of MSCs from their surrounding environment or the selection of MSCs with high stemness. It should be noted that the marker expression of MSCs is not in a stable level. Culture conditions have potential influence on the phenotype of MSCs and that such influence may contribute to the contradictory reports on marker expression. Particularly, some antigens may be artificially induced by in vitro manipulation and culturing, such as the induction of SSEA-4 by FCS [75]. Culture confluence can also induce certain markers, such as CD49d, CD200, or CD106, or diminish them, such as CD49f and PODXL [36]. Certain growth factors and cytokines, such as fibroblast growth factor and interferon-γ, or disease conditions such as inflammation, may also modulate the phenotype of MSCs (Table 7). This therefore emphasizes the importance of a standard operation procedure for in vitro MSC expansion and validation of the markers in vivo.

As shown in the above analysis, there is no sole marker that is truly MSC-specific. Among the known MSC markers, CD146 may be the most appropriate stemness marker, as it is universally detected in the MSC population isolated from various tissues, and enriches cells with clonogenicity and multipotency. On the other hand, SSEA-3 may be a more immature stemness marker which represents an ESCs-like phenotype. This is consistent with the proposed in vivo identity of MSCs as pericytes, as CD146 is also a pericyte marker. Interestingly, CD146 is highly expressed in both MSCs and pericytes, but not in dermal fibroblasts [35], while FSP-1, a fibroblast marker, is lowly expressed in MSCs and pericytes [86], lending support to the closer association of MSCs with pericytes. However, this theory cannot explain the non-pericytic origin of MSCs suggested in a number of reports. Further investigation to

understand the niche components of MSCs in vivo is therefore demanded to validate the theory.

Accompanied by this dilemma is an emerging theory proposing a neuroectodermal origin for MSCs, represented by the expression of nestin [115]. We have not included nestin, Sox2, or Oct4, in the analysis, since these are intracellular proteins but not surface markers. However, we noticed that nestin(+)/CD271(−)/Stro-1(−) MSCs derived from human ESCs were reported to differentiate into representative derivatives of all three embryonic germ layers [116]. Therefore, compared with CD271 and Stro-1, nestin positivity may represent a more primitive phenotype of MSCs. An interesting hypothesis is that CD146(+) MSCs may be a lineage of nestin(+) MSCs, since pericytes within several tissues were reported to be derived from neural crest derivatives [117]. While nestin is a intracellular protein which may complicate the isolation of nestin(+) MSCs, a recent paper suggests that nestin (+) MSCs can be isolated by PDGFRalpha and CD51 double positivity [118], which may facilitate the future investigation of the properties of nestin(+) MSCs.

Acknowledgements

This work was supported by Small Project Funding of the University of Hong Kong (201209176179), General Funding from National Science Foundation of China (NSFC, No. 81371993), and by the Commonwealth of Pennsylvania, Department of Health.

Author Contributions

F.-J.L.: conception and design, collection and/or assembly of data, interpretation and analysis of data, manuscript writing, and final approval of manuscript; R.S.T.: interpretation and analysis of data, manuscript writing, and final approval of manuscript; K.M.C.C. and V.Y.L.L.: financial support, administrative support, manuscript writing, and final approval of manuscript.

Disclosure of Potential Conflict of Interest

The authors indicate no potential conflicts of interest.

References

1 Manuguerra-Gagne R, Boulos PR, Ammar A et al. Transplantation of mesenchymal stem cells promotes tissue regeneration in a glaucoma model through laser-induced paracrine factor secretion and progenitor cell recruitment. Stem Cells 2013;31:1136–1148.

2 Yang F, Leung VY, Luk KD et al. Mesenchymal stem cells arrest intervertebral disc degeneration through chondrocytic differentiation and stimulation of endogenous cells. Mol Ther 2009;17:1959–1966.

3 Noth U, Steinert AF, Tuan RS. Technology insight: Adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheumatol 2008;4:371–380.

4 Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. Arthritis Res Ther 2008;10:223.

5 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

6 Jones EA, Kinsey SE, English A et al. Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis Rheum 2002;46:3349–3360.

7 Buhring HJ, Battula VL, Treml S et al. Novel markers for the prospective isolation of human MSC. Ann N Y Acad Sci 2007;1106: 262–271.

8 Eirin A, Zhu XY, Krier JD et al. Adipose tissue-derived mesenchymal stem cells improve revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. Stem Cells 2012;30:1030–1041.

9 Fukuchi Y, Nakajima H, Sugiyama D et al. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells 2004;22:649–658.

10 Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.

11 Hermida-Gomez T, Fuentes-Boquete I, Gimeno-Longas MJ et al. Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes. J Rheumatol 2011;38:339–349.

12 Tondreau T, Meuleman N, Delforge A et al. Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: Proliferation, Oct4 expression, and plasticity. Stem Cells 2005;23: 1105–1112.

13 Park JC, Kim JM, Jung IH et al. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: In vitro and in vivo evaluations. J Clin Periodontol 2011;38:721–731.

14 Schwab KE, Hutchinson P, Gargett CE. Identification of surface markers for prospective isolation of human endometrial stromal colony-forming cells. Hum Reprod 2008;23: 934–943.

15 Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 2007;25:1384–1392.

16 Sarugaser R, Lickorish D, Baksh D et al. Human umbilical cord perivascular (HUCPV) cells: A source of mesenchymal progenitors. Stem Cells 2005;23:220–229.

17 Martin-Rendon E, Sweeney D, Lu F et al. 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. Vox Sang 2008;95:137–148.

18 Crisan M, Yap S, Casteilla L et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3: 301–313.

19 Russell KC, Phinney DG, Lacey MR et al. In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells 2010;28:788–798.

20 Lv F, Lu M, Cheung KM et al. Intrinsic properties of mesemchymal stem cells from human bone marrow, umbilical cord and umbilical cord blood comparing the different sources of MSC. Curr Stem Cell Res Ther 2012; 7:389–399.

21 Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 2000;113(Pt 7): 1161–1166.

22 Batouli S, Miura M, Brahim J et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 2003;82:976–981.

23 Kuroda Y, Kitada M, Wakao S et al. Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci USA 2010;107:8639–8643.

24 Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood 1991;78:55–62.

25 Psaltis PJ, Paton S, See F et al. Enrichment for STRO-1 expression enhances the cardiovascular paracrine activity of human bone marrow-derived mesenchymal cell populations. J Cell Physiol 2010;223:530–540.

26 Bensidhoum M, Chapel A, Francois S et al. Homing of in vitro expanded Stro-1− or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. Blood 2004;103:3313–3319.

27 Diaz-Prado S, Muinos-Lopez E, Hermida-Gomez T et al. Multilineage differentiation potential of cells isolated from the human amniotic membrane. J Cell Biochem 2010;111: 846–857.

28 Chen FG, Zhang WJ, Bi D et al. Clonal analysis of nestin(−) vimentin(+) multipotent fibroblasts isolated from human dermis. J Cell Sci 2007;120:2875–2883.

29 Quirici N, Soligo D, Bossolasco P et al. Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. Exp Hematol 2002;30:783–791.

30 Jarocha D, Lukasiewicz E, Majka M. Advantage of mesenchymal stem cells (MSC) expansion directly from purified bone marrow CD105+ and CD271+ cells. Folia Histochem Cytobiol 2008;46:307–314.
31 Battula VL, Treml S, Abele H et al. Prospective isolation and characterization of mesenchymal stem cells from human placenta using a frizzled-9-specific monoclonal antibody. Differentiation 2008;76:326–336.
32 Gang EJ, Bosnakovski D, Figueiredo CA et al. SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood 2007;109: 1743–1751.
33 Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod 2007;22:2903–2911.
34 Sorrentino A, Ferracin M, Castelli G et al. Isolation and characterization of CD146+ multipotent mesenchymal stromal cells. Exp Hematol 2008;36:1035–1046.
35 Sacchetti B, Funari A, Michienzi S et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007;131:324–336.
36 Lee RH, Seo MJ, Pulin AA et al. The CD34-like protein PODXL and alpha6-integrin (CD49f) identify early progenitor MSCs with increased clonogenicity and migration to infarcted heart in mice. Blood 2009;113:816–826.
37 Nystedt J, Anderson H, Tikkanen J et al. Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. Stem Cells 2013;31:317–326.
38 Martinez C, Hofmann TJ, Marino R et al. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: A novel surface marker for the identification of MSCs. Blood 2007;109:4245–4248.
39 Castrechini NM, Murthi P, Qin S et al. Decidua parietalis-derived mesenchymal stromal cells reside in a vascular niche within the choriodecidua. Reprod Sci 2012;19:1302–1314.
40 Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003;18:696–704.
41 Masuda H, Anwar SS, Buhring HJ et al. A novel marker of human endometrial mesenchymal stem-like cells. Cell Transplant 2012; 21:2201–2214.
42 Gronthos S, McCarty R, Mrozik K et al. Heat shock protein-90 beta is expressed at the surface of multipotential mesenchymal precursor cells: Generation of a novel monoclonal antibody, STRO-4, with specificity for mesenchymal precursor cells from human and ovine tissues. Stem Cells Dev 2009;18:1253–1262.
43 Battula VL, Treml S, Bareiss PM et al. Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. Haematologica 2009;94:173–184.
44 Delorme B, Ringe J, Gallay N et al. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood 2008; 111:2631–2635.
45 Larson BL, Ylostalo J, Lee RH et al. Sox11 is expressed in early progenitor human multipotent stromal cells and decreases with extensive expansion of the cells. Tissue Eng Part A 2010;16:3385–3394.
46 Bae S, Shim SH, Park CW et al. Combined omics analysis identifies transmembrane 4 L6 family member 1 as a surface protein marker specific to human mesenchymal stem cells. Stem Cells Dev 2011;20:197–203.
47 Van Landuyt KB, Jones EA, McGonagle D et al. Flow cytometric characterization of freshly isolated and culture expanded human synovial cell populations in patients with chronic arthritis. Arthritis Res Ther 2010;12:R15.
48 Mustapha Zeddou AB, Relic B, Josse C et al. The umbilical cord matrix is a better source of mesenchymal stem cells (MSC) than the umbilical cord blood. Cell Biol Int 2010;34: 693–701.
49 Zhang X, Hirai M, Cantero S et al. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: Reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. J Cell Biochem 2011;112: 1206–1218.
50 Buhring HJ, Treml S, Cerabona F et al. Phenotypic characterization of distinct human bone marrow-derived MSC subsets. Ann N Y Acad Sci 2009;1176:124–134.
51 Wagner W, Wein F, Seckinger A et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005;33:1402–1416.
52 Sobiesiak M, Sivasubramaniyan K, Hermann C et al. The mesenchymal stem cell antigen MSCA-1 is identical to tissue nonspecific alkaline phosphatase. Stem Cells Dev 2010;19:669–677.
53 Battula VL, Bareiss PM, Treml S et al. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. Differentiation 2007;75:279–291.
54 Gronthos S, Franklin DM, Leddy HA et al. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001;189:54–63.
55 Quirici N, Scavullo C, de Girolamo L et al. Anti-L-NGFR and -CD34 monoclonal antibodies identify multipotent mesenchymal stem cells in human adipose tissue. Stem Cells Dev 2010; 19:915–925.
56 Schaffler A, Buchler C. Concise review: Adipose tissue-derived stromal cells—Basic and clinical implications for novel cell-based therapies. Stem Cells 2007;25:818–827.
57 Rosada C, Justesen J, Melsvik D et al. The human umbilical cord blood: A potential source for osteoblast progenitor cells. Calcif Tissue Int 2003;72:135–142.
58 Watson JT, Foo T, Wu J et al. CD271 as a marker for mesenchymal stem cells in bone marrow versus umbilical cord blood. Cells Tissues Organs 2013;197:496–504.
59 Kuznetsov SA, Mankani MH, Gronthos S et al. Circulating skeletal stem cells. J Cell Biol 2001;153:1133–1140.
60 Attar A PZN, Ahrari I, Khosravi Maharlooi M, Monabati A. Assessing the role of CD271 isolation from GCSF mobilized peripheral blood in isolation of MSCs. Cell J 2011;12:131.
61 Kawanabe N, Murata S, Fukushima H et al. Stage-specific embryonic antigen-4 identifies human dental pulp stem cells. Exp Cell Res 2012;318:453–463.
62 Hiwase SD, Dyson PG, To LB et al. Cotransplantation of placental mesenchymal stromal cells enhances single and double cord blood engraftment in nonobese diabetic/severe combined immune deficient mice. Stem Cells 2009;27:2293–2300.
63 Pilz GA, Ulrich C, Ruh M et al. Human term placenta-derived mesenchymal stromal cells are less prone to osteogenic differentiation than bone marrow-derived mesenchymal stromal cells. Stem Cells Dev 2011;20:635–646.
64 Yen BL, Huang HI, Chien CC et al. Isolation of multipotent cells from human term placenta. Stem Cells 2005;23:3–9.
65 De Bari C, Dell'Accio F, Tylzanowski P et al. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928–1942.
66 Karystinou A, Dell'Accio F, Kurth TB et al. Distinct mesenchymal progenitor cell subsets in the adult human synovium. Rheumatology (Oxford) 2009;48:1057–1064.
67 Arufe MC, De la Fuente A, Fuentes I et al. Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes. J Cell Biochem 2010;111:834–845.
68 Vaculik C, Schuster C, Bauer W et al. Human dermis harbors distinct mesenchymal stromal cell subsets. J Invest Dermatol 2012; 132:563–574.
69 Barkhordarian A, Sison J, Cayabyab R et al. Epigenetic regulation of osteogenesis: Human embryonic palatal mesenchymal cells. Bioinformation 2010;5:278–281.
70 Martens TP, See F, Schuster MD et al. Mesenchymal lineage precursor cells induce vascular network formation in ischemic myocardium. Nat Clin Pract Cardiovasc Med 2006; 3(Suppl 1):S18–S22.
71 Flores-Torales E, Orozco-Barocio A, Gonzalez-Ramella OR et al. The CD271 expression could be alone for establisher phenotypic marker in bone marrow derived mesenchymal stem cells. Folia Histochem Cytobiol 2010;48: 682–686.
72 Kuci S, Kuci Z, Kreyenberg H et al. CD271 antigen defines a subset of multipotent stromal cells with immunosuppressive and lymphohematopoietic engraftment-promoting properties. Haematologica 2010;95:651–659.
73 Hermida-Gomez T, Fuentes-Boquete I, Gimeno-Longas MJ et al. Bone marrow cells immunomagnetically selected for CD271+ antigen promote in vitro the repair of articular cartilage defects. Tissue Eng Part A 2011;17: 1169–1179.
74 Trubiani O, Zalzal SF, Paganelli R et al. Expression profile of the embryonic markers nanog, OCT-4, SSEA-1, SSEA-4, and frizzled-9 receptor in human periodontal ligament mesenchymal stem cells. J Cell Physiol 2010;225: 123–131.
75 Suila H, Pitkanen V, Hirvonen T et al. Are globoseries glycosphingolipids SSEA-3 and −4 markers for stem cells derived from human umbilical cord blood? J Mol Cell Biol 2011;3:99–107.
76 Tormin A, Li O, Brune JC et al. CD146 expression on primary nonhematopoietic

bone marrow stem cells is correlated with in situ localization. Blood 2011;117:5067–5077.
77 Huang S, Leung VY, Long D et al. Coupling of small leucine-rich proteoglycans to hypoxic survival of a progenitor cell-like subpopulation in *Rhesus Macaque* intervertebral disc. Biomaterials 2013;34:6548–6558.
78 Yu KR, Yang SR, Jung JW et al. CD49f enhances multipotency and maintains stemness through the direct regulation of OCT4 and SOX2. Stem Cells 2012;30:876–887.
79 Notta F, Doulatov S, Laurenti E et al. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science 2011;333:218–221.
80 Zhidkova OV, Petrov NS, Popov BV. [Production and characteristics of the growth and marker properties of mesenchymal stem cells of urinary bladder]. Zh Evol Biokhim Fiziol 2013;49:67–77.
81 Tran TC, Kimura K, Nagano M et al. Identification of human placenta-derived mesenchymal stem cells involved in re-endothelialization. J Cell Physiol 2011;226: 224–235.
82 Xu J, Liao W, Gu D et al. Neural ganglioside GD2 identifies a subpopulation of mesenchymal stem cells in umbilical cord. Cell Physiol Biochem 2009;23:415–424.
83 Khan WS, Adesida AB, Tew SR et al. Bone marrow-derived mesenchymal stem cells express the pericyte marker 3G5 in culture and show enhanced chondrogenesis in hypoxic conditions. J Orthop Res 2010;28: 834–840.
84 Wakao S, Kitada M, Kuroda Y et al. Multilineage-differentiating stress-enduring (Muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. Proc Natl Acad Sci USA 2011;108:9875–9880.
85 Sivasubramaniyan K, Harichandan A, Schumann S et al. Prospective isolation of mesenchymal stem cells from human bone marrow using novel antibodies directed against Sushi domain containing 2. Stem Cells Dev 2013;22:1944–1954.
86 Covas DT, Panepucci RA, Fontes AM et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Exp Hematol 2008;36:642–654.
87 Kozanoglu I, Boga C, Ozdogu H et al. Human bone marrow mesenchymal cells express NG2: Possible increase in discriminative ability of flow cytometry during mesenchymal stromal cell identification. Cytotherapy 2009;11:527–533.
88 Maijenburg MW, Kleijer M, Vermeul K et al. The composition of the mesenchymal stromal cell compartment in human bone marrow changes during development and aging. Haematologica 2012;97:179–183.
89 Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol 2006;6:93–106.
90 Haniffa MA, Collin MP, Buckley CD et al. Mesenchymal stem cells: The fibroblasts' new clothes? Haematologica 2009;94:258–263.
91 Lorenz K, Sicker M, Schmelzer E et al. Multilineage differentiation potential of human dermal skin-derived fibroblasts. Exp Dermatol 2008;17:925–932.
92 Junker JP, Sommar P, Skog M et al. Adipogenic, chondrogenic and osteogenic differentiation of clonally derived human dermal fibroblasts. Cells Tissues Organs 2010;191: 105–118.
93 Jiang XX, Zhang Y, Liu B et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005;105:4120–4126.
94 Haniffa MA, Wang XN, Holtick U et al. Adult human fibroblasts are potent immunoregulatory cells and functionally equivalent to mesenchymal stem cells. J Immunol 2007;179: 1595–1604.
95 da Silva Meirelles L, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. Stem Cells 2008;26:2287–2299.
96 Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol 1995;11:73–91.
97 Doherty MJ, Canfield AE. Gene expression during vascular pericyte differentiation. Crit Rev Eukaryot Gene Expr 1999;9:1–17.
98 Nayak RC, Berman AB, George KL et al. A monoclonal antibody (3G5)-defined ganglioside antigen is expressed on the cell surface of microvascular pericytes. J Exp Med 1988;167: 1003–1015.
99 Tintut Y, Alfonso Z, Saini T et al. Multilineage potential of cells from the artery wall. Circulation 2003;108:2505–2510.
100 Bexell D, Gunnarsson S, Tormin A et al. Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas. Mol Ther 2009;17:183–190.
101 Blasi A, Martino C, Balducci L et al. Dermal fibroblasts display similar phenotypic and differentiation capacity to fat-derived mesenchymal stem cells, but differ in anti-inflammatory and angiogenic potential. Vasc Cell 2011;3:5.
102 Bae S, Ahn JH, Park CW et al. Gene and microRNA expression signatures of human mesenchymal stromal cells in comparison to fibroblasts. Cell Tissue Res 2009;335:565–573.
103 Pretzel D, Linss S, Rochler S et al. Relative percentage and zonal distribution of mesenchymal progenitor cells in human osteoarthritic and normal cartilage. Arthritis Res Ther 2011;13:R64.
104 Feng J, Mantesso A, De Bari C et al. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proc Natl Acad Sci USA 2011;108:6503–6508.
105 Castrechini NM, Murthi P, Gude NM et al. Mesenchymal stem cells in human placental chorionic villi reside in a vascular Niche. Placenta 2010;31:203–212.
106 Pereira LO, Rubini MR, Silva JR et al. Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. Int Endod J 2012;45:1080–1090.
107 Alongi DJ, Yamaza T, Song Y et al. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regen Med 2010;5:617–631.
108 Li N, Liu N, Zhou J et al. Inflammatory environment induces gingival tissue-specific mesenchymal stem cells to differentiate towards a pro-fibrotic phenotype. Biol Cell 2013;105:261–275.
109 Romieu-Mourez R, Francois M, Boivin MN et al. Regulation of MHC class II expression and antigen processing in murine and human mesenchymal stromal cells by IFN-gamma, TGF-beta, and cell density. J Immunol 2007;179:1549–1558.
110 Le Blanc K, Tammik C, Rosendahl K et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31:890–896.
111 Bocelli-Tyndall C, Zajac P, Di Maggio N et al. Fibroblast growth factor 2 and platelet-derived growth factor, but not platelet lysate, induce proliferation-dependent, functional class II major histocompatibility complex antigen in human mesenchymal stem cells. Arthritis Rheum 2010;62:3815–3825.
112 Croitoru-Lamoury J, Lamoury FM, Caristo M et al. Interferon-gamma regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). PLoS One 2011; 6:e14698.
113 Delcroix GJ, Curtis KM, Schiller PC et al. EGF and bFGF pre-treatment enhances neural specification and the response to neuronal commitment of MIAMI cells. Differentiation 2010;80:213–227.
114 Gharibi B, Hughes FJ. Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells. Stem Cells Transl Med 2012;1: 771–782.
115 Wislet-Gendebien S, Hans G, Leprince P et al. Plasticity of cultured mesenchymal stem cells: Switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 2005;23: 392–402.
116 Wu R, Gu B, Zhao X et al. Derivation of multipotent nestin(+)/CD271(−)/STRO-1(−) mesenchymal-like precursors from human embryonic stem cells in chemically defined conditions. Hum Cell 2013;26:19–27.
117 Muller SM, Stolt CC, Terszowski G et al. Neural crest origin of perivascular mesenchyme in the adult thymus. J Immunol 2008; 180:5344–5351.
118 Pinho S, Lacombe J, Hanoun M et al. PDGFRalpha and CD51 mark human Nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J Exp Med 2013;210:1351–1367.

