

REVIEW

Chondrogenesis of mesenchymal stem cells: role of tissue source and inducing factors

Stephane Boeuf and Wiltrud Richter*

Abstract

Multipotent mesenchymal stromal cells (MSCs) are an attractive cell source for cell therapy in cartilage. Although their therapeutic potential is clear, the requirements and conditions for effective induction of chondrogenesis in MSCs and for the production of a stable cartilaginous tissue by these cells are far from being understood. Different sources of MSCs have been considered for cartilage tissue engineering, mainly based on criteria of availability, as for adipose tissue, or of proximity to cartilage and the joint environment *in vivo*, as for bone marrow and synovial tissues. Focussing on human MSCs, this review will provide an overview of studies featuring comparative analysis of the chondrogenic differentiation of MSCs from different sources. In particular, it will examine the influence of the cells' origin on the requirements for the induction of chondrogenesis and on the phenotype achieved by the cells after differentiation.

Introduction

Articular hyaline cartilage has poor regenerative capacity, and the loss of its function is, in the long term, often painful and debilitating. Therefore, attempts have been made to intervene in cartilage defects with the objective of supporting biological repair of tissue. Alongside cell-based strategies for *in situ* regeneration [1], autologous chondrocyte transplantation was initiated as the first cell therapy for cartilage [2]. The requirement for biopsies from a healthy area of the cartilage cap and the necessity of surgical intervention prior to transplantation are evident disadvantages of this therapy, and multipotent mesenchymal stromal cells (MSCs) represent an appealing alternative cell source for cartilage repair.

The therapeutic potential of MSCs for cartilage repair is clear; however, the requirements and conditions for effective induction of chondrogenesis in MSCs and for the production of a stable cartilaginous tissue by these cells are far from being understood. Different sources of MSCs have been considered for cartilage tissue engineering, mainly based on criteria of availability, as for adipose tissue (AT), or of proximity to cartilage and the joint environment *in vivo*, as for bone marrow (BM) and synovial tissues. Focussing on human MSCs, this review will provide an overview of studies featuring comparative analysis of the chondrogenic differentiation of MSCs from different sources.

Definition of multipotent mesenchymal stromal cells

The presence of cells with osteochondral differentiation potential in BM was shown in the late 1960s [3]. Their isolation as the adherent mononuclear cell fraction of BM and *ex vivo* cultivation allowed their further characterization as colony-forming unit fibroblasts (CFU-Fs) [4]. This pioneering work in guinea-pig was followed by the identification of human BM CFU-Fs [5] and the demonstration of their osteogenic potential in diffusion chambers [6]. The *in vitro* differentiation of cloned MSC populations along the osteogenic, chondrogenic and adipogenic lineages demonstrated the multilineage potential of these cells [7].

The differentiation potential of MSCs was the feature that fostered their discovery and characterization. *In vivo*, MSCs function to support the homeostasis of mesenchymal tissues, and this mesengenic activity bears high therapeutic potential. However, it has been recognized in recent years that the potential therapeutic benefits of MSCs do not reside solely in their ability to differentiate towards multiple lineages but also in paracrine mechanisms [8]. In particular, the cardiovascular reparative effects attributed to MSCs appear to be mediated predominantly through the secretion of factors targeting cells at the site of repair [9]. Indeed, MSCs secrete a variety of bioactive molecules with trophic, immunomodulatory, anti-scarring and chemo-attractant activities [10]. New therapeutic strategies thus

*Correspondence: wiltrud.richter@med.uni-heidelberg.de
Research Centre for Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg, Schlierbacher Landstrasse 200a, 69118 Heidelberg, Germany

include transplantation of MSCs for the promotion of haematopoietic engraftment or for immunosuppression in graft-versus-host disease [11]. Whether beneficial effects can be expected from the immunomodulatory activities of MSCs for the treatment of rheumatic arthritis is still under debate [12].

The term 'mesenchymal stem cells' was proposed by Caplan [13] on the basis of the ability of these cell populations to differentiate towards tissues of mesenchymal origin. Based on different isolation methods to obtain MSCs or subpopulations of MSCs, investigators have given different names, such as bone marrow stromal cells (BMSCs) [14], marrow-isolated adult multipotent inducible (MIAMIs) cells [15] or multipotent adult progenitor cells (MAPCs) [16] to these cell populations. The International Society for Cellular Therapy proposed the term 'multipotent mesenchymal stromal cell' for the plastic-adherent cell population isolated from BM or other tissues, thus avoiding use of the term 'stem cells' to designate a population that does not consist entirely of such cells [17].

Indeed, early evidence for heterogeneity of MSC populations in terms of morphology, growth characteristics and differentiation potential has been reported [18]. MSC populations are heterogeneous cell populations whose composition depends on isolation methods and expansion conditions that differ largely among investigators. A recent publication on cloned populations of MSCs showed that nearly 50% of CFU-Fs from BM were tripotent MSCs while the remaining population of cells showed varied phenotypes [19].

So far, no clear marker for MSCs has been identified. The criteria for the definition of MSCs, as set by the International Society for Cellular Therapy, are the ability of MSCs to adhere to plastic in standard culture conditions, their phenotypical characterization based on the expression of a set of surface antigens and their *in vitro* differentiation along the osteogenic, the adipogenic and the chondrogenic lineages [20]. The ability to form CFU-Fs is another commonly accepted criterion. However, none of these criteria is unequivocal and only their combination can be used to define MSC populations.

Sources of MSCs in different organs

While it was the characterization of BM stromal cells that introduced the concept of MSCs, analysis of progenitor cell populations isolated from other tissues showed that they shared the properties ascribed to BM MSCs. This was not restricted to mesodermal tissues, on which this review will focus, as MSCs have also been isolated from ectodermal tissues, such as skin or hair follicles, as well as from perinatal tissue and umbilical cord blood [21].

The multilineage differentiation potential of a cell population can be due to a mixture of different

committed progenitor cells. Therefore, to demonstrate the presence of multipotent cells in a cell population, it is essential to show the multilineage potential of cloned cell populations. A tissue other than BM whose osteogenic properties were described at an early stage was the periosteum [22], in which the presence of clonogenic multipotent cells has been demonstrated [23]. The presence of MSCs was also demonstrated in stromal cells isolated from AT [24]. The synovial membrane (SM) appeared a particularly interesting source of cells for cartilage tissue engineering owing to its proximity to articular cartilage. The presence of MSCs with multilineage potential was shown in the SM of healthy and osteoarthritic patients [25]. A more extended analysis of clonal populations of SM MSCs distinguished two populations: 30% of cells were tripotent while the remainder displayed only osteo-chondral differentiation potential [26]. This heterogeneity could be linked to the presence of synovial fibroblasts among SM MSC populations. The presence of MSCs has also been demonstrated in the synovial fluid of healthy and arthritic patients [27]. Dedifferentiated chondrocytes from articular cartilage of healthy and osteoarthritic donors have been shown to exhibit MSC characteristics. The reported rates of tripotent cloned cell populations arising from them varies from 10% to 30% [28,29]. In trabecular bone, cell populations with chondrogenic, osteogenic and adipogenic differentiation potential have been isolated [30], but the multidifferentiation potential of cloned populations has been shown only along the adipogenic and osteogenic lineages [31].

Putative MSC populations have also been isolated from other tissues, for which, to our knowledge, the demonstration of clonal multilineage potential has not been provided. These tissues include muscle [32] as well as joint-related tissues such as meniscus, intra-articular ligament [33] and infrapatellar fat pad [34].

Origin of MSCs *in vivo*

MSCs are defined and characterized as cultured cell populations. The low numbers of initial cells isolated from tissue and the lack of unequivocal markers hampered the investigation of their physiological location and function *in vivo*. Pericytes, a cell type found in close proximity to endothelial cells in capillaries and microvessels, have been reported to possess stem cell properties [35,36] and this gave rise to the hypothesis that MSCs and pericytes might represent one cell type. Markers for pericytes include the adhesion molecule CD146, whose expression in BM is restricted to adventitial reticular cells in the subendothelial layer of sinusoids [37]. A CD146-positive population of subendothelial cells from human BM stroma is the first and so far only MSC entity for which true self-renewing capacity has been demonstrated [37].

The possible identity of MSCs and pericytes is not restricted to BM. Based on the expression of CD146 and other markers, pericytes have been identified in multiple human organs, including skeletal muscle, pancreas, adipose tissue and placenta, and clonogenic populations from these cell populations have been found to display osteogenic, chondrogenic and adipogenic differentiation potential [38]. Possibly, MSCs derived from all vascularized tissues, such as BM, AT, trabecular bone, periosteum and SM, could share a common origin as perivascular cells. Evidence for this common origin of MSCs is so far based on similarities between MSCs and pericytes and on the shared expression of marker genes. Further investigations are needed to test this hypothesis.

However, cells with MSC characteristics have also been isolated from articular cartilage, which is an avascular tissue [28,29]. Provided the tissue of origin was not contaminated, a perivascular origin of these cells can be excluded. It is likely, therefore, that cells other than perivascular cells can contribute to multipotent MSC populations.

Numbers of MSCs in different tissues

In relation to tissue mass, yields of adherent stromal cells from BM and AT have been described as similar, with an average 2×10^5 cells per gram of tissue [39]. As AT MSCs are most frequently isolated from lipoaspirates, no correlation to initial tissue mass is possible. The total number of nucleated cells is much higher in BM than in AT and, accordingly, investigators have reported higher amounts of CFU-Fs per total cell number in AT [33,40]. Identical yields of CFU-Fs per total cell number were shown from periosteum and from AT [41], while more CFU-Fs could be isolated from SM than from subcutaneous fat [41,42] or infrapatellar fat [43].

The analysis of differences in the growth kinetics of MSCs from different sources would require precise monitoring of initial cell numbers. It is not clear whether longer growth potential of AT MSCs than of BM MSCs before senescence, as suggested by some studies [40,44,45], can be convincingly demonstrated. The better growth characteristics sometimes reported for AT MSCs may, instead, be linked to higher initial cell numbers. In terms of accessibility and yield of adherent cells, AT indeed appears one of the most attractive sources of MSCs for therapeutic use.

Molecular characterization of MSCs

No specific marker combination for MSCs has been identified so far. However, immunophenotypical profiles of expanded MSCs from different sources have generally been found to be very similar. The analysis of MSCs from BM, AT, SM and periosteum showed that these cells can be characterized by the absence of expression of surface

Table 1. Surface markers for which different expression profiles in human MSCs from adipose tissue (AT), bone marrow (BM) and synovial membrane (SM) have been reported

AT > BM*	BM > AT	SM > BM	BM > SM
CD34 [51]	CD106 [40,61] CD146 [61] HLA-ABC [61]	CD140a [64]	CD90 [48]

*Higher expression in multipotent mesenchymal stromal cells from adipose tissue than from bone marrow. AT, adipose tissue; BM, bone marrow; HLA, human leukocyte antigen; SM, synovial membrane.

markers for the haematopoietic lineage, such as the cluster of differentiation (CD) molecules CD14, CD31 or CD45, and by positivity for a panel of markers, including CD13, CD29, CD44, CD73, CD90, CD105, CD147 or CD166 [33,40,41,44,46-53]. Results from flow cytometric analysis of MSCs showing significant differences between different sources are summarized in Table 1.

While absence of CD34 is generally considered as a criterion for the definition of MSCs [20], some investigators have reported low expression in AT MSCs [46,51,53] and one group described selected cell populations with multidifferentiation potential from AT using CD34 [54]. The presence of a pericytic CD34-positive subpopulation in AT has been shown, but it has not yet been determined by cloning studies whether these cells indeed bear stem cell characteristics [55,56]. While the stem cell marker CD133 is not expressed in expanded MSC populations obtained by adhesion to plastic [40,47], the isolation of CD133-positive cell populations from blood and BM with high proliferation potential and multilineage potential, including mesodermal lineages, has been described [57,58]. CD271, a marker that is highly expressed in BM and AT MSCs and allows the isolation of MSC populations from primary tissues [54,59], has been reported not to be expressed in SM MSCs [26,60]. However, to our knowledge, no direct comparison of stem cell populations from different sources has been performed for this marker. Several studies have pointed to higher expression of CD106 in BM than in AT [40,46,53,61]. The vascular cell adhesion molecule CD106/VCAM1 has been shown to be involved in homing of haematopoietic stem cells (HSCs) [62]. This difference may, therefore, be related to the specific microenvironment in BM and has indeed been correlated with a functional difference between AT and BM MSCs, the latter showing a higher capacity to maintain long-term cultures of primary HSCs [63]. Another marker with potential functional relevance is the platelet-derived growth factor receptor CD140a/PDGFR α , which is involved in proliferation and migration of MSCs and osteoblasts and has been

described in one study to be expressed more highly in SM than in BM [64].

Comparative array analysis of expanded MSCs from different sources was published by several groups and provided, overall, very similar expression profiles of MSC populations [44,47,48,65-67]. Interestingly, intra-articular MSCs from SM and MSC-like cells from anterior cruciate ligament and meniscus were found to cluster separately from AT, BM and muscle MSCs [33]. Similar results were found by two-dimensional gel electrophoresis analysis of the proteome of MSCs, where the expression profiles of AT and BM MSCs were closer to each other than either was to SM MSCs [53]. While the functional heterogeneity of SM MSCs has been characterized [26], it remains unknown for other intra-articular sources of cells. It is not clear, therefore, whether the separate clustering of SM MSCs is due to particular characteristics of MSCs from the joint environment or to a higher heterogeneity of these populations. Altogether, the comparative transcriptome analyses of MSCs from different sources have revealed few differences, suggesting that these cell populations contain a common population of similar cells.

Epigenetic characterization of MSCs

Large-scale analysis of DNA methylation in embryonic and adult stem cells has shown that embryonic stem (ES) cells can clearly be discriminated from MSCs by specific hypermethylation of numerous genes. In contrast, the comparison of AT and BM MSCs revealed few differences [66]. A comparison of DNA methylation profiles in MSCs from AT, BM and muscle and in HSCs also revealed specific hypermethylation of numerous genes in HSCs while the methylation patterns of MSCs from different sources were very similar. Most promoters specifying mesodermal, endodermal and ectodermal differentiation were hypomethylated in all MSC populations [68]. This suggests that promoter hypomethylation is not predictive for the differentiation potential of cells, while hypermethylation sets restrictions that define frames for differentiation potentials, distinguishing MSCs from ES cells or HSCs.

Accordingly, genes related to the adipogenic and myogenic lineage were found to be equally hypomethylated in MSCs from AT, BM or muscle [69], and in an analysis of the methylation patterns in the promoters of *COL2A1* (collagen type II gene) and *COL10A1* (collagen type X gene) in MSCs we found no differences between BM- and AT-derived MSCs [70]. However, two cytosines in the *COL10A1* promoter were consistently hypomethylated in MSCs in comparison with articular chondrocytes, correlating to the inducibility of *COL10A1* expression and hypertrophy during *in vitro* chondrogenesis of MSCs [70]. Differences between the DNA methylation patterns of differentiated cells originating

from embryonic precursors and MSCs could thus be of functional relevance for tissue engineering.

Post-translational histone modifications have been mapped in ES cells and in MSCs and have been recognized to play an important role in transcriptional regulation in stem cells [71]. To date, no comparative analysis of MSCs from different sources has been published. Histone modifications and histone-modifying molecules are regulated, while MSCs enter senescence *in vitro* and could be involved in the ensuing loss of differentiation potential [72]. They are also actively involved in differentiation. Several studies have indicated that histone deacetylases, in particular HDAC4, may represent important regulators of chondrogenesis [73,74].

MicroRNAs represent a further epigenetic regulation mechanism relevant for stem cell biology [75]. The comparison of the microRNA expression profiles of MSCs from BM and AT revealed that only one microRNA was differentially expressed while the differences with ES cells were high [66]. Studies have shown regulation of the expression of microRNAs in MSC senescence [76] and chondrogenic differentiation [77], but the functional mechanisms are unknown. The epigenetic characterization of MSCs is a relatively new field of investigation that has so far revealed only minor differences between MSCs from different sources at all levels. Refinement of the analysis of profiles may, however, lead to an epigenetic definition of MSCs, which could have the advantage of correlating with the functional potential of the cells.

Induction of chondrogenic differentiation of MSCs *in vitro*

During embryogenesis the development of cartilage is initiated by a phase of condensation of mesenchymal precursor cells, and the cell-cell contact arising from condensation appears to be crucial for the onset of chondrogenesis [78]. N-cadherin seems to be involved in cell-cell contact in pre-cartilage condensations, and functional N-cadherin was necessary for chondrogenesis of chick limb mesenchymal cells *in vitro* and *in vivo* [79]. In human MSCs, N-cadherin is strongly up-regulated during the condensation phase during the first few days of chondrogenic induction *in vitro* [80]. When MSCs are submitted to chondrogenic conditions in monolayer culture, they begin to condensate in response to the stimulus and form high-density three-dimensional cell aggregates [65]. However, proper chondrogenic differentiation occurs also for MSCs embedded in gel-like biomaterials that keep cells apart from each other and thus limit direct cell-cell contact [81]. This suggests that, although cell-cell contact facilitates chondrogenic induction of MSCs compared with monolayer culture, it does not represent an absolute requirement for *in vitro*

Table 2. Growth factors reported as full inducers (I) or promoters (P) of chondrogenic differentiation of human MSCs in pellet culture *in vitro*

Factor	BM MSC		AT MSC		SM MSC	
	I	P	I	P	I	P
TGFβ1	[84,96,98]					
TGFβ2	[84]					
TGFβ3	[40,61,65,83, 89,90,99]		[40]			
BMP2	[85,109]	[85,90]	[110]	[89]		[41,105]
BMP4		[90,91]		[89]		
BMP6		[45,87,90,92]	[86]	[45,89]		[52]
BMP7		[90]				[111]
IGF1		[87,90,97]		[103]		[112]

I, factors inducing proteoglycan and collagen type II deposition in pellets according to histology when added as single factors to chondrogenic basal medium; P, factors promoting proteoglycan and collagen type II deposition in pellets when added in combination with TGFβ to chondrogenic basal medium. The list of publications is not exhaustive. AT, adipose tissue; BM, bone marrow; BMP, bone morphogenic protein; IGF, insulin-like growth factor; MSC, multipotent mesenchymal stromal cell; SM, synovial membrane; TGF, transforming growth factor.

chondrogenic differentiation of human MSCs in a three-dimensional structure.

One of the most widely applied culture systems for chondrogenesis is pellet culture, alternatively termed aggregate or spheroid culture [82,83]. Pellets comprising between 200,000 and 500,000 cells, depending on the investigators, are submitted to chondrogenic induction with a basal medium containing, conventionally, dexamethasone, ascorbate, insulin, transferrin and selenous acid [82,83]. The classic growth factor supplementation for this medium is 10 ng/ml of transforming growth factor (TGF)β. TGFβ1, 2 and 3 are the only well-established full inducers of chondrogenesis that lead to deposition of proteoglycan and collagen type II when added as single factors [83,84]. Although other inducers of chondrogenesis, such as the bone morphogenic proteins BMP2 for BM MSCs and BMP6 for AT MSCs, have been described [85,86], this has not been confirmed by other investigators [65,87-90] and may apply only to MSCs from selected donors. Rather, BMP2 [85], BMP4 [91], BMP6 [92] and the insulin-like growth factor IGF1 [87] may be regarded as promoters of chondrogenesis in MSCs when used together with the inducer TGFβ. Table 2 gives an overview of studies characterizing growth factors as full inducers or promoters of chondrogenesis in MSCs. Besides soluble factors, environmental factors such as mechanical stimulation [93] and hypoxia [94,95] have also been reported to modulate chondrogenesis of MSCs *in vitro*.

With some exceptions [40], most studies undertaking a direct comparison of BM and AT MSCs have described a lower chondrogenic differentiation potential of AT MSCs in pellet culture under induction with TGFβ1 or 3 alone [61,65,96] or with TGFβ2 and IGF1 [97], including studies using cells isolated from the same patients [98,99]. Cultures in alginate beads [50,100], hyaluronic

acid scaffolds [101] and cartilage-derived matrix [100] also showed a lower response of AT MSCs to TGFβ-driven chondrogenic induction.

Looking for factors that might explain the reduced inducibility of AT MSCs with TGFβ, we analysed the expression of relevant growth factors in expanded MSCs from BM and AT and found reduced expression of BMP2, 4, 6 and the TGFβ receptor 1 (TGFBR1) in AT MSCs [89] and enhanced levels of the integral membrane protein 2A (*ITM2A*) gene [102]. The high expression of *ITM2A* during the early phase of the induction of chondrogenesis correlated to inhibition of chondrogenesis, and forced overexpression of *ITM2A* was indeed able to inhibit chondrogenesis in a mouse cell line [102].

One strategy explored for the enhancement of chondrogenesis in AT MSCs was to increase concentrations of TGFβ. While we found that concentrations up to 50 ng/ml did not enhance the chondrogenesis of AT MSCs [89], another group found that a combination of 25 ng/ml TGFβ2 and 500 ng/ml IGF1 induced a chondrogenic phenotype in AT MSCs similar to that induced by 5 ng/ml TGFβ2 in BM MSCs [103]. A second strategy arising from the differences in the growth factor repertoire of AT and BM MSCs was to add BMPs for the induction of chondrogenesis. The addition of BMPs at a concentration of 10 ng/ml indeed enhanced the chondrogenic differentiation of AT MSCs. Among the BMPs the most potent inducer was BMP6, which eliminated the differences in differentiation potential between AT and BM MSCs [45,89,100].

The third source of MSCs often considered for applications in cartilage tissue engineering is the SM. While the chondrogenic potential of SM MSCs was initially described with TGFβ1 as inducing factor [25,104], another laboratory found no induction of chondrogenesis in pellet cultures with TGFβ3 alone, but

only with a supplementary high-dose BMP2 treatment [105]. Under these conditions with TGF β 3 and high-dose BMP2, chondrogenic differentiation was higher in MSCs from BM, SM and periosteum than in those from muscle and subcutaneous AT [41,42]. We found the response of SM MSCs to chondrogenic induction with TGF β 3 alone to be higher than that of AT MSCs, but lower than that of BM MSCs. While TGF β 3 was able to induce chondrogenesis in only 50% of SM MSC populations from distinct donors, 100% of SM MSCs responded when TGF β 3 was combined with 10 ng/ml BMP6 [52].

The requirements for the induction of chondrogenesis in MSCs from different sources thus appear to differ in terms of growth factors. The comparative analysis of AT and BM suggests these different requirements may be related to differences in the growth factor repertoires expressed by the cells or to active pathways at the time point of the initiation of chondrogenesis, which may depend on their microenvironment *in vivo*.

Hypertrophic differentiation of MSCs *in vitro*

Despite differences in the conditions necessary for effective induction of chondrogenesis, the chondrogenic phenotype and molecular profile achieved by AT and BM MSCs under appropriate conditions were found to be similar [65,89]. The chondrogenic induction of MSCs in pellet culture with TGF β 3 is accompanied by an undesired up-regulation of hypertrophy-associated marker molecules, such as collagen type X and the matrix metalloproteinase MMP13, and by an activation of alkaline phosphatase (ALP) activity *in vitro* [65,96,100,106]. After ectopic transplantation into subcutaneous pouches of severe combined immunodeficient (SCID) mice, the hypertrophic phenotype of differentiated pellets of both AT and BM MSCs leads to pronounced matrix calcification accompanied by vascular invasion and even micro-ossicle formation [89,106]. Common *in vitro* protocols of chondrogenesis thus produce MSC-derived chondrocytes that undergo premature hypertrophy and develop into transient endochondral cartilage, instead of stable articular cartilage-like tissue. As AT MSCs, in spite of their origin, mineralized their surrounding matrix *in vivo* to an extent similar to BM MSCs, the predisposition for osteogenesis and matrix calcification does not appear to be due to an origin of MSCs from bone.

In vitro, SM MSCs showed a tendency identical to those of MSCs from AT and BM to induce expression of osteogenic genes [105] and collagen type X after chondrogenic differentiation [52]. Under TGF β 3 and BMP6, the mean up-regulation of ALP activity was lower in SM MSCs than in AT and BM MSCs, but ALP activity in SM MSCs cell populations displayed extremely high donor variability compared with other MSCs, ranging from negative to very strong signals [52]. *In vivo*, cell

populations that showed low ALP activity *in vitro* displayed low calcification, and this was surprisingly accompanied by a loss of already deposited collagen type II protein, possibly due to high MMP2, 3 and 13 activity. The MSCs in these transplants thus lost their differentiated phenotype, while SM MSCs from other donors, which displayed high ALP activity *in vitro*, showed calcification in a similar way as AT and BM MSCs [52]. The cause of this variability of phenotypes in SM MSCs after chondrogenic induction is unknown. Although SM MSCs show a different phenotype than AT and BM MSCs after chondrogenesis *in vitro*, their origin from the joint environment appears not to be sufficient to program them towards a stable chondrogenic phenotype.

Undesired hypertrophic development of expanded MSCs seems no concern *in vivo* in a cartilage microenvironment where expanded animal MSCs spontaneously mature into collagen type II-positive and collagen type X-negative chondrocytes [107]. *In vitro* co-culture experiments with articular chondrocytes demonstrate that the hypertrophic differentiation of MSCs can be inhibited by soluble factors secreted from chondrocytes, and parathyroid hormone-related protein (PTHrP) may be a candidate molecule for involvement in this inhibition [108]. PTHrP₁₋₃₄ displays an inhibitory action on the TGF β -induced hypertrophic differentiation of MSCs *in vitro* [90]. As a counteractor of Indian hedgehog, which is up-regulated during chondrogenesis of MSCs, it could represent an important factor for the stabilization of an articular phenotype in MSCs. Further *in vivo* and co-culture experiments may enable identification of factors that are active in the microenvironment of cartilage and have the ability to lock cells in a hyaline chondrogenic stage.

Conclusion

Despite the growth of knowledge on the origin and composition of MSC populations from different tissues, their heterogeneity is poorly understood. Transcriptional and epigenetic analyses of different MSC populations reveal very similar profiles. However, differences in expressed growth factors or active pathways between MSCs from different sources could explain the different requirements for the induction of chondrogenesis. Conditions allowing efficient chondrogenic *in vitro* differentiation of MSCs from AT, BM and SM have been described. For long-lasting cell therapy in cartilage it is, however, essential to be able to achieve a stable chondrogenic phenotype. The endochondral pathway triggered in MSCs during *in vitro* chondrogenesis and the fibrous dedifferentiation observed for some SM MSC populations therefore merit more thorough analysis. A better understanding of articular cartilage differentiation should permit determination of the conditions necessary for stable chondrogenic differentiation of MSCs.

Abbreviations

ALP = alkaline phosphatase; AT = adipose tissue; BM = bone marrow; BMP = bone morphogenic protein; CD = cluster of differentiation; CFU-F = colony-forming unit fibroblast; ES = embryonic stem; HSC = haematopoietic stem cell; IGF = insulin-like growth factor; MMP = matrix metalloproteinase; MSC = multipotent mesenchymal stromal cell; PTHrP = parathyroid hormone-related protein; SM = synovial membrane; TGF = transforming growth factor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors contributed to the writing of the manuscript and read and approved the final version.

Acknowledgments

This work was financially supported by the Deutsche Forschungsgemeinschaft (grant number RI707/7-1) and by the EuroBoNeT consortium, a European Commission FP-6 granted Network of Excellence for studying the pathology and genetics of bone tumours.

Published: 13 October 2010

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doi:10.1186/srct31

Cite this article as: Boeuf S, Richter W: Chondrogenesis of mesenchymal stem cells: role of tissue source and inducing factors. *Stem Cell Research & Therapy* 2010, **1**:31.