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Review Article

The Impact of Mesenchymal Stem Cells on Differentiation of Hematopoietic Stem Cells

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Abstract

Bone marrow microenvironment contains cellular and acellular compartments. The cellular compartment includes hematopoietic stem cells, mesenchymal stem cells and some other stromal cell types, while the acellular compartment is composed of scaffold proteins known as the extra cellular matrix. Direct cell-cell contact as well as cytokines secreted by mesenchymal stem cells during coculture of hematopoietic stem cells and mesenchymal stem cells play a critical role in hematopoiesis, and determines the fate of hematopoietic stem cells. Several studies have demonstrated the impact of mesenchymal stem cells on self-renewal, expansion, proliferation and differentiation of hematopoietic stem cells in vitro, which have shown different and contradictory results. In this paper, we will investigate the effect of mesenchymal stem cells on differentiation of hematopoietic stem cells in vitro.

Introduction

Based on embryonic anatomy, primary hematopoiesis begins in embryonic yolk sac and then transiently in placenta and liver, and is finally stabilized in embryonic bone marrow.[1]

Two groups of stem cells are presented in the bone marrow, including hematopoietic stem cells (from which RBC and WBC are produced) and mesenchymal stem cells generating chondrocytes (cartilage), osteoblasts (bone), fat and skeletal muscle cells.[2]

Hematopoietic cells grow and expand in the bone cavity and remain there up to their maturity, and are released into vascular system after maturity.[3,4] Hematopoietic stem cells (HSC) and their progenitors in the bone marrow are surrounded by stromal cells. Mesenchymal stem cells (MSC) reside in the bone cavity form the majority of stromal cells in BM, including chondrocytes, osteoblasts, adipocytes, endothelial cells and myocyte.[5-7]

Hematopoietic Stem Cells (HSC)

All hematopoietic cells are derived from a small population of hematopoietic stem cells.[8] Functional hematopoietic stem cell lack the surface markers of differentiated cells or mature blood cells but express high levels of CD34 and Kit^+.[9,10] On the basis of their self-renewal ability, hematopoietic stem cells are divided to long-term and short term HSC.

LT-HSC(long term-HCS) are capable of unlimited self-renewal and can preserve long term self-renewal capacity while ST-HSC(short term-HSC)have limited self-renewal potential and maintain hematopoiesis only for a limited period in vivo.[8,11-13]

Hematopoietic stem and progenitor cells have specific markers that distinguish them from other cells. CD34 is an adhesion molecule expressed on progenitor cells and HSC.CD90 is another important cell surface marker exclusively expressed on early hematopoietic cells. The absence of CD38 on a cell indicates the most primitive hematopoiesis stage. CD10 and CD7 are important markers of early lymphoid lineage. CD123 (IL-3 receptor) and CD135 (FLT3) are related to myeloid lineage and CD110 (thrombopoietin receptor) is a platelet lineage marker.[14]

Hematopoietic stem cells possess self-renewal property to maintain stem cell repertoire as well as multipotential differentiation capacity to other hematopoietic lineages, while hematopoietic progenitor cells (HPC) are not capable of self-renewal, and can only differentiate to a specific lineage. HSC and HPC are distinguished by their specific surface markers.[15] CD150 differentiates HSC from HPC. HSC are $CD150^+$, $CD244^-$ and $CD48^-$ while hematopoietic multipotent progenitors (MPP) are $CD150^-$, $CD48^-$, $CD244^+$ and HPC are $CD48^+$, $CD244^+$ and $CD150^-$.[16]

Mesenchymal Stem Cells (MSC)

Mesenchymal stem cells are fusiform cells with plastic adhesion property and mutilineage differentiation capacity isolated from bone marrow, fat and other tissues

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in laboratory conditions.[17] In tissue culture, MSCs expand with limited half-life and doubling capacity of 15-50 times. These cells differentiate to adipocytes, chondrocytes and osteoblasts in vivo and on exposure to proper stimuli in vitro.[18,19]

Mesenchymal stem cells have no specific unique markers.There is a general agreement that human MSC do not express the hematopoietic markers such as CD45, CD34 nor co-stimulatory molecules of CD80, CD86 and CD40.However, they express variable levels of CD105 (endoglin), CD73 (ecto-5-endonucleotidase), CD44, CD90 (THY-1), CD71 (transferrin receptor), GD2 Ganglioside and CD271 (low affinity nerve growth factor receptor), and are detected by STRO-1 monoclonal antibody. Changing expression of these markers is likely to be due to interspecies differences, tissue of origin and culture conditions.[20]

Stem cells secrete several cytokines, summarized in a paper by Azizi et al.[21] The majority of cytokines are related to proliferation and differentiation of HSCs, including IL-6, FLT3L, SCF, GCSF and GMCSF.MSCs express a number of adhesion molecules (CD166, CD54, CD3 and CD106) and integrins (CD49, CD29, CD11 and β4 integrins).[19] MSCs support LTC-ICs (long-term culture-initiating cells), and can function as a feeder layer for HSC.[22]

Bone marrow MSCs can maintain long term hematopoiesis in vitro, and support the expansion and proliferation of hematopoietic colony forming cells in combination with added exogenous cytokines (e.g. SCF, FLT3L and TPO).[23]

Differentiation of hematopoietic cells

ST-HSCs are derived from LT-HSC. These cells change to common myeloid progenitors (CMP) and common lymphoid progenitors (CLP). CLP are the origin of lineage committed T- and B-lymphocytes. Erythroid/megakaryocyte progenitor cells generate megakaryocyte–erythroidprogenitor cells (MEP) and granulocyte-macrophage progenitor cells (GMP), which produce mast cells, eosinophils, neutrophils and macrophages.[24]

Signaling pathways involved in hematopoietic stem cell fate

Wnt, Notch, Hedgehog and TGFb/SMAD signaling pathways involved in hematopoiesis have been conserved in various creatures during the evolution. In addition, several other pathways regulate self-renewal, differentiation, proliferation, apoptosis and aging of hematopoietic stem cells.[25]

Several factors are involved in the interaction between hematopoietic and mesenchymal stem cells. Hematopoietic stem cells bind MSCs via adhesion molecules like N-cadherin and β-integrins. Soluble cytokines secreted by MSCs such as Kit-L, SDF-1 and Ang1 support the growth and differentiation of HSCs via Kit, CXCR4 and Tie2 receptors. When HSCs are exposed to MSCs, the expression of Notch ligands (Jagged and Delta like) in MSC is increased through Wnt pathway. Furthermore, the expression of Notch receptors in HSC is increased by sonic Hedgehog (Shh) in HSC and MSC. VEGF can increase the expression of Notch receptor in autocrine or paracrine mode, and increased activity of Notch signaling pathway induces the expression of downstream target genes, including Hes1. The expression of BMP is increased in MSC through Shh signaling pathway.[26]

On the other hand, activation of Notch1 inhibits the differentiation of HSCs both in vivo and in vitro,[27] and studies indicate the role of Notch signaling pathway in survival and expansion of HSCs.[28]

The inhibitory effect of Notch signaling in vitro shows that Notch1 ligands may support the proliferation of HSCs.[29] Inhibition of Notch signaling pathway increases HSC differentiation in vitro, and decreases the repertoire of hematopoietic stem cells in vivo.[30]

Wnt signaling pathway blocks multilineage differentiation of MSCs and maintains them in an undifferentiated state.[31] This signaling pathway plays a vital role in self-renewal, survival and proliferation of HSCs in vitro.[32,33]

MAPK kinase signaling pathway plays critical roles in cellular physiology, and organizes several cellular behaviors, including cellular processes and differentiation.[34] In hematopoietic system, activation of MAPK1 plays an important role in proliferation and differentiation to macrophage/ granulocyte series.[35]

Effect of MSC on differentiation of HSCs

Mesenchymal stem cells in BM in adults generate signals for differentiation and proliferation of hematopoietic stem cells and their progenitors through direct cell-cell contact.[36] These cells also release cytokines and factors meant for growth of HSCs.[37-39]

In a study, cord blood hematopoietic stem cells were co-cultured with mesenchymal stem cells in vitro. Three culture conditions were provided for them: (1) Co-culture of HSCs with cytokines along with MSCs as the feeder layer; (2) Culture with cytokines without MSC; (3) Co-culture with MSC without cytokines. The cytokine included SCF, TPO and FLT3L. The highest apoptosis rate and the lowest number of cells was observed in G0/G1 phase in culture with cytokine without mesenchymal stem cells. The expansion of umbilical cord HSCs on mesenchymal stem cell feeder layer results in high proliferation and reduced apoptosis. This study showed that application of MSCs as the feeder layer for umbilical cord blood HSC reduces the apoptosis rate in expanded cells, rendering them in G0/G1 phase.[40]

Da silva et al cultured umbilical cord blood $CD34^+$ cells in serum free medium enriched with SCF, BFGF, LIF and FLT3 in the presence and absence of stromal cells, and observed that the stromal cells support the expansion process without inducing differentiation and exhaustion of initial stem cells.[41]

It has also been shown that MSCs act as a feeder layer maintaining HSCs in an undifferentiated state.[42] Differentiation of HSCs during their expansion increases the process of cell aging and death,[43] but direct contact between the stem cells and microenvironment of the cell represents their essential role in hematopoiesis versus differentiation.[44]

Mesenchymal stem cells produce a variety of cytokines and factors affecting hematopoiesis.[45,46] IL-6, Flt3-L (FL), SCF, G-CSF, M-CSF, GM-CSF, TPO, CXCL-12 (SDF1) and IL-11 are among these factors.[22,47,48]

IL-6 plays a vital role in immune response and hematopoiesis, and is a factor for the differentiation of B cells. IL-6 induces megakaryocytic maturity, and increases the expansion of HSCs through the induction of IL-3.[49] IL-6 and TPO affect the proliferation and differentiation of HSCs.[50,51] TPO is the primary cytokine regulating the growth of platelets and megakaryocytes. TPO affects primary hematopoietic cells via its MPL receptor and other receptors.[52]

FL is an important growth factor for immature myeloid and stem cells, and can expand $CD34^+$cells both in vivo and in vitro.[53] FL maintains the proliferation and self-renewal capacity of HSCs and regulates the hematopoietic expansion.[47] IL-11 shows outstanding thrombopoietic activity, which is assessed in clinical tests.[54] GM-CSF is a hematopoietic growth factor involved in the production of granulocytes, macrophages and dendritic cells from HPC.[55] GM-CSF regulates the transplantation of HSCs.[56]

CXCL12 regulates the adherence, migration and implantation of HSCs.[47,57,58] SDF1 reduces the production of inflammatory chemokines and cytokines.[59] SCF maintains the proliferation and self-renewal of HSCs, regulating hematopoietic growth and transplantation of HSC.[47,56,60]

Apart from production of these cytokines and growth factors, close contact between mesenchymal stem cells and hematopoietic stem cells supports the differentiation of hematopoietic stem cells towards mature red cells, and this has been discussed in various papers.[61] Apoptosis of umbilical cord HSCs is inhibited in co-culture with MSCs, and addition of BM derived MSCs improves the expansion of adult HSCs collected from various sources.[62,63]

Hematopoietic stem cells preserve a large number of cells with $CD34^+/CD38^-$ phenotype with support from BM stromal mesenchymal stem cells while the umbilical cord MSCs increase differentiation. In this study, a three-dimensional culture system based on collagen (collagen gel containing mesenchymal stem cells or cell free collagen gel) was used, and was subject to co-culture in four conditions of HSCs: (1) Without mesenchymal stem cells (2) On a single layer of mesenchymal cells (3) On cell free collagen gel and (4) On collagen gel with embedded MSC. In this study, hematopoietic stem cells show differentiation towards myeloid series ($CD13^+$, CAE^+ and MPO^+) and are also $CD45^+$.[64] Myeloid differentiation of HSCs in all the conditions in the presence of MSCs was significantly increased compared to stromal cell free conditions.[65]

Research shows that cord blood mesenchymal stem cells are not appropriate to maintain the $CD34^+/CD38^-$ phenotype during long term culture. However, cord blood MSCs significantly increase the proliferation of HSCs and promote their differentiation.[64]

Mesenchymal stem cells support the cell proliferation in high passages while they maintain the $CD34^+$ phenotype in early passages in high number of cell divisions, indicating increased self-renewal versus differentiation in early passages, and are thus more appropriate for the expansion of $CD34^+$ HSCs in vitro. $CD34^+$ cell differentiation in co-culture with stromal cells occurs only after several cell divisions, and it is noteworthy that this is related to asymmetric cell division.[65]

Mesenchymal stem cell feeder layer has been shown to support the self-renewal and subsequently differentiation of HSCs after 7 days of culture promote the proliferation and differentiation of mesenchymal stem cell feeder layer[66] and also plays a crucial role in the development and differentiation organization of the hematopoietic stem cells.[67]

Another study showed that mesenchymal stem/progenitor cells from cord blood promote the proliferation and differentiation of hematopoietic stem/progenitor cells in the same tissue but the relationship between mesenchymal stem/progenitor cells and hematopoietic stem/progenitor cells in human cord blood during growth has not been elucidated.[68]

Cord blood HSC have been subject to co-culture with bone marrow MSC exposed to cytokine cocktail of CSF, FLT3L, bFGF and LIF in vitro. The differentiation potential of HSCs mainly tended towards myeloid series in presence of these cytokines, but a large number of these cells maintained their initial lymphocyte potential ($CD7^+$cells).[69]

Conclusion

MSCs play a supportive role in bone marrow. Since these cells produce hematopoietic growth factors effective upon hematopoiesis, induction of the differentiation effect of these cells on HSCs is quite possible. Moreover, the interaction of these cell lines and the signaling pathways activated by this interaction enables proliferation and expansion of HSCs in undifferentiated state.

Several studies have been conducted on co-culture of MSC and HSC with different and sometimes conflicting results. In most studies on co-culture of mesenchymal stem cells and hematopoietic stem cells under different conditions, it has been stated that hematopoietic stem cells differentiate towards myeloid series but this requires further studies in vitro, and their erythroid differentiation remains controversial. In these studies, differentiation mechanisms towards different series have not been dealt with in detail, and have only been cited. In some studies, the contribution of MSCs to expansion and proliferation of hematopoietic cells has been mentioned.

The effect of MSCs on differentiation of HSCs requires further studies in the future.

Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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