Mesenchymal Stem Cells and Their Cell Surface Receptors

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Abstract: Daily increasing evidence indicates that stem cells can be found in nearly every tissue. Mesenchymal stem cells (MSCs) are adult stem cells, which reside in the bone marrow and other mesenchymal tissues. MSCs can be expanded to large numbers and can be driven into diverse mesenchymal cell lineages, including chondrocytes. Therefore, MSCs have attracted the attention of the biomedical community as very promising tools for repair of joint tissues, such as articular cartilage. This review will outline the MSC surface receptors and will focus on receptors that deliver important signals for chondrogenic differentiation of MSCs. Finally, the role of receptors in the progression of cartilage degeneration disorders, such as osteoarthritis (OA), will be discussed.

Keywords: Mesenchymal stem cells (MSCs), receptors, chondrogenic differentiation, osteoarthritis (OA).

INTRODUCTION

Legal and moral controversies regarding the therapeutic and clinical use of pluripotent embryonic stem cells have prompted the quest for progenitor cells harboured within adult tissues [1]. Mesenchymal stem cells (MSCs) are adult stem cells that can be isolated from a variety of tissues, most commonly from the bone marrow (BM). Although, MSCs are found in very small quantities *in vivo*, they can be easily expanded *in vitro*. MSCs are multipotent and, as such, they can give rise to a variety of mesenchymal phenotypes, including chondrogenic cells. Up to now, no definitive characteristic marker has been identified for MSCs. However, MSCs express a large number of surface receptors associated with their function, and therefore this review will start with a brief description of major receptor groups found on MSCs.

SURFACE RECEPTORS DETECTED ON MSCs

Phenotypic Receptors

MSCs constitute a heterogeneous population of cells in terms of their morphology and expression of surface antigens [2, 3]. Hence, no surface antigen individually, or in combination, has been unified in the literature as a "supreme" MSC marker for MSC identification and enrichment. Furthermore, the majority of data concerning the phenotypic properties of MSCs is based on analyses of *in vitro* expanded cells, whereas little is known about their *in vivo* phenotype.

Several antibodies have been raised against MSCs in an effort to better characterize them. For instance, the monoclonal Stro-1 antibody was shown to react with non-haematopoietic BM cells [4]. Stro-1 recognizes a trypsin-resistant cell surface antigen present only on a subpopulation of MSCs that is capable of osteogenic differentiation [5]. The antibodies against CD73 (membrane-bound ecto-5′-nucleotisidase), CD90 (Thy-1), CD105 (endoglin) and CD166 (ALCAM) were also reported to react with undifferentiated MSCs and thus seem suitable for isolation of more pure MSC population [6-8]. CD271 (low affinity nerve growth factor receptor, LNGFR) was also used for the enrichment of MSCs, particularly from BM [9]. Interestingly, CD271 expression disappears upon *in vitro* cultivation of MSCs [10], suggesting that the inductive stimuli are absent in the standard culture media and that CD271 may have a morphogenic role in the development of the BM stroma. Moreover, a very recent study searching for additional MSC markers [11], has shown that established, as well as, novel MSC antibodies (W1C3, W3D5, 9A3 and etc.) recognize only the CD271-positive population but no other BM cells. Thus, the authors concluded that CD271 is the most specific marker for BM-derived MSCs, so far. Other intriguing reports proposed that perivascular cells might be the MSC precursors and that CD146 (MCAM) can be also employed as a phenotypic marker for MSCs [12, 13]. A very recent report from Bianco's group [14] showed that indeed CD146 expression distinguishes BM-derived MSCs from other osteogenic and non-osteogenic cell strains.

Unfortunately, all these antigens can also be found on other cell types and furthermore some can be differently expressed depending of the source of MSCs. For example, adipose tissue-derived MSCs have much lower CD106 expression and lack Stro-1 antigen in comparison to MSCs that have originated from BM [15, 16]. MSCs do not express haematopoietic and endothelial cell markers: CD11, CD14, CD31 (PECAM-1), CD33, CD34, CD45 and CD133 [8, 17].

Without a definitive marker and better distinction of the MSC subtypes, it remains an obstacle to firstly, generalize the already accumulated research data on MSC and secondly, study the *in vivo* behaviour of endogenous or implanted MSCs. In this respect, one attempt to standardise the phenotypic characterization of MSCs came for the International Society for Cellular Therapy (ISCT). The ISCT has proposed that MSC populations must be positive at least for several antigens, such as, CD73, CD90 and CD105. Additionally, these cells must lack the expression of haematopoietic antigens like CD45, CD34 and markers for monocytes, macrophages and B cells [18].

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Growth Factor Receptors

MSCs can be influenced *via* a multitude of growth factor receptors that have been identified on their surface. EGFR, bFGFR, IGFR, PDGFR, TGFβRI and RII have been reported to be important for MSCs self-renewal and differentiation [19]. Apart of TGFβ receptors, the other growth factor receptors mentioned belong to the family of receptor tyrosine kinases (RTKs). Most RTKs are single subunit receptors but some, namely IGFR, exist as multimeric complexes [20]. Both types of TGFβ receptors are single-pass transmembrane serine/threonine kinases. The TGFβRII is a constitutively active kinase capable of both autophosphorylating and transphosphorylating an associated TGFβRI. This results in the formation of a heteromeric TGFβ complex and type I receptor activation of downstream signalling molecules [21].

Chemokine Receptors

Several studies have underlined the pivotal role of chemokines and their corresponding receptors in homing, migration and engraftment of MSC to sites of injury but also to sites of neoplasia. The chemokine receptors are classified as G-protein-coupled receptors for CXC, CC, C or CX3C chemokines [22]. One characteristic feature of chemokines is that several chemokines bind to more than one receptor and the majority of chemokine receptors have multiple possible ligands. To date, MSCs are known to express CCR1, CCR2, CCR4, CCR6, CCR7, CCR9, CCR10, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6 and CX3CR1 receptors, and to secrete a variety of chemokines [23, 24]. However, the reported chemokine receptor repertoire of MSCs has been inconsistent. This might be due to the heterogenic nature of the MSC population. Nonetheless, the stimulating role of some chemokines, for instance CXCL12 (also known as SDF-1), has been demonstrated not only *in vitro* but also in injury models *in vivo*. For example, Abbott *et al.* [25] administered MSCs in mice with induced myocardial infarction and *via* antagonizing CXCR4, the receptor for CXCL12, the migration of MSCs to the infarct zone was inhibited. Furthermore, Ji *et al.* [26] have observed in a rat nerve injury model that MSCs get targeted to the avulsed hypoglossal nucleus also by the CXCL12 – CXCR4 axis. Using the chemokine trajectories, MSCs can be misled to sites of *de novo* tissue formation and thus can contribute to tumour outgrowth. Nakamizo *et al.* [27] observed *in vivo* integration of MSCs into human glioma xenografts and matrigel invasion assays showed that conditioned media from gliomas support the migration of MSCs. When the conditioned media were treated with a blocking antibody cocktail, including anti-CXCL12 antibody, the migration potential of MSCs was significantly attenuated. Recently, the group of Weinberg [28], has demonstrated that MSCs can greatly increase the metastatic potency of breast carcinoma cells. At the bottom of this cell affair, the authors discover CCL5, which was produced by the MSCs and in a paracrine manner led to an augmented motility of the cancer cells.

In contrast to haematopoietic stem cells, a wide range of soluble factors exert significant chemotactic activity on MSCs. Recently, it was shown that MSCs constitutively express hepatocyte growth factor receptor (HGFR, c-met) and that HGF exerts a very strong chemotactic stimulus on MSCs [29, 30]. Ponte *et al.* [31] have shown that PDGF-AB and IGF-1 are better chemoattractants than some chemokines, namely CCL5 (RANTES), CCL22 (MDC) and CXCL12 (SDF-1). In addition, the authors observed that inflammatory cytokines, such as TNFα, are able to increase the sensitivity of MSCs to chemokines.

Cytokine Receptors

MSCs express a wide set of cytokine receptors: IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, IFNγR and TNFI and IIR (reviewed in [32]). These receptors differ in their structural organization and signalling mechanisms, and are therefore segregated into type I and type II cytokine, and TNF receptor families. Most of the receptors that recognize interleukins belong to type I cytokine receptor family, while interferon receptors are type II cytokine receptor proteins [33, 34].

Recently, Ries *et al.* [35] have demonstrated that MSCs can be indeed directly stimulated by TNFα and IL-1 to invade and migrate through basement membrane-like matrigels. Moreover, Croitoru-Lamoury *et al.* [36] provided evidence that TNFα and IFNγ, alone or in combination, exhibit regulatory effects on the expression of chemokines and their receptors in MSCs. For example, TNFα-primed MSCs upregulated the gene transcription of chemokines CCL2, CCl3, CCL4, CCL5, CXCL8 and CXCL10, and cytokines IL-1β and IL-6. In brief, these results suggest that MSCs have the ability to respond to local environmental signals and to access damaged tissues. MSCs can also produce cytokines and chemokines, and thus, can promote trophic support and regeneration of the damaged tissues by autocrine or paracrine signalling.

Cell-Matrix Receptors

Expression of specific integrins by MSCs can also play a role in homing to sites of injury. The integrins are heterodimeric receptors consisting of two non-covalently bound subunits – α and β. Integrins mediate cell-matrix and cell-cell adhesion and affect many cellular processes like cell attachment and spreading, motility, proliferation, differentiation and death. Integrin subunits α1, α2, α3, α5, α6, αV, β1, β3 and β5 have been identified on the surface of MSCs among others [37]. Furthermore, many studies have analysed their role for the attachment and survival of MSCs on different natural and artificial substrates, as well as their importance for the MSC entrance into a specific differentiation program [37]. During MSC chondrogenic differentiation and chondrocyte de-differentiation changes in integrin expression have been examined and will be discussed later in the review.

CD44 is another important receptor that is involved in cell-matrix interactions and it has been shown to be expressed on MSCs [6, 38]. This single transmembrane receptor is responsible for binding to hyaluronan. Zhu *et al.* [39] have research into the role of CD44-hyaluronan interactions for MSC migration. They have found that upon PDGF stimulation, the cells elevated CD44 expression and that their adhesion and migration on hyaluronan was indeed dependent on CD44, since it can be blocked by either CD44 antibody or small interfering RNA. CD44 is also abundantly found on chondrocyte cell surfaces and it is an important factor in maintaining cartilage homeostasis [40].

Cell-Cell Receptors

MSCs engage in cell-cell contacts *via* several types of receptors. Oldershaw *et al.* [41] have investigated the gene expression of Notch receptors and their ligands (Jagged-1, Jagged-2, DLL-1, DLL-3 and DLL-4) in MSCs. The authors reported Notch 1, 2 and 3 presence in MSCs. Jagged 1 was the only Notch ligand expressed at significant level in these cells. Notch receptors are single-pass receptors. Activation of Notch, by its ligands, triggers proteolytic cleavage and release of the Notch intracellular domain, which enters the cell nucleus and alters gene expression [42].

Surface molecules of the immunoglobulin superfamily are also involved in cell-cell interactions, e.g. within the BM compartment. Flow cytometry analysis on MSCs determined that they express ICAM-1 and -2, VCAM-1 and ALCAM [43]. The ligands of these surface molecules are present on mature cells of the haematopoietic lineage. Majumdar *et al.* looked into the role of VCAM-1 – integrin α4 binding in the interactions between MSCs and T lymphocytes, and observed a tremendous inhibition of T cell attachment to MSCs when blocking antibody against integrin α4 was used. This report also provided evidence that the binding between the two cell types also resulted in antigen presentation and cytokine production, suggesting an *in vivo* role for MSCs influencing both haematopoietic and immune functions.

Immuno-Modulating Receptors

Indeed, MSCs have been shown to inhibit the immune function of T and B lymphocytes, and NK cells. MSCs express intermediate levels of MHC class I molecules, while class II molecules can be induced only after stimulation with IFNγ. However, the immuno-suppressive effect is independent of the presence of these molecules, since MSCs devoid or expressing both antigen classes are still able to inhibit the activation of T lymphocytes (reviewed in [44]). MSCs do not express co-stimulatory molecules CD40, CD80 (B7-1) and CD86 (B7-2), and probably therefore, do not activate alloreactive T cells. Furthermore, several soluble factors, such as indoleamine 2,3-dioxygenase (IDO), IL-6 and IL-10 have been implicated in the immuno-modulating role of MSCs [45]. Interestingly, MSCs present on their surface toll-like receptors (TLRs) 1, 2, 3, 4, 5, 6 and 9 [46]. TLRs are single membrane-spanning non-catalytic receptors that recognize unrelated molecules shed from both pathogens and injured tissues. Upon binding to their antagonist, TLRs activate signalling pathways which trigger secretion of cytokines and chemokines. Thus TLRs can specifically drive the recruitment, migration and immuno-modulating function of MSCs at injured sites.

MSC RECEPTORS INVOLVED IN CHONDROGENIC DIFFERENTIATION

TGFβs, IGF, and FGFs have been implicated in MSC chondrogenesis [47]. Goessler *et al.* [48] showed that during chondrogenic differentiation, the MSCs constantly express TGFβ1-4, while Hennig *et al.* [49] determined that MSC present on their surface both, TGFβ-RI and TGFβ-RII receptors. TGFβ signal through binding to TGFβ-RII, which in turn leads to phosphorylation of TGFβ-RI, and thereby Smad-dependent or independent cascades are turned on [50]. Furthermore, it has been shown that MSCs cultured at high density in a defined serum-free medium, in presence of TGFβs, express a chondrogenic potential. With regard to promoting chondrogenesis, TGFβ2 and TGFβ3 were superior to TGFβ1, causing a two-fold greater accumulation of glycosaminoglycans [51]. Besides TGFβs, IGF-1 has also been shown to enhance extracellular matrix production by MSCs [52]. However, the positive effect of IGF-1 on chondrogenesis has been controversial. Indrawattana *et al.* [53] showed in human MSCs IGF-1 induction of chondrocyte marker expression only in the presence of TGFβ3 was. Next, Kawamura *et al.* [54] used adenoviral-expression system, also concluded that TGFβ1 but not IGF-1 stimulates chondrogenic differentiation of human MSCs in pellet cultures. Oppositely, Longobardi *et al.* [55] showed in mouse MSCs, that IGF-1 chondro-inductive actions were equally potent as TGFβ1, and that the two growth factors had additive effects. Possible reasons for the observed differences can be the different amounts of insulin used and that the MSCs in these studies originated from different species. Other growth factors promoting chondrogenesis are FGF-2 and FGF-18. Based on mouse models for human chondrodysplasia, FGFs have been identified as ligands for: FGFR1 in hypertrophic chondrocytes, FGFR3 in resting and proliferating chondrocytes, and FGFR2 in the preichondrium and periosteum of long bones [56]. FGF-2 was initially found to only exhibit a strong mitogenic effect on MSCs [57, 58], but Solchaga *et al.* showed that it can also promote MSC chondrogenesis [59]. With regard to FGF-18, Davidson *et al.* [60] used FGFR3 knockout mice as a source for MSCs and determined firstly, that FGF-18 is a selective ligand for this receptor, and secondly, that it suppressed proliferation and promoted MSC differentiation as well as production of cartilage matrix. Interestingly, MSCs express also EGFR [61] and respond to EGF or heparin-bound EGF (HB-EGF) with augmenting their motility and proliferation. Although the *in vivo* role of EGFR and its ligands in MSCs is not yet clear, the EGF and HB-EGF could be used as mitogens for *in vitro* expansion and self-renewal maintenance of MSCs for the purposes of tissue engineering and regenerative medicine [62].

BMPs belong to the TGFβ superfamily and upon binding to their receptors, Smad or MAPK pathways are activated. BMP receptors are heterodimeric transmembrane serine-threonine kinase receptor complexes. There are three BMP type I receptors, BMPR-IA, BMPR-IB and ActR-I, and three BMP type II receptors, BMPR-II, ActR-IIA and ActR-IIB [63]. BMPs can induce or enhance MSC chondrogenic differentiation alone or synergized with other growth factors. Sekiya *et al.* [64] compared the effect of BMP-2, -4 and -6, all in combination with TGFβ3, on *in vitro* cartilage formation of MSCs. However, Knippenberg *et al.* [65] have shown that a short treatment of MSCs with BMP-2 stimulated Runx-2 and osteopontin gene expression. This problem is not necessarily insurmountable. Hanada *et al.* [66] demonstrated that hypertrophic differentiation of MSCs, following BMP-2 administration, was inhibited by co-treatment with TGFβ1, suggesting that combinational treatments could be applied to achieve an appropriate phenotype.

The members of the Wnt family are important regulators of skeletogenesis. Wnts signal through Frizzeled receptors (Fz). Fz is a family of G-protein-coupled receptor proteins that integrate into the plasmalemma with seven transmembrane regions. In an activated state, Fz receptors bind to their

low-density lipoprotein co-receptors (LRP 5 and 6) and signal *via* β-catenin-TCF/LEF transcriptional unity. MSCs are equipped with several Wnt receptors (Fz2, Fz3, Fz4, Fz5 and Fz6) [67] and some of their ligands have been reported to have a direct, mostly suppressing, effect on chondrogenesis of MSCs. For instance, Church *et al.* [68] observed that Wnt4a blocked the initiation of chondrogenesis and accelerated terminal chondrocyte differentiation. In contrast, Wnt5a and Wnt5b promoted early chondrogenesis but inhibit terminal differentiation. Wnt7a clearly blocked chondrogenesis [69], while Wnt3a had been shown as both positive and negative regulator of chondrogenesis. Fischer *et al.* [70] determined that Wnt3a has the capacity to enhance BMP-2-mediated MSC chondrogenesis *via* N-cadherin-mediated adhesion, while Hwang *et al.* [71] showed Wnt3a to inhibit chondrogenesis throughout the Jun/AP1 pathway.

Besides, the cell-cell signalling, cell-matrix interactions can also alter cell behaviour and thus influence the commitment of MSCs into chondrocyte lineage. For instance, Goessler *et al.* [72] investigated the expression of integrins during MSC chondrogenic differentiation in comparison with de-differentiating human chondrocytes. A similar study, also based on microarray technology, was performed by Djouad *et al.* [73]. On the adhesion molecules, the authors presented evidence that the mRNA levels of many CAM molecules (VCAM-1, ALCAM, etc.) and integrin subunits (α1, α5, β1 and etc.) increased during MSC chondrogenesis. Another study dealing with integrins, but more from the perspective of engineering artificial cartilage substitutes, demonstrated that integrin β1 is essential for the attachment and survival of MSCs and chondrocytes on biodegradable polymers [74].

Finally, we will discus the pronounced effect of two unrelated receptor types, prolactin receptor (PRLR) and EP (receptor binding to prostaglandin (PGE)), on MSC chondrogenesis.

PRLR belongs to the type I cytokine receptor family. This receptor and its ligand prolactin (PRL) have been linked to many developmental processes, including bone formation but also to diseases, such as arthritis. Ogueta *et al.* [75] purified PRL from synovial liquid, demonstrated that MSC have PRLR and further investigated its role during MSC acquisition of chondrogenic phenotype.

Prostaglandins (PGEs) are other soluble factors that are involved in chondrogenesis and chondrocyte maturation. PGEs signal *via* binding to EP receptors. The EP receptors are coupled to G-proteins and display canonical seven transmembrane domains [76]. Clark *et al.* [77] identified on MSC surface four PGE receptor isoforms, EP1, EP2, EP3 and EP4.

CONCLUDING REMARKS

OA is one of the key targets of regenerative medicine. Due to their chondrogenic potential, *in vitro* expanded MSCs are potential candidates for stem cell therapy of OA. We reviewed here MSC receptors that play a positive role in the transition of MSCs towards the chondrocyte phenotype. Furthermore, since MSCs are equipped with various receptors, and some receptors are involved in OA, the therapeutic use of these cells must be carefully planned. Interactions between the surface receptors of MSCs and the molecules comprising the scaffolds applied in tissue engineering have to be thoroughly examined.

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