MSC Populations for Cartilage Regeneration

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Roberto Narcisi, Mairéad A. Cleary, Kavitha Sivasubramaniyan, Pieter A.J. Brama, and Gerjo J.V.M. van Osch

Abstract

Adult mesenchymal stem cells (MSCs) have an excellent capacity to repair tissues since they can proliferate and differentiate to form various tissues, cartilage included. Moreover, MSCs are potentially accessible in high quantities with low donor site morbidity and reasonable cartilage-forming capacity. In 1998, Johnstone et al. (Exp Cell Res 238(1):265–272) were the first that proposed an effective protocol to chondrogenically differentiate MSCs by using transforming growth factor-β (TGF-β), now used by many groups in the world and since then hardly changed. However, MSCs are a heterogeneous population, and the amount

R. Narcisi • K. Sivasubramaniyan
Department of Orthopaedics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
e-mail: r.narcisi@erasmusmc.nl; k.sivasubramaniyan@erasmusmc.nl

M.A. Cleary
Department of Orthopaedics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Section of Veterinary Clinical Sciences, School of Veterinary Medicine, University College Dublin, Belfied, Dublin 4, Ireland
e-mail: mairead.cleary@ucdconnect.ie

P.A.J. Brama
Section of Veterinary Clinical Sciences, School of Veterinary Medicine, University College Dublin, Belfied, Dublin 4, Ireland
e-mail: pieter.brama@ucd.ie

G.J.V.M. van Osch (✉)
Department of Orthopaedics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Department of Otorhinolaryngology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
e-mail: g.vanosch@erasmusmc.nl

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and type of cartilage formed are strongly influenced by intra- and inter-donor variation. In this chapter, we mainly focused on surface markers and their modulation by growth factors. We aim to first clarify the characteristics and the embryonic origin of cartilage progenitor cells (chondroprogenitor), then to summarize the characteristics and the contribution to cartilage repair by MSCs from different origins both in vivo and in vitro, and finally, to show a few examples of promoting articular cartilage phenotype by growth factor administration, in relation to the modulation of surface marker expression. With the exception of the next section focused on embryology, our interest was posed specifically on MSCs from human origin.

2.1 Embryonic Origin of Chondroprogenitor Cells (CPCs)

Adult joints contain progenitor cells (Im 2016). Some insight into the origin, fate, and function of such progenitor cells, including those associated with articular cartilage, has been gained through multiple studies utilizing various conditional reporter mice. Whether these adult progenitor cells are derived from interzone cells, the compact layer of mesenchymal cells from which synovial joints and their specialized tissues arise (Holder 1977), remains to be clarified.

Interzone cells emerge at sites previously occupied by chondrocytes (Craig et al. 1987; Nalin et al. 1995). This led to the hypothesis that interzone cells represent dedifferentiated chondrocytes. Sox9 and Dcx lineage-tracking studies using mouse embryos, however, demonstrate that while articular and growth plate chondrocytes arise from a common population of mesenchymal progenitors, articular chondrocytes arise from distinct populations of these mesenchymal progenitor cells (Soeda et al. 2010; Zhang et al. 2011). This hypothesis is supported by the observed migration of joint progenitor cells into the prospective joint site from flanking regions (Hyde et al. 2008; Pacifici et al. 2006; Koyama et al. 2007; Li et al. 2013).

Indian hedgehog null ($Ihh^{-/-}$) mouse embryos lack joints in their skeletal elements, which remain completely cartilaginous (Koyama et al. 2007). In $Ihh^{-/-}$;Gdf5 mouse embryos, in which Gdf5 is selectively expressed by interzone cells, Gdf5-expressing cells were observed at respective joint sites, but not within the future joint site itself; instead they flanked and surrounded the uninterrupted joint site (Koyama et al. 2007; Storm and Kingsley 1996). The absence of a joint in $Ihh^{-/-}$ mouse embryos may arise from the inability of the Gdf5-expressing cells to migrate into the prospective joint area.

Recruitment and immigration of surrounding cells into the interzone are also supported by studies involving the TGF-β type II receptor that is essential for joint formation, especially in the hands/feet (Spagnoli et al. 2007; Li et al. 2013). At E13.5, Tgfbr2-positive cells are present in the dorsal and ventral regions of the joint but completely absent from the central region of the interzone. By E16.5 and postnatally, positive cells are present in various joint tissues, although not in articular cartilage. Tgfbr2-deficient mouse embryos display fusion of digit joints, a

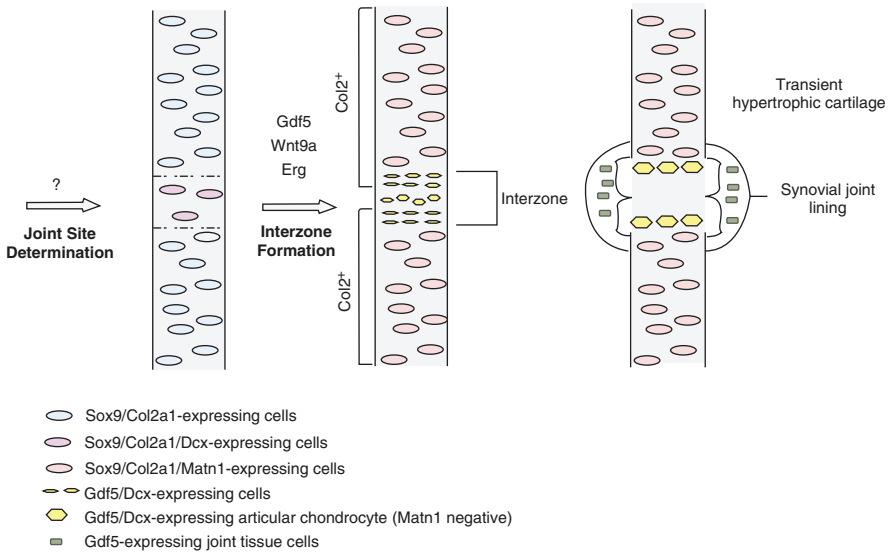

Fig. 2.1 Model of joint development. Unknown upstream mediators determine the location of the future joint site along *Sox9/Col2a1/Dcx*-expressing cells within the uninterrupted cartilaginous template. Soon after, the interzone mesenchymal population is defined and morphologically observable among *Sox9/Col2a1/Matn1*-expressing cells. *Gdf5/Dcx*-expressing cells differentiate to form the articular chondrocytes of the synovial joint. Remaining interzone cells are involved in forming other joint tissues and structures (Adapted from Decker et al. 2014)

phenotype which can be rescued by blocking the MCP-5 receptor CCR2, further supporting the need for low expression of Mcp5 for interzone and joint formation (Longobardi et al. 2012). Moreover, Tgfbr2-expressing cells present in the adult joint are observed, through BrdU-labeling experiments, to constitute slow-cycling stem/progenitor cells (Li et al. 2013).

Thus, along the initial uninterrupted Sox9/Col2/Dcx-expressing cartilaginous anlagen, the future joint site develops, the site itself identified by as yet unknown upstream morphogenetic and determination mechanisms. Soon after, the interzone mesenchymal population is specified through Gdf5 expression with concomitant cell recruitment and maintenance of Dcx expression. Dorsal and ventral flanking cells activate Tgfbr2 expression and articular chondrocytes and then arise from Gdf5/Dcx-expressing cells with a Sox9/Col2a1+/Matn1 history (Fig. 2.1).

2.2 In Vivo Presence of MSCs in Adult Joints

Endogenous stem or progenitor cells contribute to maintenance of healthy tissues by acting as reservoirs of repair cells or as immunomodulatory sentinels to reduce inflammation. In a joint environment, these cell populations exist in tissues adjacent to articular cartilage, including the articular cartilage itself, bone marrow, synovium,

synovial fluid, and infrapatellar fat pad (Im 2016). A great deal has been learnt in recent years about the isolation and characterization of endogenous MSCs from these tissues. The characteristics of endogenous stem or progenitor cell populations from these tissues are discussed below.

2.2.1 Characteristics of MSCs In Vivo

2.2.1.1 Articular Cartilage-Derived Chondroprogenitor cells (CPCs)

Chondrocytes are the most abundant cells within articular cartilage. A small population of these chondrocytes constitutes the chondroprogenitors (Dowthwaite et al. 2004; Khan et al. 2009). CD166 is the most broadly used biomarker for the identification and localization of these progenitor cells in human articular cartilage with high chondrogenic potential (Pretzel et al. 2011; Swart 2002). One study investigated the zonal distribution of $CD166^{+}$ CPCs in articular cartilage, where it was verified that these cells were almost exclusively located in the superficial and middle zones (Pretzel et al. 2011). Notably, 99% of the mesenchymal progenitor cells in that study co-expressed CD166 with another surface marker: the TGF-β co-receptor CD105. Indeed, the superficial zone seems to be of central importance for the growth of articular cartilage in young animals (Hunziker et al. 2007) and presumably relies on a progenitor cell population located in this zone (Hayes et al. 2001). Apart from CD166, also expression of Notch-1 (Dowthwaite et al. 2004) or triple positivity for CD44/CD151/CD49c (Grogan et al. 2007) or CD9/CD90/CD166 (Fickert et al. 2004) has been shown to isolate chondroprogenitors from articular cartilage.

2.2.1.2 Bone Marrow-Derived MSCs (BMSCs)

Bone marrow (BM)-derived MSCs are most extensively studied and best characterized. Several markers were identified that are suitable to isolate MSCs directly from fresh bone marrow samples including antibodies specific for a variety of cell surface molecules, CD49a, CD63, CD73 (SH3/SH4), CD90, CD105 (SH2), CD106, CD140b, CD146, CD200, CD271, CD349, TNAP, Hsp90, GD2, TM4SF1, and NG2 as well as orphan antigens defined by antibodies STRO-1 and 3G5 (Lv et al. 2014; Harichandan et al. 2013; Harichandan and Buhring 2011). In other approaches, BMSCs were enriched using negative selection, by depletion of hematopoietic cells, employing markers such as CD14, CD34, CD45, and/or CD235 (glycophorin A) and other lineage-negative markers (Harichandan and Buhring 2011).

CD271 is the most widely used marker for the characterization and purification of primary human bone marrow MSCs (Alvarez-Viejo et al. 2015). MSCs reside in $CD271^{bright}$ cells, while $CD271^{dim}$ cells comprise $CD45^{+}$ hematopoietic cells (Buhring et al. 2007). More selective markers for MSC isolation than CD271 are SUSD2 (W5C5) and CD140b, as they are expressed on $CD271^{bright}$, but not $CD271^{dim}$ cells (Sivasubramaniyan et al. 2012, 2013). Another well-known marker for BMSCs is Stro-1. But it is unsuitable as a sole marker to separate MSCs from its harboring tissue, as greater than 95% of Stro-1^{+} cells in the human BM are glycophorin A expressing nucleated erythroid cells (Simmons and Torok-Storb 1991). Delorme

et al. (2008) reported CD73, CD130, CD146, CD200, and integrin a5/β5 as markers to enrich colony-forming unit fibroblasts (CFU-Fs) from bone marrow-derived mononuclear cells (MNCs), while other known MSC markers CD49b, CD90, and CD105 showed less enrichment. The neural ganglioside GD2 was introduced by Martinez et al. (2007) as a stand-alone marker to isolate BMSCs. However, in another study, GD2 was found to be expressed only in cultured MSCs and not in primary MSCs (Sivasubramaniyan et al. 2013).

Several groups have reported that BMSCs are heterogeneous with respect to their growth and differentiation potential (Lv et al. 2014). However, little information exists about markers that discriminate between anatomically and functionally distinct MSC subsets. A few studies have defined CD146 and SSEA-3 as markers of perivascular BMSCs while CD56 and CD166 as markers of endosteal BMSCs (Sivasubramaniyan et al. 2012; Tormin et al. 2011). In addition, $CD56^+$ BMSCs give rise to osteoblasts and chondrocytes but not to adipocytes (Battula et al. 2009), while CD56-BMSCs give rise to osteoblasts and adipocytes but poorly to chondrocytes. This suggests that apart from the distinct surface antigen expression profile and differentiation potential, bone-lining and perivascular MSCs may also have distinct, yet to be identified, functional characteristics/properties.

2.2.1.3 Synovium-Derived MSCs (SM-MSCs)

In 2001, De Bari et al. introduced synovium as a source of MSCs that possess chondrogenic potential (De Bari et al. 2001). Recent work has shown subpopulations of MSCs to express different surface markers, such as CD105, CD166, CD90, CD9, and CD271 (Chang et al. 2013; Ogata et al. 2015; Van Landuyt et al. 2010). Chang et al. (2013) reported that CD105- and CD166-enriched cells derived from human synovium may be valuable sources for cartilage regeneration due to their enhanced chondrogenic potential. The most widely used BMSC marker CD271 is shown to be present in a specific subpopulation of inflamed synovium but not at all or barely present in expanded cell populations from the same patients (Van Landuyt et al. 2010; Ogata et al. 2015). In another study, Van Landuyt et al. (2010) reported the detection of CD34 expression on a subpopulation of $CD271^+$ MSCs, but the chondrogenic differentiation ability of this population is not known. So far, CD90 is the most commonly used marker for isolation of SM-MSCs. It has been used in combination with CD9/CD166 or CD271 to isolate a subpopulation of highly chondrogenic SM-MSCs (Ogata et al. 2015; Fickert et al. 2003). But these studies did not characterize the chondrogenic ability of all the MSC subpopulations from synovium, thus questioning the reliability of CD90 or CD271 in isolating highly chondrogenic SM-MSCs.

2.2.1.4 Synovial Fluid-Derived MSCs (SF-MSCs)

SF-MSCs form a pool of highly clonogenic cells with chondrogenic potential and are present both in healthy and OA joints (de Sousa et al. 2014; Jones et al. 2008). The exact origin of SF-MSCs is unclear. Morito et al. showed that they originate neither from BM nor from circulating MSCs but probably from the synovium or cartilage (Morito et al. 2008; Lee et al. 2012). The phenotype of freshly isolated human SF-MSCs is not yet known.

2.2.1.5 Infrapatellar Fat Pad-Derived MSCs (IF-MSCs)

Subcutaneous human adipose tissue is an interesting source of multipotent progenitors (Zuk et al. 2002). In recent days, also human infrapatellar fat pad is newly gathering attention as a source of MSCs (Khan et al. 2012). Jurgens et al. (2009) characterized stem cells in the freshly isolated stromal fraction based on the presence of the early marker CD34 and the absence of the endothelial marker CD31. These $CD34^{+}CD31^{-}$ cells were characterized further and shown to be additionally positive for the stem cell-associated markers CD29, CD54, CD90, CD105, and CD166. Others have also characterized the phenotype of IF-MSCs but only looked into culture-passaged cells. Thereby, certain markers such as CD34 were found to be negative, whereas in fact they are present on the surface of freshly isolated cells and lost upon culturing (Wickham et al. 2003). In another study, a small subpopulation of human IF-MSCs has been shown to express the pericyte marker 3G5, and anatomically these cells are localized to the perivascular region (Khan et al. 2008). In a recent study, CD44 was used to isolate IF-MSCs, and these freshly isolated IF-MSCs have been shown to have enhanced chondrogenic potential in vivo when seeded on a cartilage-extracellular matrix-derived scaffold (Almeida et al. 2015). These findings indicate that infrapatellar fat pad may be a promising cell source for endogenous MSCs.

2.2.2 Contribution to Repair by MSCs In Vivo

Until now, there is no clear blueprint of the host cell sources that participate in in situ cartilage regeneration. But more new knowledge is being gained in this field starting from the work of Hunziker and Rosenberg (1996) where for the first time a combination of fibrin, cells, and growth factors was used to repair a cartilage defect. For instance, Lee et al. (2010) showed that the articular surface of the synovial joint can be regenerated with TGF-β3 infused in a bioscaffold composite of poly-ε-caprolactone and hydroxyapatite. Though the source of endogenous stem cells bringing about the repair is not known, they speculate that the endogenous cells are derived from stem or progenitor cells of synovium, bone marrow, infrapatellar fat pad, and perhaps vasculature.

First approaches to heal cartilage by in situ regeneration date back as early as 1959 (Muller and Kohn 1999). The Pridie technique was directed at recruitment of BMSCs to cartilage defects by drilling small holes into the subchondral bone that underlies regions of damaged cartilage. It was refined later on, by reducing the size of the perforations, and was called microfracture technique which is now a frequently performed and well-studied procedure (Richter 2009; Steadman et al. 2001). While microfracture is restricted to lesions of 1–4 cm^2 size, autologous matrix scaffolds that induce chondrogenesis were developed and used to treat larger defects in combination with microfracture (Kramer et al. 2006) (for details see Chap. 5). Development of scaffolds with different materials and biophysical properties containing bioactive factors aiming to attract endogenous MSCs, especially BMSCs, is a growing field of research. In a study, Koelling et al. (2009) found some

evidence that chondrogenic cells migrate from the bone marrow to the cartilage defect through breaks in the tidemark. Hence, for full-thickness defects, the contribution of BMSCs is most likely, but for partial thickness defects, where direct access of BMSCs to the joint cavity is restricted, MSCs from synovium (Hunziker and Rosenberg 1996) and joint fat pads could be involved.

Cells derived from human synovium were shown to have the highest chondrogenic potential among the various mesenchymal tissue-derived cells, indicating a possible source for cartilage repair (Sakaguchi et al. 2005). Nishimura et al. (1999) showed that explants of synovium embedded in agarose undergo chondrogenesis when cultured in the presence of TGF-β1. The data indicate a possible synovial origin for the chondrocytic cells. Furthermore, these data are consistent with the clinical findings of synovial chondrogenesis leading to synovial chondromatosis (Nishimura et al. 1999). It was recently demonstrated by Kurth et al. (2011) that there is an in vivo presence of slow-cycling SMSCs in synovium of murine knee joints. Through iododeoxyuridine labeling and surface staining, these cells were shown to increase in number after cartilage injury, showing their activation in response to cartilage damage. Hence, homing of stem cells from synovium to sites of cartilage injury is an appealing concept that warrants further investigation.

Cartilage-derived progenitors have been observed in human, equine, and bovine articular cartilage (Jiang and Tuan 2015) and have been identified as slow-cycling cells by pulse-chase experiments in a murine model (Candela et al. 2014). In a recent study, Tong et al. (2015) confirmed the existence of CPCs in situ for the first time in murine model, both in normal and OA articular cartilage, and showed that these CPCs were activated from resting state in OA. Regarding the ability of articular cartilage to self-repair, they speculate that this ability probably depends on the presence of CPCs, as the number of transient-proliferating CPCs is synchronous with the OA progression in the early stage.

In the infrapatellar fat pad, MSCs are localized in the perivascular region, and hence, they can infiltrate into the synovial fluid through the synovium and participate in cartilage repair. The direct involvement of IF-MSCs in cartilage repair in situ is yet to be explored.

MSCs are present in the SF from knee joints of healthy individuals as well as from individuals with OA, RA, ligament injury, and meniscal injury (Jones et al. 2004, 2008; Matsukura et al. 2014; Morito et al. 2008). Diseased or injured joint SF has greater MSC numbers than healthy joint SF (Jones et al. 2004; Matsukura et al. 2014; Morito et al. 2008), with total number positively correlating with the period of injury (Morito et al. 2008) or severity of OA (Jones et al. 2004; Lee et al. 2012; Sekiya et al. 2012). These SF-MSCs are possibly derived from dislodged synovial fragments. This is particularly noteworthy since synovial stem cells have been proposed to contribute to spontaneous cartilage repair (Hunziker and Rosenberg 1996; Kurth et al. 2011), and synovial fluid could therefore be viewed as a possible conduit for their passage (Jones et al. 2008). Taken together, these findings provide a platform to explore the role of SF-MSCs in superficial cartilage homeostasis and repair.

2.3 MSCs In Vitro

It has been proposed that the best markers for identification of progenitor cells may be different between freshly isolated and culture-expanded cells. The first definitive markers of MSCs in vitro were proposed in the pioneering study of Pittenger et al. (1999) and included CD105 (SH2) and CD73 (SH3). These two markers alongside CD90 remain the primary molecules used to identify MSCs by the International Society for Cellular Therapy (ISCT) which also advices that MSCs should be negative for the expression of CD11b or CD14, CD19 or CD79a, CD34, CD45, and HLA-DR (Dominici et al. 2006). This is primarily to allow the exclusion of hematopoietic cells which may contaminate MSC cultures.

Cultured MSCs, however, are uniformly and strongly positive/negative for many markers regardless of their passage or time in culture. That many markers are expressed at similar levels in early- and late-passage MSCs indicates their value may be limited to basic MSC characterization only. The following is a concise review of in vitro markers of MSCs from different tissue sources (Table 2.1) and their correlation with chondrogenic potential.

2.3.1 Articular Cartilage-Derived MSCs (AC-MSCs)

Barbero et al. (2003) were the first to describe the existence of a subpopulation of cells within the dedifferentiated adult human articular chondrocyte population that, at clonal level, possess properties of MSCs, including tri-lineage differentiation potential. Hoechst 33342 dye uptake (Grogan et al. 2009) and adhesion to fibronectin (Williams et al. 2010) are utilized in an attempt to isolate this population of cells from the chondrocyte population. Equally, these cells can be isolated by their ability to migrate out of explants in vitro, although only from damaged cartilage (Koelling et al. 2009), in which they are present in higher numbers (Alsalameh et al. 2004).

AC-MSCs migrate from diseased cartilage in response to nerve growth factor (NGF) (Jiang et al. 2015) raising the possibility that these NGF-responsive cells are native $CD271^{+}$ cells, as CD271 expression is not expressed during in vitro expansion (Jiang et al. 2015; Koelling et al. 2009). In contrast, almost all clonal cells obtained by adhesion to fibronectin (Williams et al. 2010) and that have high chondrogenic capacity express CD49e (Grogan et al. 2007). Clonal cells with high chondrogenic capacity also express CD49c, CD49f, CD166, CD44, CD90, and CD151 higher than clonal cells with low chondrogenic capacity (Grogan et al. 2007); singly sorted $CD49c^{Bright}$ and $CD44^{Bright}$ cells have greater chondrogenic capacity than unsorted chondrocyte populations (Grogan et al. 2007).

Notch-1, Stro-1, and CD106 are highly expressed on clonal cells compared to articular chondrocytes (Ustunel et al. 2008; Williams et al. 2010); however, they are too widely expressed on mature chondrocytes in vivo to be useful (Grogan et al. 2009). Similarly, despite a CD105/CD166-enriched adherent population having chondrogenic capacity comparable to BMSCs, these markers are also expressed in vivo (Alsalameh et al. 2004). The combination of CD9/CD90/CD166 to obtain cells with chondrogenic differentiation capacity after culture may prove useful; however, further investigation is warranted (Fickert et al. 2004).

Table 2.1 Summary of the markers expressed by human MSCs in vitro

Marker	Percentage of positive cells and reference
B2microglobulin	BM: +++ [7]
BMPR1A	SF: – [16]
CXCR4	SF: – [16] FP: – [1]
D7-FIB	BM: +++ [7–8] SM: ++ [12] SF: ++ [16] FP: +++ [2]
FLK-1	BM: – [15] ± [9] SM: ± [9] SF: – [16]
Glycophorin A	BM: – [7] SM: – [11]
HLA-I	BM: +++ [7]
HLA-II	BM: – [7]
HLA-DR	BM: – [4] SM: – [11] SF: – [4] FP: ± [3–4]
KDR	SM: – [15] FP: – [1]
STRO-1	AC: ± [19] BM: – to + [15], ± to +++ [9] SM: – [11], – to + [15], ± to +++ [9] SF: + to ++ [16]
TGFβR11	BM:– [8] FP: – [2]
CD3	BM: – [7]
CD9	BM: +++ [7] FP: + [3]
CD10	BM: – to + [15], ± to +++ [9] SM: – [15], +/1 to ++ [9], ± to + [11], + to ++ [15] SF: – to + [16] FP: [3]
CD11a	SM: – [11] FP: – [3]
CD11b	BM: – [7] FP: – [3]
CD11c	FP: – [3]
CD13	BM: ± to + [7], +++ [4,8] SM: + to +++ [11], ++ to +++ [12] SF: +++ [4] FP: +++ [2,5]
CD14	BM: – [7] SM: – [11] FP: – [3]

(continued)

Table 2.1 (continued)

Marker	Percentage of positive cells and reference
CD15	BM: − [7]
CD16	BM: − [7]
CD18	BM: − [7] FP: − [3]
CD19	BM: − [7]
CD28	BM: − [7]
CD29	BM: +++ [7] SM: ++ to +++ [15] FP: ++ [3,5]
CD31	BM: −[7,10], − to ± [4,9] SM: − [11], − to ± [9] SF: − [4,16] FP: − [3,4], ± [5]
CD34	BM: − [4, 6–10], ± [9] SM: − [13], − to ± [9], − to + [11] SF: − [16,18], − to ± [4], + [17] FP: − [2], ± [1,3,4,6], + [5]
CD36	BM: − [4,7] SF: − [4] FP: ± [4]
CD38	BM: − [7]
CD44	BM: +++ [6,7,8,9,10] SM: ++ [10,14], ++ to +++ [11,15], +++ [9, 13] SF: ++ to +++ [16,18], +++ [4, 17] FP: +++ [2,3,4,5,6]
CD45	BM: − [6,7,8,9,10] SM: − [10,11,13], − to + [12], ± [9] SF: − [16,18] FP: − [1,2,3,5]. ± [6]
CD49a	BM: ++ to +++ [7] SM: − to ++ [15], ± to + [11]
CD49b	BM: ± to + [7]
CD49c	BM: ++ to +++ [7], +++ [4] SF: ++ to +++ [4] FP: +++ [4]
CD49d	BM: + to ++ [4] SM: − [11] SF: ++ [4] FP: +++ [4]
CD49e	BM: ++ to +++ [7] FP: ++ [3]
CD49f	BM: − [7], + [4] SF: + [4] FP: + [4]
CD50	BM: − [7] FP: ± [3]

Table 2.1 (continued)

Marker	Percentage of positive cells and reference
CD51	BM: +++ [7]
CD54	BM: ± to ++ [7], ± to +++ [9], + [4,10] SM: ± to +++ [9], + [15] SF: + [4], ++ [16] FP: ± [3], + to ++ [4]
CD55	BM: +++ [7] SM: − [11]
CD56	BM: − [7], ± [6] SM: − [11] FP: −[6], ± [3]
CD58	BM: ++ to +++ [7]
CD59	BM: +++ [4,7] SF: +++ [4] FP: +++ [3,4]
CD61	BM: − [7]
CD62	SM: − [11]
CD62e	BM: − [7] FP: + [3]
CD62L	BM: − [7]
CD62P	BM: − [7]
CD68	SM: − [11]
CD71	BM: − [7], + to ++ [4] SF: ± [4] FP: [4]
CD73	BM: +++ [4,8] SM: + to ++ [11,13], ++ to +++ [12,14,15] SF: ++ to +++ [18], +++ [4] FP +++ [1,2,4]
CD90	AC: +++ [19] BM: +++ [4,6,7,9,10] SM: ++ [10,13,14,15], +++ [9] SF: + to ++ [16], ++ [18] FP: +++ [1,4,5,6]
CD95	BM: − to ± [7] SF: +++ [4,17,18]
CD102	BM: − [7]
CD104	BM: − [7]
CD105	AC: ++ [19] BM: ++ to +++ [7,10], +++ [4,6,8,9] SM: + to +++ [13], ++ [12], ++ to +++ [10,14,15], +++ [9,11] SF: − to + [18], + to ++ [16], +++ [4] FP: ++ [5], +++ [1,2,3,4,6]
CD106	BM: − to + [15], ± to +++ [9], ++ [4], ++ to +++ [7] SM: − [11], − to + [10, 12,15], ± to +++ [9], + [13] SF: − [16], − to ± [4] FP: ± [4], + [2,3,5]

(continued)

Table 2.1 (continued)

Marker	Percentage of positive cells and reference
CD117	BM: − [7,8,10], − to ± [4,9] SM: − [10,11], − to ± [9] SF: − [4,16] FP: − to ± [4]
CD133	BM: − [7,8] SM: − [11] FP: − [1,2]
CD133/1	BM: − [4] SF: − [4] FP: − [4]
CD140b	BM: +++ [4] SF: +++ [4] FP: +++ [4]
CD146	BM: ++ [8] SM: − to + [12] FP: + [2]
CD147	BM: ++ to +++ [15] SM: ++ to +++ [15], +++ [9] SF: ++ [16]
CD151	BM: +++ [4,8] SM: ++ to +++ [14] SF: +++ [4] FP: ++ [5], +++ [2,4]
CD166	AC: ++ [19] BM: ± to + [7], ± to +++ [9], ++ [15], +++ [4,8] SM: ± [11], ± to +++ [9], + to ++ [10,13], ++ to +++ [12,15], ++ [14] SF: − to + [16], +++ [4] FP: ++ [3,5], +++ [2,4]
CD271	BM: − [8], − to ± [4,7,9,10], + [15] SM: − to ± [9], − to + [10,12], + [13] SF: − [4,16] FP: − to ± [4], + [2]

Percentage of positive cells: (+++) = 80–100%, (++) = 40–80%, (+) = 15–40%, (±) = 5–15%, (−) = 0–5%. When the authors reported different percentage of expression following the MSC expansion in vitro, a range of expression is indicated in the table. Due to the large number of literature available, for some of the listed markers only a representative selection of the literature is reported. References (see the reference paragraph for more details): [1] Lopez-Ruiz E. et al., *Osteoarthritis and Cartilage* (2013), [2] Jones E.A. et al., *Arthritis Rheum* (2004) and *Arthritis Rheum* (2008), [3] Wickham M.Q. et al., *Clin Orthop Relat Res* (2003), [4] Alegre-Aguaron E. et al., *Cells Tissues Organs* (2012), [5] Lopa S. et al., *Eur Cell Mater* (2014), [6] Ding D.C. et al., *Cell Transplant* (2015), [7] de la Fuente R. et al., *Exp Cell Res* (2004), [8] English A. et al., *Rheumatology* (2007), [9] Shirasawa S. et al., *J Cell Biochem* (2006), [10] Sakaguchi Y. et al., *Arthritis Rheum* (2005), [11] Jo C.H. et al., *Cytotherapy* (2007), [12] Karystinou A. et al., *Rheumatology* (2009), [13] Arufe M.C. et al., *J Cell Biochem* (2010), [14] Jones E. et al., *Ann Rheum Dis* (2010), [15] Han H.S. et al., *J Orthop Res* (2014), [16] Morito T. et al., *Rheumatology* (2008), [17] Lee D.H. et al., *Osteoarthritis and Cartilage* (2012), [18] Matsukura Y. et al., *Clin Orthop Relat Res* (2014), [19] Ozbey O. et al., *Acta Histochem* (2014).

AC articular cartilage-derived MSCs, *BM* bone marrow-derived MSCs, *SM* synovium membrane-derived MSCs, *SF* synovial fluid-derived MSCs, *FP* infrapatellar fat pad-derived MSCs

2.3.2 Bone Marrow-Derived MSCs (BMSCs)

The studies of Johnstone and Pittenger were the first to demonstrate and characterize individual clonal BMSCs and their chondrogenic or multi-lineage potential (Johnstone et al. 1998; Pittenger et al. 1999), establishing that not all clonal cells are capable of chondrogenesis. Colony cells were uniformly positive for CD105 (SH2), CD73 (SH3), CD29, CD44, CD71, CD90, CD106, CD120a, and CD124 and negative for CD14, CD34, and CD45, similar to their parent culture. From the beginning, therefore, these surface markers appeared not suitable for exclusive identification of BMSCs with high chondrogenic potential. Indeed, many of the surface markers characterized for BMSCs are acquired during culture and remain consistently expressed (Alegre-Aguaron et al. 2012). This is in contrast to the chondrogenic potential of these cells, which decreases with culture (Banfi et al. 2000; Bonab et al. 2006). Furthermore, CD105-sorted populations have been shown to possess similar chondrogenic potential and are comparable to the total, unsorted populations (Majumdar et al. 2000; Cleary et al. 2016).

Conversely, other markers, such as CD106, have been identified which appear more sensitive to culture conditions, including passage number, with declining expression in later passages (Fukiage et al. 2008). Moreover, CD106 may be a marker of MSC differentiation potential as it is strongly downregulated after chondrogenic differentiation (Gronthos et al. 2003). CD271 expression, present on a small percentage of freshly isolated BMSCs, also decreases with culture. However, this decrease occurs almost immediately during the first passage (Jones et al. 2006; Quirici et al. 2002; Mifune et al. 2013). Both $CD271^+$ and $CD271^-$ BMSCs, though, are capable of healing chondral defects in vivo, although $CD271^-$ repair ECM is less rich in proteoglycans (Hermida-Gomez et al. 2011; Mifune et al. 2013). Similarly, $CD146^+$ MSCs derived from bone marrow have higher CFU-F ability and proteoglycan content when compared to the total or $CD146^-$ population (Hagmann et al. 2014; Sacchetti et al. 2007; Kaltz et al. 2010). These observations require further investigation, however, as no difference in CFU-F potential and differentiation potential has been reported (Espagnolle et al. 2014).

2.3.3 Synovium-Derived MSCs (SM-MSCs)

Although similar to MSCs derived from other sources, synovium-derived MSCs often seem to possess superior chondrogenic potential and higher CFU-F numbers (Jo et al. 2007; Sakaguchi et al. 2005; Shirasawa et al. 2006). In vitro, chondrogenically differentiated synovium-derived MSCs acquire the expression of markers associated with the stable cartilage phenotype of articular cartilage-derived cells. This phenotype, however, is only transient, and chondrogenically differentiated SM-MSCs fail to form stable cartilage in vivo (De Bari et al. 2004).

Surface marker expression is characteristic of MSCs and is relatively stable during culture, with the exception of the first passage; CD14, CD34, CD45, and HLA-DR disappear, CD105 and CD166 appear, and CD10, CD13, CD44, CD49a,

and CD73 increase. Thereafter, only mild fluctuations in expression occur (Jo et al. 2007; Nagase et al. 2008). A $CD9^+/CD90^+/CD166^+$ population of synovium-derived cells, the total frequency of which increases with culture, represents an osteochondral progenitor population. There is, however, no difference between this and the unsorted population (Fickert et al. 2003). The $CD34^-/CD44^+/CD90^+$ subpopulation too possesses multipotent differentiation potential (Lee et al. 2012).

CD44 and CD90 expression alone correlates positively with chondrogenic potential (Jo et al. 2007; Jones et al. 2010), with CD90 expression correlating with pellet weight (Jo et al. 2007). CD90 is also expressed on >80% of $CD271^+$ MSCs from synovium (20–30% of the total population) which, along with $CD73^+$ cells, have better chondrogenic potential (Arufe et al. 2010; Harvanova et al. 2011). Indeed, $CD73^+$ cells also displayed the best osteogenic phenotype indicating that this surface protein is likely a marker of osteochondral cells. $CD105^+$ subpopulations also show chondrogenic potential, with expression also on >80% of $CD271^+$ cells (Arufe et al. 2009, 2010). A population of $CD271^+/CD90^+$ in combination with CD73, CD44, or CD105, therefore, may prove the most chondrogenic. Moreover, $CD14^+$ cells dampen the chondrogenic capacity of SM-MSCs. Depletion of this subpopulation is, therefore, likely to improve the chondrogenic potential of the starting population (Han et al. 2014).

2.3.4 Synovial Fluid-Derived MSCs (SF-MSCs)

Several studies have demonstrated no difference in chondrogenic potential for MSCs derived from SF, BM, and SM (Kurose et al. 2010; Lee et al. 2012). Indeed, SF-MSCs are assumed to be derived from either BM or SM, with current evidence weighing in the favor of an SM origin (Jones et al. 2004; Matsukura et al. 2014; Morito et al. 2008; Sekiya et al. 2012). However, others indicate that differences in chondrogenic potential may exist between SF-derived MSCs and MSCs derived from other sources, including having weaker chondrogenic potential than BM- or IF-derived MSCs (Alegre-Aguaron et al. 2012).

Akin to MSCs derived from other tissue sources, a proportion of cultured SF-MSCs can be $CD271^+$ (Jones et al. 2004), although at levels lower than MSCs from BM (Alegre-Aguaron et al. 2012; Jones et al. 2004). Similarly, cultured SF-MSCs express different levels of CD117, CD106, CD71, CD54, CD49c, HLA-DR, CD34, CD166, and CD133/1 to BMSCs (Alegre-Aguaron et al. 2012); SF-MSCs increase their expression of CD34 and CD49d, while decreasing their expression of CD71, CD106, and CD271 (Alegre-Aguaron et al. 2012).

While no difference is observed in the ability of $CD90^+$ and $CD90^-$ SF-MSCs to undergo osteogenesis or adipogenesis, greater chondrogenic potential exists in the $CD90^+$ fraction. This potential, however, is not superior to that of the total, unsorted population (Krawetz et al. 2012). $CD105^+$ SF-MSCs are also capable of tri-lineage differentiation (Harvanova et al. 2011), but further studies are required to understand whether this fraction is superior to the total or negative fraction.

2.3.5 Infrapatellar Fat Pad-Derived MSCs (IF-MSCs)

The presence of MSC-like cells within a cellular population derived from the infrapatellar fat pad (IPFP) was first described by Wickham et al. (2003). Such cells can be isolated in higher quantities from the IPFP than from other tissue sources (Khan et al. 2007) and are phenotypically comparable to MSCs, including their differentiation and CFU-F ability (English et al. 2007). IF-MSCs, however, have been described to maintain their chondrogenic differentiation capacity longer in culture than MSCs derived from other tissues (English et al. 2007). Moreover, several studies have identified higher chondrogenic capacity in this MSC source (Ding et al. 2015; English et al. 2007; Khan et al. 2008; Lopa et al. 2014). IF-MSCs seem to lack age-related declines in proliferative and differentiation potential (Khan et al. 2008), and similar to other MSC populations, when expanded in the presence of fibroblast growth factor-2 (FGF2), proliferation and chondrogenic potential is enhanced (Khan et al. 2008).

Cell surface marker expression of these cells indicates a profile similar, although not identical, to other MSC populations; IF-MSCs express relatively higher levels of CD34, CD45 (Ding et al. 2015), CD271 (English et al. 2007), and CD106 (Mochizuki et al. 2006; Lopa et al. 2014), with lower expression of CD146 (English et al. 2007) and CD10 (Mochizuki et al. 2006). The maintenance of CD271 expression on a subpopulation of cultured IF-MSCs raises the possibility that in vivo, MSCs present in the infrapatellar fat pad also express CD271 (English et al. 2007). This observation remains unclear, however, as CD271, CD45, and CD34 expression on cultured IF-MSCs is often described as negative (Khan et al. 2007, 2012; English et al. 2007). 3G5 is also observed on a subpopulation of these cultured IF-MSCs (Khan et al. 2007, 2008, 2012) showing that pericytes are present in the infrapatellar fat pad.

2.4 Manipulation of Joint Stem Cell Phenotype In Vitro by Growth Factors

Growth factors are often used as media supplement for MSC expansion. The first growth factor tested on human MSCs was FGF2 in 1997 (Martin et al. 1997), and few years later, its role in enhancing chondrogenic capacity was established (Mastrogiacomo et al. 2001; Tsutsumi et al. 2001). Although the capacity of FGF2 to improve proliferation and chondrogenic capacity of MSCs is evident and acknowledged by the scientific community, very little is known about the role of FGF2 and other growth factors in influencing surface marker expression.

Addition of FGF2 decreases the expression of CD146 and alkaline phosphatase (ALP) on BMSCs, and the effect seems reversible after removal of the growth factor, possibly indicating a direct effect of FGF2 on CD146 expression. The effect of FGF2 on CD146 and ALP expression was observed on both the whole population of BMSCs (Gharibi and Hughes 2012; Hagmann et al. 2013b) and on the CD146+ subpopulation (Sacchetti et al. 2007). The group of Paolo Bianco further studied the

effect of FGF2 and other growth factors on preselected CD146+ BMSCs, observing that FGF2 and platelet-derived growth factor-BB (PDGF-BB) also reduce the expression of CD105 and CD49a, and TGF-β enhances CD63 and α-SMA expression (Sacchetti et al. 2007). Epidermal growth factor (EGF) slightly increases ALP expression, and WNT3a treatment did not significantly influence cell surface marker expression (Gharibi and Hughes 2012). Surprisingly, none of those studies related the effect of growth factors on surface marker expression with the chondrogenic capacity of the cells. This was evaluated by Hagmann et al. (2013a) in a study comparing the use of different expansion media on BMSCs. However, the media used were supplemented with several different factors, and therefore, it is not possible to conclude on the effect of a single growth factor on surface marker expression or chondrogenesis. Only more recently, we observed that the expansion of BMSCs in the presence of WNT3a influenced the chondrogenic capacity of the cells and also their surface marker expression (Narcisi et al. 2015). However, the only direct effect observed was an enhanced number of CD271+ cells found in the cells treated with WNT3a. All the other markers tested were either unchanged by the treatment (CD73 and CD146) or maintained longer during the expansion in vitro (CD90, CD105, CD166), but they were not regulated directly. The ability of the BMSCs treated with WNT3a to enhance or retain the expression of certain surface markers in culture was linked by the authors to the capacity of the WNT3a-stimulated BMSCs to maintain, over multiple passages in vitro, a robust chondrogenic capacity.

Considering the other cell sources, to our knowledge only one article underlined the effect of growth factors on surface marker expression, demonstrating that FGF2 does not alter the expression of CD13, CD29, CD44, CD90, CD105, 3G5, STRO-1, CD34, and CD56 in IF-MSCs (Khan et al. 2008). However, again, no correlation with the chondrogenic capacity was reported.

Conclusion

In this chapter we provided an overview of the relation between surface marker expression and chondrogenic capacity of human adult MSCs from different sources. Since a large number of studies are available, in particular for BMSCs, we had to make a selection of the literature. However, two clear messages should be evident: (1) surface marker expression of cells in vivo strongly depends on tissue and cell localization, and (2) in vitro surface marker expression of expanded MSCs with high chondrogenic capacity is different between MSCs isolated from different sources. Several attempts to isolate, characterize, and purify populations of MSCs either directly from the tissue or after expansion in culture have not led to a clear description of MSCs with enhanced chondrogenic capacity. Moreover, especially for the culture-expanded MSCs, the use of different isolation techniques or expansion media could strongly influence the outcome. It is also interesting to note that MSCs derived from cartilage (CPCs), infrapatellar fat pad (IF-MSCs), and synovium (SD-MSCs) are still poorly characterized compared to BMSCs, and, moreover, only a very limited number of studies have directly compared the chondrogenic capacity of selected populations from different sources. Despite the use of surface markers for cell selection, the resulting

populations are still heterogeneous, which might explain the lack of success in finding the most suitable marker to select the best chondrogenic population of cells directly from the native tissue or after expansion in vitro. Therefore, researchers are continuously looking for new surface markers or new combinations of markers. However, we are convinced that additional efforts in exploring the use of alternative systems to purify or select chondrogenic cells with methods not necessarily based on surface marker expression might be required.

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