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Phenotypic Characterization of Mesenchymal Stem Cells from Various Tissues

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Key Words
MSC · Flow cytometry · CD271 · STRO-1 · SSEA-4 · CD105 · CD73

Summary
Research on expanded human stem cells has become an increasing field of interest during the last decade. The increasing interest in adult stem cells, especially mesenchymal stem and mesenchymal stromal cells, in hematology and regenerative medicine is also based on the simplicity of isolation and ex vivo expansion of these cells. These processes require an adequate quality control of source and product. In this review, we summarize various different attempts to characterize mesenchymal stem cells based on surface protein expression by flow cytometry and to define multipotent subpopulations of mesenchymal stem cells for prospective isolation. The importance of defining functional assays and a unique marker panel to characterize mesenchymal stem cells for clinical trials as well as the factors that can modulate the marker expression is discussed.

Schlüsselwörter
MSC · Durchflusszytometrie · CD271 · STRO-1 · SSEA-4 · CD105 · CD73

Zusammenfassung
In den letzten Jahren ist im Bereich der humanen Stammzellforschung ein zunehmendes Interesse zu verzeichnen. Mesenchymale Stammzellen und mesenchymale Stromazellen finden auf Grund der unproblematischen Zugänglichkeit und Expandierbarkeit verstärkt Einzug in die Hämatologie und regenerative Medizin. Diese Vorgänge erfordern eine angemessene Qualitätskontrolle des Ausgangsmaterials und Endprodukts. In dieser Übersichtsarbeit beschreiben wir die verschiedenen Versuche der durchflusszytometrischen Charakterisierung mesenchymaler Stammzellen sowie das Bestreben, Subpopulationen mesenchymaler Stammzellen durch die Expression spezifischer Oberflächenproteine zu definieren. Die Notwendigkeit einer einheitlichen Definition mesenchymaler Stammzellen für klinische Studien durch funktionelle Testung und durchflusszytometrische Bestimmung charakteristischer Oberflächenprotein-Expressionsprofile unabhängig von dem Gewebe und die Einflussgrößen auf diese Marker-Expression werden diskutiert.

Introduction

The use of human stem cells offers a powerful tool for diverse therapies from hematopoietic stem cell transplantation, to regenerative medicine, to tissue-specific directed gene delivery. One attempt is to find potent stem cells from adult tissue and thus avoid ethical questions, and to overcome the potential problem of tumor formation when using human embryonic stem cells. One type of multipotent stem cells are mesenchymal stem cells (MSC) that are candidates for several clinical applications, from use as simple bone glue to organ repair (liver, pancreas, tension replacement), wound healing, and

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Table 1. Expression of markers on MSC derived from indicated tissues (detected by flow cytometry or immunofluorescence if not indicated otherwise)

Marker	Synonym	Tissue	Expression	Reference
CD1a	HTA-1	BM	–	[2, 37]
		AM	–	[2]
		CM	–	[2]
CD3		BM	–	[2]
		AM	–	[2]
		CM	–	[2]
CD4	T4	BM	–	[38]
CD5	Ly-1	BM	–	[7]
CD9	DRAP-1	BM	+	[38]
		PLA	+	[39]
CD10	CALLA	BM	+	[13, 40]
		BM	–	[7]
		PLA	varies	[39]
CD11a	integrin αL	BM	–	[38, 41]
CD13	APN	BM	+	[2, 7, 13, 40, 42–44]
		CB	+	[10, 44]
		PLA	+	[45]
		PLA	varies	[39]
		AM	+	[2]
		CM	+	[2]
		CV	+	[45]
		ATSVF	+	[46]
		PMSC	+	[47]
CD14		BM	–	[2, 3, 7, 13, 16, 37, 38, 42, 44, 48–52]
		mPB	–	[16]
		FB	–	[53]
		CB	–	[10, 16, 44]
		AM	–	[2, 54]
		CM	–	[2]
		DPSC	–	[49]
		AT	–	[55]
CD15	LewisX	BM	–	[38]
CD18	integrin β2	BM	–	[38, 41]
CD25	IL-2R	BM	–	[38]
CD26	ADA-BP	PLA	varies	[39]
CD29	integrin β1	BM	+	[2, 3, 5, 7, 8, 37, 38, 41, 42, 44, 49, 56]
		FB	+	[53]
		CB	+	[44, 57]}
		AM	+	[2, 54]
		CM	+	[2]
		ENDO	+	[58]
		ATSVF	+	[46]
		PMSC	+	[47]
		DPSC	+	[49]
CD31		BM	–	[7, 37, 42, 44, 59]
		PLA	–	[45]
		CM	–	[44]
		CV	–	[45]
		ATSVF	–	[38, 46]
		AT	–	[55]
CD33	gp67	BM	–	[52]
CD34		BM	+	[11]
CD34		BM	–	[2, 6–9, 11, 13, 16, 37, 38, 42–44, 49–52, 56, 59–61]
CD34		mPB	–	[16, 62]
		FB	–	[53]
		CB	–	[16, 44, 57]
		PLA	–	[45]
		AM	–	[2, 54]
		CM	–	[2]
		CV	–	[45]

Table 1 continued on next page

Table 1. Continued

Marker	Synonym	Tissue	Expression	Reference
		ENDO	–	[58]
		ATSVF	–	[46]
		PMSC	–	[47]
		DPSC	–	[49]
CD38	ADP-RC	BM	–	[44]
		CB	–	[44, 57]
		ATSVF	–	[46]
CD44	H-CAM	BM	+	[2, 3, 5, 8, 38, 44, 49–51, 56, 59, 63]
		FB	+	[53]
		CB	+	[44, 57]
		PLA	+	[45]
		AM	+	[2, 54]
		CM	+	[2]
		CV	+	[45]
		ENDO	+	[58]
		ATSVF	+	[46]
		AT	+	[55]
		PMSC	+	[47]
		DPSC	+	[49]
CD44H		BM	+	[37]
CD45	LCA	BM	+	[11]
CD45		BM	low	[11–13]
		BM	–	[2, 3, 6–9, 11, 16, 37, 38, 42, 44, 48–52, 56, 60, 61]
		mPB	–	[16, 62]
		FB	–	[53]
		CB	–	[10, 16, 44]
		PLA	–	[45]
		AM	–	[2, 54]
		CM	–	[2]
		CV	–	[45]
		ENDO	–	[58]
		ATSVF	–	[46]
		AT	–	[55]
		DPSC	–	[49]
CD49a	integrin α1	BM	+	[12, 38, 41, 48]
CD49b	integrin α2	BM	weak	[41]
		BM	+	[38]
CD49c	integrin α3	BM	weak	[41]
		BM	+	[38]
CD49d	integrin α4	BM	–	[38, 41]
		BM	+	[37]
CD49e	integrin α5	BM	+	[37, 38, 41]
CD49f	integrin α6	BM	weak	[41]
CD50	ICAM-3	BM	–	[38, 41]
CD51	integrin αv	BM	weak	[37, 41]
		BM	+	[38]
CD54	ICAM-1	BM	–	[41]
		BM	+	[2, 37, 38]
		AM	+	[2]
		CM	+	[2]
CD56	NCAM	BM	–	[7, 37, 51]
CD58	LFA-3	BM	+	[38, 41]
CD59	MIRL	BM	+	[60]
CD61	integrin β3	BM	weak	[37, 41]
		BM	+	[38]
CD62E	E-selectin	BM	–	[38, 41]
CD62L	L-selectin	BM	+	[38]
CD62P	P-selectin	BM	–	[38, 41]
CD63	LIMP	PLA	+	[39]
CD68	macrosialin	FB	–	[53]

Table 1 continued on next page

Table 1. Continued

Marker	Synonym	Tissue	Expression	Reference
CD71	transferrin receptor	BM	–	[8]
		CB	–	[38, 57]
CD73	ecto-5'-nuclotidase	BM	+	[2, 6–8, 16, 37, 40, 42, 44, 48, 51, 52, 61, 64, 65]
		FB	+	[53]
		CB	+	[10, 44]
		PLA	+	[45]
		AM	+	[2, 54]
		CM	+	[2]
		CV	+	[45]
		ENDO	+	[58]
		ATSVF	+	[46]
CD90	Thy-1	BM	+	[2, 3, 5–7, 9, 13, 38, 42–44, 48, 51, 60]
		mPB	+	[62]
		CB	+	[10, 44]
		PLA	+	[39, 45]
		AM	+	[2]
		CM	+	[2]
		CV	+	[45]
		ENDO	+	[58]
		ATSVF	+	[46]
		AT	+	[55]
CD95	Fas	CB	+	[57]
CD102	ICAM-2	BM	+	[38]
		BM	–	[41]
CD104	integrin β4	BM	weak	[41]
		BM	+	[38]
CD105	endoglin	BM	+	[2, 3, 5–9, 13, 16, 37, 38, 40, 42–44, 48, 50–52, 59–61, 63, 65, 66]
		mPB	+	[16, 62]
		FB	+	[53]
		CB	+	[16, 44, 57]
		PLA	+	[45]
		AM	+	[2, 54]
		CM	+	[2]
		CV	+	[45]
		ENDO	+	[58]
		ATSVF	+	[46]
		AT	+	[55]
CD106	VCAM-1	BM	varies	[3]
		BM	+	[6, 37, 38, 41, 44, 49, 63]
		CB	weak	[44]
		AT	weak	[55]
		DPSC	+	[49]
CD109		BM	subset	[67]
CD117	c-kit	BM	–	[13]
		BM	50%	[43]
CD119	IFNγR	BM	+	[38]
CD120a	TNFIR	BM	+	[38]
CD120b	TNFIIR	BM	+	[38]
CD121a	IL-1R	BM	+	[38]
CD123	IL-3R-α	BM	–	[38, 42]
CD124	IL-4R	BM	+	[38]
CD126	IL-6R	BM	+	[38]
CD127	IL-7R	BM	+	[38]
CD133	HSCA	BM	–	[2, 13, 42, 59]
		mPB	+	[16]
		CB	+	[16]
		AM	–	[2]
		CM	–	[2]
		ATSVF	–	[46]

Table 1 continued on next page

Table 1. Continued

Marker	Synonym	Tissue	Expression	Reference
CD140a	PDGFRα	BM	+	[38]
CD140b	PDGFRβ	BM	+	[40]
		ENDO	+	[58]
CD144	Cadherin-5	BM	–	[6, 38, 61]
CD146	MCAM	BM	+	[7, 42]
		ENDO	+	[58]
		AT	+	[55]
CD166	ALCAM	BM	varies	[3]
		BM	+	[2, 5, 38, 44, 50, 56]
		CB	+	[44]
		AM	+	[2, 54]
		CM	+	[2]
		ATSVF	+	[46]
		AT	+	[55]
CD178	FasL	BM	–	[38]
CD271	NGFR	BM	+	[13, 40, 68]
		PLA	low	[39]
		ATSVF	–	[46]
CD340	HER-2/erb-2	BM	+	[40]
CD349	frizzled-9	BM	+	[40, 69]
		PLA	+	[39, 69]
3G5		AT	weak	[55]
α Smooth muscle actin		FB	+	[53]
Actin		BM	+	[63]
Alkaline phosphatase		BM	+	[49]
		AT	+	[55]
		DPSC	+	[49]
β-Tubulin		BM	+	[59]
bFGFR		BM	+	[38]
BMPRIA		BM	+	[13]
BS1 lectin		BM	+	[7]
CCR2[a]		BM	+	[50]
CCR8[a]		BM	+	[50]
Collagen I		BM	+	[49]
		PMSC	+	[47]
		DPSC	+	[49]
Collagen II		BM	–	[49]
		BM	+	[59]
		DPSC	–	[49]
Collagen III		BM	+	[49]
		PMSC	+	[47]
		DPSC	+	[49]
Collagen IV		BM	+	[63]
CXCR1[a]		BM	+	[50]
CXCR2[a]		BM	+	[50]
CXCR3[a]		BM	+	[50]
Cytokeratin (pan)		BM	+	[37]
Cytokeratin 18		BM	+	[37]
Cytokeratin 19		BM	+	[37]
D7-FIB		BM	+	[13]
Desmin		BM	+	[59]
EGFR-3		BM	–	[38]
FGF-1		BM	+	[70]
FGF-2		BM	+	[49, 70]
		DPSC	+	[49]
FGF-3		BM	+	[70]
FGF-4		BM	+	[70]
Fibronectin		BM	+	[63]

Table 1 continued on next page

Table 1. Continued

Marker	Synonym	Tissue	Expression	Reference
		mPB	+	[62]
		FB	+	[53]
HLA-A,B		BM	+	[7, 42]
HLA-A,B,C		BM	+	[6, 8, 9, 41, 44]
		CB	+	[10, 44, 57]
		PLA	+	[45]
		CV	+	[45]
HLA-DR		BM	+	[13]
HLA-DR		BM	–	[6–9, 16, 41, 42, 44, 51, 56]
		mPB	–	[16]
		FB	–	[53]
		CB	–	[10, 16, 44, 57]
		PLA	–	[45]
		PMSC	–	[47]
Laminin		FB	+	[53]
Vimentin		mPB	+	[62]
MyoD		BM	–	[49]
		DPSC	–	[49]
Neurofilament		BM	–	[49]
		DPSC	–	[49]
Osteocalcin		BM	+	[49]
		DPSC	+	[49]
PPARγ		BM	–	[49]
		DPSC	–	[49]
Prolyl-4-hydrolase		FB	+	[53]
SSEA-4		BM	–	[1]
		BM	+	[69, 71]
		PLA	+	[69]
STRO-1		BM	+	[8, 13, 61, 70, 72, 73]
		AT	–	[55]
		ENDO	–	[58]
TGFβIIR		BM	+	[38]
TGFβIR		BM	+	[38]
VEGFR-2		BM	–	[42]
Vimentin		FB	+	[53]
vWF		BM	–	[38]
		BM	+	[37]
		FB	–	[53]
		PMSC	–	[47]
W8B2		BM	+	[40]

BM = Bone marrow; mPB = G-CSF mobilized peripheral blood; FB = fetal blood; CB = cord blood; PLA = placenta; AM amniotic membrane; CM = chorionic membrane; CV = chorionic villi; ENDO = endometrium; ATSVF = adipose tissue stromal vascular fraction, AT = adipose tissue; PMSC = pancreas mesenchymal stem cells; DPSC = dental pulp stem cells; [a]Detection by real time PCR.

graft-versus-host disease suppression. MSC were first isolated from bone marrow (BM). Multipotent MSC-like cells were also isolated from adipose tissue, dental pulp, cord blood (CB), placenta, and many other tissues. The broad field of sources from which MSC can be isolated and all the different potential applications render necessary an extensive characterization and quality control, especially as MSC isolated from different tissues do not represent a homogenous cell population. There still is no simple way to define MSC by a single marker or a simple set of markers. In this review, we will summarize published literature and our own experience on expression of surface markers of MSC from various sources and in various stages of differentiation and culture.

Characterization of Mesenchymal Stem Cells

The most common surface marker molecules used to describe native or expanded MSC are listed in table 1 and figure 1. The proteins normally analyzed (absence or presence) for the characterization of MSC are markers naturally expressed on stem cell precursor cells, endothelial or epithelial cells,

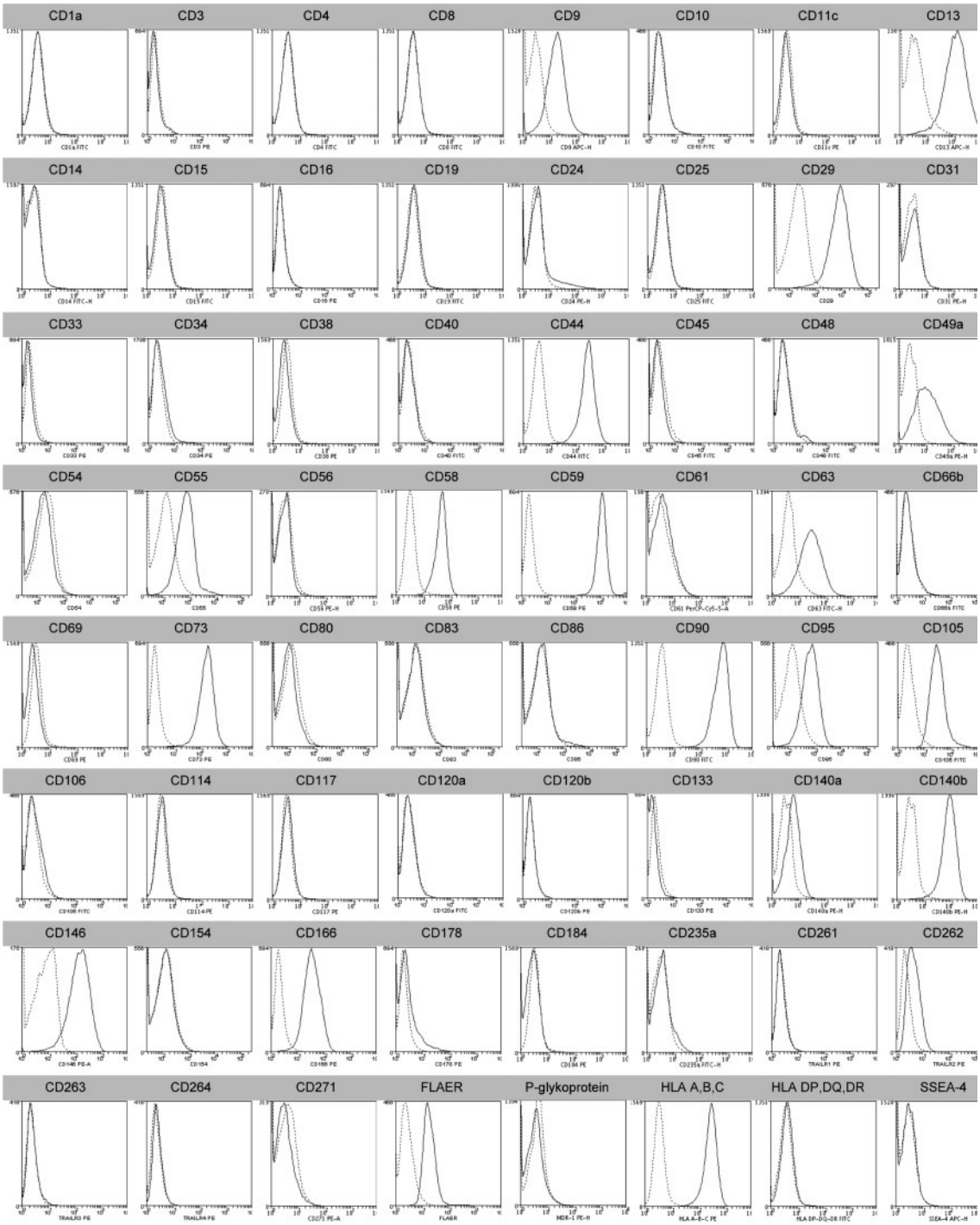

Fig. 1. Representative flow cytometry data to characterize expanded BM-derived MSC. Fluorescence intensities of 50,000–100,000 cells were acquired after applying a standard antibody-staining protocol for cell lines as previously described [86, 87]. Discrepancies of this figure and data from table 2 are discussed in the text.

T-lymphocytes, B-lymphocytes, natural killer (NK) cells, macrophages/monocytes, granulocytes, dendritic cells, platelets, and erythrocytes (table 2). These markers represent cellular adhesion molecules (CD44, CD50, CD54, CD56, CD102, CD106, CD146, CD166), integrins (CD11a, CD18, CD29, CD49a-f, CD51, CD61, CD104), selectins (CD62E, CD62L, CD62P), chemokine receptors (CD117, CD119, CD121a, CD123, CD124, CD126, CD127, CD140a, CD140b), or membrane-bound receptors involved in apoptosis or necrosis (CD95, CD178, CD120a, CD12b). Figure 1 shows representative immunophenotype of ex vivo-expanded BM-derived MSC. Although such a huge number of different surface molecules has been analyzed on MSC, there is no general guiding principle to which classes of markers are expressed on MSC.

Discrepancies in Surface Marker Expression Profiles

The differences in various surface marker expressions observed by different investigators might be due to several factors.

Table 2. Tissue expression of surface markers used for characterization of MSC

Stem cell precursor cells CD10, CD13, CD33, CD34, CD38, CD45, CD49b, CD49d, CD49e, CD49f, CD50, CD71, CD90, CD104, CD105, CD109, CD117, CD123, CD124, CD126, CD127, CD133, CD271
Endothelial or epithelial cells CD9, CD10, CD13, CD26, CD29, CD31, CD34, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD54, CD58, CD61, CD62E, CD62P, CD63, CD71, CD90, CD102, CD104, CD105, CD109, CD119, CD120a, CD120b, CD121a, CD123, CD124, CD133, CD140a, CD140b, CD144, CD146, CD166, CD178
T lymphocytes CD3, CD4, CD5, CD11a, CD18, CD26, CD29, CD31, CD38, CD44, CD45, CD49c, CD49d, CD49e, CD49f, CD50, CD54, CD56, CD58, CD59, CD62L, CD73, CD95, CD102, CD119, CD120a, CD120b, CD121a, CD124, CD126, CD127
B lymphocytes CD5, CD9, CD11a, CD18, CD25, CD26, CD29, CD31, CD38, CD44, CD45, CD49b, CD49c, CD49d, CD50, CD54, CD58, CD62L, CD73, CD95, CD102, CD119, CD120a, CD120b, CD124, CD166
NK cells CD11a, CD18, CD25, CD26, CD29, CD31, CD38, CD45, CD49b, CD49d, CD49e, CD50, CD56, CD58, CD59, CD62L, CD95, CD119, CD120a, CD120b, CD178
Macrophages/monocytes CD4, CD9, CD11a, CD13, CD14, CD15, CD18, CD26, CD29, CD31, CD33, CD38, CD44, CD45, CD49a, CD49b, CD49c, CD49e, CD49f, CD50, CD51, CD54, CD58, CD59, CD61, CD62L, CD63, CD95, CD102, CD119, CD120a, CD120b, CD123, CD124, CD127
Granulocytes CD4, CD9, CD11a, CD13, CD14, CD15, CD18, CD29, CD31, CD33, CD44, CD45, CD50, CD58, CD59, CD63, CD95, CD119, CD120a, CD120b, CD123, CD178
Dendritic cells CD1a, CD33, CD45, CD49d, CD49e, CD58, CD73, CD120a, CD120b, CD123, CD271
Platelets CD9, CD29, CD31, CD44, CD49b, CD49f, CD51, CD61, CD62P, CD63, CD102, CD120a, CD120b, CD140a
Erythrocytes CD49e, CD58, CD59, CD235a

Impact of MSC Source

Most obviously, the tissue from which MSC are derived may play an important role for surface marker expression. Markers like Oct-4, Nanog, Rex-1, SSEA-3, SSEA-4, Tra-1-60, and Tra-1-81 are expressed on MSC from human first-trimester fetal blood, liver, and BM but not in expanded MSC derived from adult BM [1]. Furthermore, CD271 was shown to be present on a small population of unprocessed BM cells yielding high potent MSC by ex vivo expansion, but to our knowledge no report exists on the successful isolation of CB-derived MSC by this method. Whether CD271 is expressed on expanded BM-derived MSC from adults, is still not quite clear. In a recent work, Soncini et al. [2] published the isolation and characterization of BM-, amnion membrane-, and chorionic membrane-derived MSC. The expression profile of MSC isolated from all tissues was comparable [2]. However, there were variations in the percentage of positive cells after 4 passages (plastic adherence method for isolation) expressing positive markers, mainly CD73, CD105, and CD166. BM-derived MSC showed higher percentages of CD73-expressing cells than amnion membrane- and chorionic membrane-derived MSC, whereas percentages of CD105- and CD166-positive cells were lower in amnion membrane-derived MSC only. CD271 was present on less than 1% of BM, about 15% of amnion, and about 20% of chorionic preparations. CD271 expression disappeared during in vitro expansion.

Impact of Donor Age

Age and sex of MSC donors may play an important role. There is no systematic report on differences in surface marker expression. The potential of differentiation has been described to be dependent on the age of MSC donors. CD45 and CD14 expression was quite high for BM-derived MSC preparations from older patients (15 and 13%) at passage 1, compared to pediatric donors (3.6 and 2.2%). In contrast, expression of CD45 and CD14 decreased in older donors, whereas a slight increase was observed in pediatric donors [3]. However, these high values in older patients might be due to limited donor number or experimental layout and analysis of only 200–500 cells in flow cytometry. Besides, it is not clear whether MSC were positive for CD45 and/or CD14 or whether CD45+ or

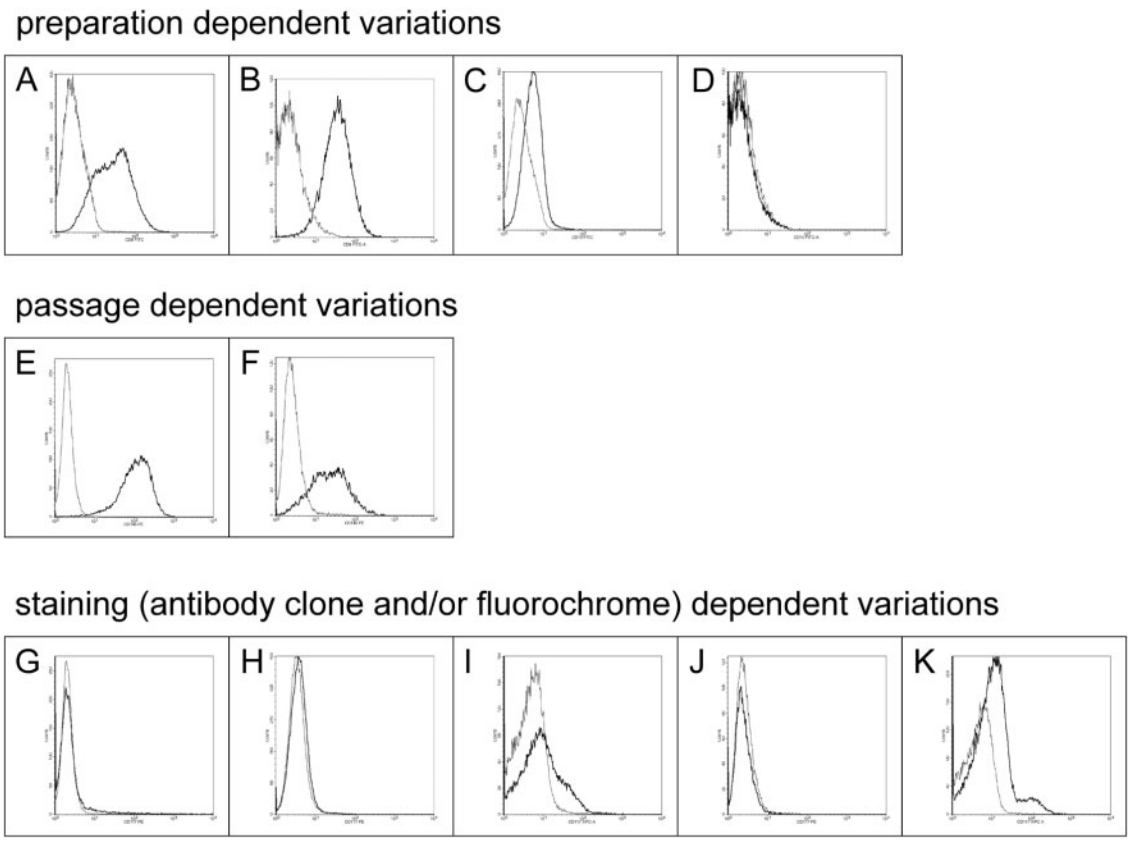

Fig. 2. Variations in surface marker expression might be based on different factors, among which are MSC preparation specific intrinsic factors, passaging dependent effects due to plastic adherence or aging artifacts or the choice of antibody clones or fluorochromes for flow cytometric detection of the same protein. **A**+**B** and **C**+**D** show variations of surface markers CD9 and CD10, respectively. The corresponding BM derived MCS-preparations had comparable passage numbers but were from different donors (**A**, **C** from UL-SARK03 passage 4; **B** and **D** from UL-IKT03 passage 5). Passage-dependent decrease and appearance of a second population of surface marker CD146 is demonstrated in **E** and **F**. The data were acquired from the same BM-derived MSC preparation (UL-SARK02) but from different passages (passage 2 for **E**, passage 10 for **F**). The choice of antibody clone and fluorochrome also might influence expression profiling (**G–K**). BM-derived MSC preparations from different donors (UL-SARK02 (**G**, **J, K**), UL-RKUCH01 (**H**), and UL-551#1 (**I**)) with comparable matched passage numbers (passage 2 (**G**), passage 5 (**H**), passage 6 (**I**), passage 10 (**J**), and passage 12 (**K**)) were stained with 2 different, commercially available antibodies to CD117. Antibodies were labeled with PE (**G, H, J** clone YN5.B8) or APC (**I, K** clone 104D2). MSC preparations were negative when stained with the PE-labeled antibody, but a subpopulation of positive cells could be detected when cells were stained with the APC-labeled antibody. Fluorescence intensities of 50,000–100,000 cells were acquired after applying a standard antibody-staining protocol for cell lines as previously described [86, 87]. Discrepancies in the data from this figure and from table 2 are discussed in the text.

CD14+ cells were accidentally co-cultured. No differentiation or functional assays were performed with isolated or expanded CD45+ or CD14+ cells alone. On the other hand, CD106 is up-regulated in MSC from pediatric donors, and a great variation has been observed for CD106 and CD166 expression in MSC from pediatric and adult donors. However, this finding is viewed controversially [4] and needs further clarification. Up-regulation of surface molecules like VCAM-1 (CD106) or ALCAM (CD166) may have important impact on factors like migration and cell adhesion.

Impact of Isolation Procedure

It is not clear to what extend the surface marker expression is affected by the method used for isolation of MSC. Manipulating MSC might result in up- or down-regulation of markers. It is not proven that the marker used for selection is expressed on all differentiation stages. There is a risk that the markers used for isolation are not expressed in immature progenitor cells which therefore are lost during the selection procedure. The resulting MSC preparation might have a reduced capacity in differentiation, migration, immune modulation, or surface marker expression.

Impact of in vitro Senescence

Senescence may play an important role during expansion of MSC for clinical purposes. Mareddy et al. [5] demonstrated recently that slow growing MSC clones may show senescence and reduced differentiation capacity but still express normal levels of standard MSC surface markers like CD29, CD44, CD90, CD105, and CD166. As demonstrated in figure 2, expression of markers might be down-regulated during expansion and aging, due to cell contact, plastic adherence, contact with (non-human) growth factors, or enzymatic manipulation (e.g. trypsin). This fact may also explain the differences in expression profiles described for adhesion molecules and chemokines. As described above, CD271 expression was de-

Table 3. Enrichment strategies of MSC from different tissues

Isolation based on	Isolation parameter	Tissue	Purpose and outcome	References
Single color cell sorting (FACS)	SSEA-4	BM	– 2–4% positive cells – low density plated SSEA-4-negative cells do not grow – low density plated SSEA-4-positive cells are plastic adherent, grow for 7 weeks without senescence – expanded cells are CD45–, maintain SSEA-4 expression, and can be differentiated into adipocytes, cartilage and osteoblasts	[71]
Single color cell sorting (FACS)	CD271	BM	– only $CD271^{bright}$ but not $CD271^{dim}$ population contain CFU-F – $CD271^{bright}$ population is positive for CD10, CD13, CD73, CD105, CD140b, CD340, CD349, W8B2 and other cell-surface antigens defined by specific antibodies	[40]
Dual color cell sorting (FACS)	selection of $STRO\text{-}1^{+}$/CD235a–	BM	– about 10% of total cells isolated – 100-fold enrichment of stromal cells in CFU-F – differentiation into adipocytes, smooth muscle cells, fibroblastic element under long-term BM culture and maintenance of self-renewal capacity – support growth of CD34+ cells	[73]
Dual color cell sorting (FACS)	anti-fibroblast micro-bead binding/$CD45^{low}$	BM	– 0.01% of mononuclear cells isolated – positive for CD105, CD271, CD10, CD13, CD90, STRO-1, BMPRIA – negative for CD14, CD34, CD117, CD133 – cells proliferate and produce adherent monolayers capable of chondrogenic, osteogenic, and adipogenic differentiation	[13]
Dual color cell sorting (FACS)	CD45–/CD14–	BM/BC	– spongious bone was filtered (100 µM nylon filter) and pooled with BC cells, ficoll was performed and suspension was sorted for CD45–/CD14– – cells after 1–3 days of culture – cells express CD73, CD49a – high CFU-F clonogenity, even though CD105+ and CD90+ subsets comprised less than half of total – normal culture conditions up-regulate CD73, CD105, CD90, CD49a	[48]
Dual color cell sorting (FACS)	CD146+/CD140b+	ENDO	– higher number of colonies in CD146+/CD140b+ – cells are positive for CD29, CD44, CD73, CD90, CD105, CD146, CD120a – cells are negative for STRO-1, CD34, CD45: CD146 expression is reduced during culture – differentiation in: osteogenic, adipogenic, myogenic, and chondrogenic	[58]
Dual color cell sorting (FACS)	CD34+ CD34– CD45+/CD34– $CD45^{high}$/CD34– $CD45^{med+low}$/CD34– CD45–/CD34–	BM	– small population of MSC from CD45+/CD34+ – large population of MSC from CD45–/CD34– – MSC from all populations could be differentiated into adipocytes and osteocytes – MSC from CD34+ and CD34– differentiated in addition into chondrocytes – during in vitro culture all fractions developed the phenotype CD45–/CD34–	[11]
Dual color cell sorting (FACS) Magnetic immunobead isolation (MACS)	CD49a+ CD49a+/$CD45^{+med,low}$	BM	– isolation of CD49a and/or CD45+ cells – all CFU-F detected in CD49a+ fraction – CD49+ cells show adipogenic, chondrogenic, osteogenic and stromatogenic differentiation – uncultured cells reveal $CD45^{med,low}$, $CD34^{low}$, MHC class II– cell population – CFU-F potential was obtained only from a CD49a+/$CD45^{med,low}$ population – when cultured, cells were CD45–, CD34–, MHC class II–and CD49a+	[12]

Table 3 continued on next page

Table 3. Continued

Isolation based on	Isolation parameter	Tissue	Purpose and outcome	References
Multicolor cell sorting (FACS)	CD349 CD10 CD29	PLA	– prospective isolation of 0.2% positive cells – cells are positive for CD9, CD63, CD90 – cells are heterogeneous for CD10, CD13, CD26 – cells show low expression of CD271 – 60 fold enriched CFU-F in CD349+/CD10+CD29+ – no CFU-F in CD349+CD10–CD29– – CD349+ express embryonic markers Oct-4, Nanog, SSEA-4, TRA-1-49/6E – CD349+ cells can be differentiated into functional adipocytes, and osteoblasts	[39]
Magnetic immunobead isolation (MACS)	STRO-1STRO-1	BM	– 10- to 20-fold increase in formation of CFU when co-cultured with CD34+/CD38+ and CD34+/CD38^{low} cells – development of myofibroblastic, adipogenic, osteogenic, and chondrogenic phenotype/potential	[72]
Magnetic immunobead isolation (MACS)	STRO-1	BM	– MSC from patients with osteoporosis – no difference in CFU-F number or percentage of alkaline phosphatase positive cells of young individuals, old individuals and patients with osteoporosis	[4]
Magnetic immunobead isolation (MACS)/ dual color cell sorting (FACS)	STRO-1^{bright} STRO-1–/CD45–/ CD235a–	BM	– adherent cells only in STRO-1^{bright} fraction – STRO-1^{bright} fraction is CD34–/CD45–/CD235a– – STRO-1^{bright} cells form osteoblasts, adipocytes, and chondrocytes – STRO-1^{bright} cells form lamellar bone structures, vascularized fibrous tissue supporting adipose formation and hematopoietic active BM – no obvious difference in growth potential or differentiation potential of STRO-1^{bright}/ CD34–, STRO-1^{bright}/ CD45– and STRO-1^{bright}/ CD235a–	[74]
Magnetic immunobead isolation (MACS)	CD105	BM	– isolation of MSC from washings of BM collection bags – expanded cells were differentiated (adipogenic, osteogenic), characterized (positive for CD44, CD105, CD90, CD73 and negative for CD45, CD14, CD34, CD56, HLA-DR and Lin1) – decreased proliferation of CD4+ T-cells in allogeneic one-way mixed lymphocyte reaction when co-cultured with MSC.	[51]
Magnetic immunobead isolation (MACS)	CD271+ or CD45–/CD235a–	BM	– CD271+ selected cells showed persistent CD271 expression and CFU-F activity (no CFU-F activity in CD271– fraction) – CD271+ cells have higher potential in adipogenic and osteoblast differentiation, fibroblast like growth and support of hematopoietic precursor growth	[68]
Magnetic immunobead isolation (MACS)	CD271 selection and CD271 depletion	BM AM CM	– percentage of CD271+ cells varies (BM: < 1%; CM 15%; AM 20%) – CFU-F were present in CD271+ enriched, but not in CD271 depleted BM-preparations – significantly less (about 30 and 50%) CFU-F colonies for CD271 depleted preparations from CM and AM, respectively	[2]
Adhesion to microbeads	fibrin micro-beads	mPB	– rotation of cells and beads during incubation – non-attached cells were removed after 48 h and beads were washed – isolated cells were negative for CD45 and CD34 – isolated cells were positive for CD90 and CD105, vimentin and fibronectin – isolated cells could be differentiated into adipocytes, chondrocytes, and osteoblasts	[62]

Table 3 continued on next page

Table 3. Continued

Isolation based on	Isolation parameter	Tissue	Purpose and outcome	References
Single cell isolation	limiting dilution	BM	– isolation of single cell-derived MSC clones from adult BM – isolation of slow and fast growing tri-potential clones – all clones express surface markers CD29, CD44, CD90, CD105, CD166 – slow growing clones showed limited differentiation potential and morphological changes associated with cellular senescence	[5]
(a) RosetteSep (b) magnetic immunobead isolation (MACS) (c) plastic adhesion	negative selection (a) RosetteSep (b) CD45/CD235a	BM	– 1.0–2.9% of cells were enriched by negative selection – isolated cells represent homogenous population of rapidly growing MSC – isolated cells: > 90% positive for CD73 and CD105 – isolated cells are negative for CD14, CD34, and CD45 – three passages of plastic adhesion were necessary to obtain the same CD105 homogeneity of cells for plastic adherent cells – differentiation to adipocytes, chondrocytes, osteocytes, and neuronal/glial cells	[52, 65]
(a) Plastic adhesion (b) positive selection (MACS)	(b) CD133	BM mPB CB	– isolated cells positive for CD73 and CD105 – isolated cells negative for CD14, CD34, CD45, HLA-DR – mPB and CG derived MSC express Oct-4 – CD133-positive fraction contained more MSC with higher proliferative potential – all preparations differentiated into adipocytes, osteocytes, chondrocytes and neuronal/glial cells	[16]
Size sieve	size	BM	– plastic culture dish comprising a plate with 3 micron pores to sieve out a homogeneous population of cells – self renewal capacity, multi-lineage potential (osteogenic, adipogenic, chondrogenic)	[75]
Magnetic immunobead isolation (MACS)	aptamer	pBM	– isolation of MSC that can be differentiated and express MSC markers – CD271 disappears during in vitro culture	[14, 15]

BM = Bone marrow; PB = peripheral blood; mPB = G-CSF mobilized peripheral blood; ENDO = endometrium, AM = amniotic membrane, CM = chorionic membrane, PLA = placenta, pBM = porcine bone marrow, MACS = magnetic immunobead-based cell sorting, FACS = fluorescence-activated cell sorting.

tectable in different amounts in freshly isolated preparations from BM, chorionic, and amniotic membranes but disappeared after culture passages [2]. CD45 was reported to be present on 15% of BM-derived MSC preparations of older patients at passage 1 but decreased to 0.5% at passage 10 [3].

Impact of Expansion Conditions

MSC phenotype might be influenced by the culture conditions for ex vivo expansion, e.g. type of supplements (fetal bovine serum, human serum, platelet lysate). For standard positive markers like CD73, CD90, CD105, CD106, CD146, and HLA-A,B,C or standard negative markers like CD34, CD45, and HLA-DR no difference could be observed so far [6, 7]. The impact of different culture media (e.g. α-MEM, DMEM, IMDM, Optimem) and medium supplements (e.g. bFGF, heparin, additional amino acids, low or high glucose, addition of deoxyribonucleotides) has never been analyzed in detail. The influence of media mainly may influence proliferation potency, but also lead to marker expression variations of CD44, STRO-1, and HLA-DR [8] and changes in differentiation potency [9]. However, the choice of initial plating densities and plastic source for first step adhesion and expansion mainly plays a role for expansion capacity [7–10]. In a recent report, Kaiser et al. [11] describe the isolation of MSC from BM by expanding fractions of CD45–/CD34– and CD45+/CD34+ cells. Using fluorescence-activated cell sorting (FACS), they obtained a CD34+ population with only 86% purity, and only 21.8% of cells grew out. Therefore, it cannot be excluded that mainly contaminating CD34– cells were analyzed. After in vitro culture, the phenotype of all isolated fractions changed into CD45–/CD34–. The majority of in vivo MSC in human BM clearly was CD34–/CD45–. These findings contradict reports predicating that most MSC derive from CD49a+/CD45$^{med.low}$ cells [12, 13] and turn into CD49a+/CD45– cells when cultured.

Table 4. Characterization of expanded MSC in publications on clinical trials

Topic of investigation	Origin	Surface antigen expression		Differentiation	References
		present	absent		
Co-transplantation to reduce GvHD	BM	CD73 CD105	CD14 CD45	–	[76]
Treatment of therapy-resistant GvHD	BM	CD29 CD44 CD73 CD105 CD166 HLA-A, B, C	CD14 CD34 CD45 HLA-DP, DQ, DR	–	[77]
Graft failure in haploidentical hematopoietic stem cell transplantation	BM	CD73 CD90 CD105	CD31 CD34 CD45	–	[78]
Hematopoietic recovery after co-infusion of autologous blood stem cells in advanced breast cancer	BM	CD73 CD105	CD14 CD45		[79]
Acute myocardial infarction	BM	N.I.	N.I.	N.I.	[80]
Myocardial infarction	BM	CD29 CD44 CD73 CD105	CD34 CD45	–	[81]
Ischemic cardiomyopathy	BM	–	–	–	[82]
Metachromatic leukodystrophy and Hurler syndrome	BM	CD73 CD105	CD14 CD45	–	[83]
Multiple sclerosis	BM	CD13 CD44 CD105 CD106	CD31 CD34 CD45	adipocytic osteocytic	[84]
MSC/fibrin spray for accelerated wound healing	BM	CD29 CD44 CD90 CD105 CD166	CD34 CD45	adipocytic osteocytic chondrogenic	[85]

GvHD = graft-versus-host disease; BM = bone marrow; N.I. = not indicated.

Impact of Detection Methods

The use of different detection methods (flow cytometry, ELISA, micro array, reverse transcription polymerase chain reaction (RT-PCR)) and individual variations within these detection systems like antibody specificity or fluorochrome (fig. 2) may also result in differences in expression profiling.

Isolation or Enrichment of Mesenchymal Stem Cells Based on Surface Marker Expression Patterns

Several attempts have been made for prospective isolation of MSC by selection of MSC-containing subpopulations from various sources (table 3) such as BM, buffy coat, endometrium, placenta, amniotic and chorionic membrane, or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood. Several cell surface markers listed in tables 1 and 2 were applied for positive or negative selection using magnetic immunobead-based or fluorescence-activated cell sorting. The cell populations isolated by these different methods show the typical MSC characteristic and differentiation capacity as far as tested in the individual attempts. Several of the positive selection markers like CD271, CD34, CD45, and CD133 disappear during ex vivo expansion. Novel attempts like aptamer-based magnetic immunobead-activated cell sorting have not yet been published for human MSC [14, 15].

Characterization of Mesenchymal Stem Cells Used for Clinical Trials

Table 4 summarizes the origin and immunophenotypic characterization of MSC used in recent clinical trials with MSC. The description of expanded MSC used for patients is either published in detail (surface antigen expression, testing of differentiation capacity), or no statement is given on the characterization of the cells. The expression of CD105 in combination with at least one additional marker was analyzed as the minimum standard in most trials, except those where no information on surface marker was available (table 4). In several trials, the expression of CD73, CD29, or CD44 and lack of expression of CD34, or CD14 and CD45 were included as criteria for expanded MSC. Despite some overlap, marker panels are rather heterogeneous. Differentiation capacity of ex vivo-expanded MSC was determined in few trials only.

The Quandary to Define Surface Markers for Mesenchymal Stem Cells

Characterization of MSC by the establishment of a clearly defined, well deliberated surface marker expression panel might be helpful to define expanded MSC for special purposes. However, characterization of MSC solely by surface antigen expression has to be reconsidered, especially as this characterization may not be helpful to identify MSC precursors or isolate MSC from the original source. Markers like CD133 [16] or CD34 [11], which generally are requested to be absent on MSC preparations (tables 1 and 2) but are described to be prospective markers, need further investigation (table 3). The discrepancy of surface antigen expression in unprocessed raw material and expanded MSC preparations used for clinical purpose has to be investigated in more detail.

Minimal Requirements for Mesenchymal Stem Cell Characterizations

All these discrepancies make it difficult to find clear and generally applicable parameters for the definition of MSC. In a first step, there has to be a clear definition on what MSC stands for. MSC has synonymously been used for different types and qualities of cells like mesenchymal stem cells, mesenchymal stromal cells, multipotent stromal cells but also mesenchymal progenitor cells, regardless from which tissue they have been isolated: BM, spongiosa, G-CSF-mobilized peripheral blood, placenta, dental pulp, pancreas, fetal blood, cord blood, adipose tissue, endometrium, or amniotic and chorionic membrane or villi. During the last years, it has become obvious that stem cell-like or progenitor-like populations with MSC features can be isolated from virtually any tissue, not only from mice [17] but most probably also from humans. Therefore, the International Society for Cellular Therapy (ISCT) published a position statement on the nomenclature for MSC claiming that only cells meeting the specific stem cell criteria should be referred to as mesenchymal stem cells, whereas all other fibroblast-like plastic-adherent cell preparations, regardless of the tissue from which they are isolated, should be termed mesenchymal stromal cells [18]. Unfortunately, the abbreviation for both mesenchymal stem cells and mesenchymal stromal cells is defined as MSC. The minimal criteria for multipotent mesenchymal stromal cells have been defined by the Mesenchymal and Tissue Stem Cell Committee of the ISCT [19]. MSC must be plastic-adherent in standard culture conditions, express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR. In addition, MSC must differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. However, these criteria are not fulfilled for all cell preparations referred to as MSC in the literature. Unfortunately, several publications on MSC and even publications on clinical trials with MSC (table 4) were done with MSC characterized by only an incomplete panel of surface markers without testing their differentiation capacity. On the other hand, MSC isolated from e.g. adipose tissue may not fulfill all criteria demanded for differentiation potential.

As quality control for ex vivo-expanded MSC, we suggest that the panel of positive markers should cover surface proteins expressed by MSC expanded from every tissue (table 1). These markers may include CD105, CD73, and CD90. In addition, expanded MSC should at least be tested negative for markers of cells that accidentally may be co-isolated and co-expanded, like CD1a or CD33 for dendritic cells, CD14 or CD33 for monocytes, CD3 or CD45 for resting lymphocytes, and CD34 for hematopoietic stem cells. Moreover, the chondrogenic, osteogenic, and adipogenic differentiation potential of ex vivo-expanded MSC has to be proven. For the clinical application of MSC as suppressor of graft-versus-host disease, the anti-proliferative role of MSC in functional assays like mixed lymphocyte reaction may be more suitable than differentiation assays to prove their suitability.

As proposed by Bensidhoum et al. [20] for the marker STRO-1, expression, lack of expression, or variation of expression levels for clearly defined markers may help to further subclassify MSC. This might help to develop a helpful tool for isolation of application-specific expandable progenitor cells for special purposes like immune suppression, enhanced engraftment, bone or cartilage formation, or neuronal repair. Stro-1+ cells may rather be used for gene delivery in tissues while Stro-1– cells may rather be used to support hematopoietic engraftment [20]. In this case, both Stro-1+ and Stro-1– cells show differentiation potency. Therefore, Stro-1 might be one of the many useful markers to further characterize phenotypic and functional subpopulations in the MSC pool, and even Stro-1+ or Stro-1– MSC might rather represent a variety of different cell qualities than a unique stem cell quality.

Another novel attempt to overcome the dilemma of characterizing MSC is the exploitation of the minimum requirements of MSC that are necessary for a specific function. Parekkadan et al. [21] used bioreactor-expanded MSC to define factors within the secretome of MSC-derived molecules in order to reverse fulminant hepatic failure in mice.

The Need for New Markers and Methods

All things considered, the known surface proteins described for the characterization of MSC are not sufficient to distinguish between subpopulations and different cell types with different intrinsic qualities of MSC. Search for surface antigens representing the pure, native MSC population within the different basic raw materials remains one of the most challenging topics of MSC research for the future. In addition, easy methods for a robust characterization of expanded MSC that do not loose pluripotency or show chromosomal abnormalities due to culturing artifacts have to be established. Time-consuming assays like in vitro differentiation into osteoblasts, chondrocytes, or adipocytes have to be replaced by faster methods. First attempts into this direction have been proposed and performed recently using proteome and microarray screening tests [22–36]. However, establishing more complex assays to characterize MSC may not achieve broad acceptance as long as the definition of MSC by surface proteins still is easier to handle, delivers faster results, and remains less costs intensive than gene expression arrays or complex proteomics.

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