Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2015, 5(2), 141-149
doi: 10.15171/apb.2015.021
http://apb.tbzmed.ac.ir

Mini Review

The Effect of Hypoxia on Mesenchymal Stem Cell Biology

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Article info
Article History:
Received: 8 June 2014
Revised: 12 September 2014
Accepted: 17 September 2014
ePublished: 1 June 2015

Keywords:
· Hypoxia
· Mesenchymal stem cells
· Hypoxia-inducible factor
· Niche

Abstract
Although physiological and pathological role of hypoxia have been appreciated in mammalians for decades however the cellular biology of hypoxia more clarified in the past 20 years. Discovery of the transcription factor hypoxia-inducible factor (HIF)-1, in the 1990s opened a new window to investigate the mechanisms behind hypoxia. In different cellular contexts HIF-1 activation show variable results by impacting various aspects of cell biology such as cell cycle, apoptosis, differentiation and etc. Mesenchymal stem cells (MSC) are unique cells which take important role in tissue regeneration. They are characterized by self-renewal capacity, multilineage potential, and immunosuppressive property. Like so many kind of cells, hypoxia induces different responses in MSCs by HIF-1 activation. The activation of this molecule changes the growth, multiplication, differentiation and gene expression profile of MSCs in their niche by a complex of signals. This article briefly discusses the most important effects of hypoxia in growth kinetics, signalling pathways, cytokine secretion profile and expression of chemokine receptors in different conditions.

Introduction

The bone marrow microenvironment has two cell types-non-haematopoietic stem cells and haematopoietic stem cells-which form a bone marrow niche.[1-3] The haematopoietic stem cells (HSCs) that settle in the bone marrow microenvironment differentiate via factors such as cytokines and the extra cellular matrix. For example, stem cell factor (SCF) and erythropoietin (EPO) are effective in erythroid stem cell maturation.[4] HSC niches can contribute to promoting haematological malignancies,[5-7] so the biology of these cells can have important clinical applications, especially in bone marrow transplantation. MSCs that play a critical role in the bone marrow niche are able to self-renew or differentiate to other lineages[8-10] and such cells have been isolated from different tissues such as brain, liver, bone marrow, adipose tissue, foetal tissues, umbilical cord (UC), Wharton's jelly, and placenta.[11-14] According to the declaration of the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, MSCs express CD13, CD44, CD73, CD90, and CD105, but CD45, CD34, CD14, and CD19 are not expressed naturally in these cells.[15] The bone marrow-derived MSCs have the highest grade of lineage plasticity and are capable of converting to all cell types following implantation into early blastocysts.[9,16] Umbilical cord blood-derived MSCs expansion is highest in comparison with bone marrow and adipose-derived MSCs.[17,18] This matter may be due in part to higher telomerase activity.[19] In bone marrow niche, an oxygen gradient exists that creates a hypoxic condition for stromal and stem cells.[20] Low oxygen tension has an effect on different cells in many tissues. Hypoxia has a strong effect on several aspects of cell biology such as metabolism, angiogenesis, innate immunity and stemness induction[21] Effects of hypoxia are usually mediated by hypoxia-inducible factors (HIFs), i.e. HIF-1α and HIF-2α.[21-24]

HIFs are members of a subfamily of basic helix-loop helix transcription factors, and contain a PAS domain recognized as the Per, Arnt, and Sim proteins.[25] HIFs alter more than one thousand target genes. These factors heterodimerize with another subunit, HIF-1β (or aryl hydrocarbon receptor nuclear translocator (ARNT)), and regulate downstream target gene expression.[26] HIFs alter more than one thousand target genes.[23,24] Recent examinations revealed expression of HIF-1α and HIF-1β to be required for normal development of the heart, blood vessels and blood cells.[27-30] The signature of hypoxia alters many conditions, signalling pathways and molecules in cells. Metabolism and enzyme kinetics is one aspect that changes when the cell is exposed to hypoxia, for example expression of metalloproteases such as matrix metalloprotease-1 (MMP-1) and MMP-3, firstly regulated by hypoxia/HIF-1α.[31] Ho IA confirmed

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that MMP-1 is a necessary factor in human bone marrow-derived mesenchymal stem cell migration towards human glioma.[32] Another enzyme examined is secreted lysyloxidase (LOX), which is required for the linkage contacts necessary for migration through focal adhesion kinase activity and cell matrix adhesion.[33] Activated LOX stimulates Twist transcription, and this transcription factor leads to the mediation of the epithelial-to-mesenchymal transition (EMT) of carcinoma cells.[34] HIF-1α regulates other lysyl oxidase-like enzymes that play a significant role in the creation of the breast cancer metastatic niche.[35]

We can also say about the signalling pathway in hypoxia, that carcinoma-associated fibroblast differentiation needs the TGF-β/SMAD signalling pathway.[36] Hypoxia alters the inhibitory function of SMAD family member 7 (SMAD7, an inhibitor of the TGF-β signalling pathway), which is a promoter of malignant cell attack.[37]

Chromatin modifiers can also be controlled via hypoxia. Stimulation of histone lysine-specific demethylase 4B (KDM4B, also known as JMJD2B) associates with cell invasion in the advanced clinical stage of cancer, for example in colorectal cancers.[38] Lack of KDM4B leads to adipogenic differentiation and reduces osteogenic differentiation of MSCs.[39] Hypoxia induces a histone methyltransferase mixed lineage leukaemia 1 (MLL1), so contributing to the differentiation of these cells.[40,41]

Different microRNAs are regulated via hypoxia/HIF-1α.[42] MiR-210 is involved in tumour initiation and metastasis by targeting various downstream molecules as well as gene expression under normoxia, but vacuole membrane protein 1 (VMP1) is regulated by hypoxia.[43,44] In a study performed on MSCs, this microRNA improved the proliferation of MSCs significantly.[45] Hypoxia and Ras-signalling pathways are controlled by three groups of microRNAs (miR-15b/16, miR-21 and miR-372/373).[46] Induction of Ras/MAPK signalling helps the osteogenic differentiation of MSCs via RUNX2 activation.[47] Mir-34a represses by hypoxia, but blocks osteoblastic differentiation of human stromal stem cells.[48,49]

Different hypoxia aspects

Hypoxia and mesenchymal stem cells

For the study of MSC proliferation, differentiation, metabolic balance and other physiological processes, their cultivation under hypoxia is an important prerequisite because it is similar to the natural microenvironment in bone marrow.[50] Thus, a diverse range of reports for in vitro cell cultures and following clinical applications recommended MSC cultivation under hypoxia (1% to 10% O_2).[51,52] This condition led them to suffer from limited nutrient and oxygen sources.[53,54]

Different functional characteristics have been confirmed for hypoxia-induced MSCs from different sources. MSCs have some immunomodulatory effects,[55] especially autocrine or paracrine diverse activity of cytokines, and growth factors of bone marrow-derived MSCs can be modulated in hypoxic conditions.[56] On the other hand, UC-derived human MSCs adjust energy consumption and metabolism during hypoxia, and hypoxia leads to an increase in UC-derived MSC growth, in parallel to reducing cellular injury.[57] The cell surface antigen expression of adherent cells derived from MSC-PBN (MSCs that collect from peripheral blood and culture in normoxia) and MSC-PBH (MSCs that collect from peripheral blood and culture in hypoxia) after two passages in culture is matched with BM MSCs. CD73 (ecto-5'-nucleotidase), CD54 (intercellular adhesion molecule-1), CD44 (homing-associate cell adhesion molecule) and CD90 (Thy-1) are positive for the cultured adherent PBN- and PBH-derived cells but CD31 (platelet-endothelial cell adhesion molecule-1), CD45 (leukocyte common antigen), CD18 (β2 integrin), CD49d (α4 integrin chain) and CD49f (α6 integrin chain) are negative. Therefore the cell surface antigen expression arrangement of PBN- and PBH-derived cells is comparable to that of BM-MSCs.[58]

Effect of hypoxia on MSC proliferation

Incubation of UC-derived MSCs with various concentrations of oxygen led to a rise in cell proliferation at hypoxia. In this condition significant levels of HIF-1α in hypoxic MSCs cultured at 2.5% or 5% O_2 can be observed.[57]

The effect of hypoxia on MSC expansion and phenotype

However, stem cells are more resistant to hypoxia than their progenies, but hypoxia stimulates cell cycle arrest in mammalian cells. This event reflects their native environment and their intrinsic inactive state. MSCs and HSCs form a distinct bone marrow niche[59] and 5% O_2 pressure in vitro is similar to the physiological conditions for MSCs. Under 5% tension of O_2 up to (Passage 1) P_1 MSCs grew slower, and earned a progressive growth advantage in the next passages.[60] Simmons showed that total cell numbers were reduced in hypoxia versus normoxia at first, while they were increased at P_1. Overall cell-doubling time was reduced by hypoxia until P_1 and increased afterwards. Fifty percent of the MSCs at P_0 under hypoxia transiently express STRO-1 and reduce afterwards.[61] Remarkably, STRO-1^+-presented cells increase expansion and multi-lineage differentiation potentialities.[62,63]

The genes that were primarily induced were not assigned to multipotency but instead belonged mostly to adhesion molecules such as the von Willebrand endothelial cell adhesion molecule and protocadherin.[64] MSC osteogenic differentiation is regulated by WNT-related transcription factor TCF1.[15] In the control of differentiation towards adipocytes, osteocytes and chondrocytes, the eight genes potentially involved were not changed by hypoxia.[15] vWF is a marker of endothelial lineage[65] and PLVAP is a leukocyte trafficking molecule,[66] which may help the transendothelial passage of MSCs from the bone marrow. Stimulation of leptin helps maintain mesenchymal progenitor cells' undifferentiated state.[67] The first stimulated gene that has a role in angiogenesis and extracellular matrix gathering is SMOC2,[68] and the Kit gene is associated with proliferation.[69] Culturing of MSC

in hypoxia impedes cell differentiation and biogenesis of mitochondria. We can say that cells in hypoxic conditions are less differentiated than cells in normoxia, the nuclei are larger and less complex, and there exist more abundant nucleoli and a higher nuclei/cytoplasm index, while the size of the cells is alike in both situations.[15,70,71]

Effect of hypoxia on MSC differentiation

In hypoxic microenvironments, haematopoietic and stromal stem cells (HSCs, MSCs) adapt themselves to hypoxia.[15,70,71] Consequently, several reports exist of the differentiation capacity of HSCs and MSCs cultured in hypoxic conditions.[60,72-80] Typical surface markers are expressed by bone marrow MSCs in human cells, and they have the potential to differentiate into adipogenic, osteogenic and chondrogenic lineages. Evaluation of adipocyte lineage-specific transcripts (LPL, PPARg) and osteocyte lineage-specific transcripts (ALPL, Runx2) show that the expression of ALPL in MSCs in severe hypoxia is higher than in normoxia. Additionally, ALPL is stimulated in hypoxic cells but Runx2 transcription does not show any noticeable alteration in normoxic MSCs. MSCs in hypoxia are more prone to osteogenic differentiation than in normoxia.[15,70,71]

Remarkably, expression of VEGF-A transcription is up to 20 times higher under hypoxic environments through osteogenesis than during adipogenesis. Additionally, analysis of PPARG expression (a key marker for adipogenesis), and Runx2 (a key marker for the osteogenic switch) demonstrated that the expression of PPARG in adipogenesis is meaningfully higher after two weeks under normoxic conditions compared to hypoxic conditions. Chemical inducers of HIF-1a facilitate the osteogenesis of human MSC, including the iron-chelating factor desferrioxamine mesylate (DFX) or the dimethyloxalylglycine (DMOG). This facilitation is observed even under normoxic conditions, but to a lesser extent than hypoxic situations.[81]

Effect of hypoxia on MSC apoptosis and necrosis

UC-derived human MSC cultured at 1.5% O_2 showed a slight rise in apoptosis. Furthermore, in 2.5% O_2 cells an augmented proliferative capability was confirmed. Comparable information was gained in bone marrow-derived MSC.[50,60] Furthermore, the level of cell injury and/or necrosis in 1.5% O_2 is meaningfully less than in normoxic control cells. These data suggest an alteration in energy requests during hypoxia. A decreased concentration of oxygen in the hypoxic milieu can lead to reduced creation and accessibility of reactive oxygen species, which are principally responsible for the augmentation of cell injury.[82,83]

Effect of hypoxia on MSC metabolism

At 1.5% O_2, consumption of glucose by MSCs and production of lactate is considerably more than in normoxic conditions. At 2.5% O_2 glucose utilization and amount of lactate production were both less than at 1.5% O_2, but still higher than that of MSCs in normoxic conditions. Glucose uptake and lactate production showed no difference between 5% O_2 compared with 21% O_2. Experiments showed an important stimulation of GLUT-1, LDHA and PDK-1 in 1.5% O_2, 2.5% O_2 and 5%O_2 in comparison with control cells (21% O_2). However, no increase was detected for G6PD in hypoxia. At 1.5% O_2, consumption of glutamine is less, and consumption at 5% O_2 is the same as the 21% O_2 controls. At 1.5%, 2.5% and 5% O_2 when compared to the 21% normoxic condition control, glutamate production is less.

This data demonstrates that MSCs, especially UC-derived mesenchymal cells, adjust their oxygen consumption and therefore their energy metabolism. Thus, oxygen consumption rates of MSCs under hypoxic situations were about three-fold less in comparison with the control group.[84-86]

Hypoxia induces VEGF, GLUT1, LDHA, PGK1, HIF-1a and HIF-1 target gene expression after 72 hours under hypoxic conditions. Note that VEGF, GLUT1, LDHA, PGK1 genes are target genes of HIF-1a.[81] Previous data showed an increase in PDK1 gene expression. These data confirm that reduced cell respiration under hypoxic conditions is an outcome of the reduction of mitochondrial oxygen consumption.[84] The utilization of pyruvate as a fuel for the Krebs cycle is suppressed by PDK1 upregulation: this mechanism is used by cells to preserve intracellular oxygen concentration and keep its homeostasis steady. These data are in agreement with animal experiments.[87]

Effect of hypoxia on MSC migration capability

One report showed that hypoxia led to the constant circulation of a small number of MSCs in the peripheral blood under inactive circumstances. Then, the circulating pool is critically increased. Significantly, this increment is moderately definite for MSCs, while HPCs exhibited no or limited increase under hypoxic situations. Some experiments determined cells similar to BM MSCs to circulate in peripheral blood from humans and animals,[88-91] while other studies led to contrary conclusions.[92,93] MSCs can be distinguished directly or indirectly in peripheral blood grafts after such a mobilization process, as several experiments have demonstrated.[90,94,95] However, this procedure is likely to be an infrequent event.[92] After G-CSF injection, CFU-Fs are not identified in the blood of many of the patients. The BM CFU-F (Colony Forming Unit-Fibroblastoid) levels were unaffected; this finding showed hypoxia to help MSCs' movement from the BM into the bloodstream. This egression, without meaningfully decreasing the BM MSC pool, shows that MSCs mobilize from other non-BM sources. However, the role of an enhanced grade of erythropoietin cannot be excluded from these experiments.[96]

Extensive examination has shown that migration of MSCs is reliant upon the different cytokine/receptor pairs SDF-1/CXCR4, SCF-c-Kit, HGF/c-Met, VEGF/VEGFR, PDGF/PDGFr, MCP-1/CCR2, and

HMGB1/RAGE.[97] For stem cell recruitment to tumours, between these cytokine/receptor pairs, stromal cell-derived factor (SDF-1) and its receptor, CXC chemokine receptor-4 (CXCR4), are significant mediators. Experiments studying the activity of secreted SDF-1 and cell surface CXCR4 of stem cells have exhibited the significance of this interaction, which is essential for stem cell migration.[98-100] The migration capability of MSCs depends on metalloproteinases (MMPs).[76] MSCs exposed to Conditioned Medium (C.M) of various tumour cells displayed suppression of matrix metalloproteinase-2 (MMP-2) and stimulation of CXCR4. Studies propose that CXCR4 and MMP-2 are involved in the multistep migration procedures of MSCs to tumours.[100] Furthermore, the appearance of MMP-2 and vascular endothelial growth factor (VEGF) in endothelial cells demonstrates their induction by hypoxia.[101,102] Determination of the key agents responsible for this procedure have clinical importance.[58]

Discussion

Hypoxia is one of the most significant environmental factors affecting cells in different ways. Hypoxia plays an important role in different aspects of cell biogenesis such as metabolism, migration, proliferation, differentiation and apoptosis. Hypoxia through some elements such as hypoxia-inducible factors (HIFs), a master transcription factor, mediates these events in cells. More than 1000 genes are targets of HIF, regulated directly or indirectly by it. For example, transcription factors, enzymes, receptors, receptor-associated kinases, and membrane proteins can be induced or suppressed by hypoxia. MSCs can be found in many tissues such as brain, liver, bone marrow, skin, adipose tissue, foetal tissues, umbilical cord, Wharton's jelly, and placenta.[11-14] These cells can differentiate to tissue types of other lineages.[8,9] MSCs and HSCs form bone marrow niches[59] and are in physiological hypoxia; thus, research performed on mesenchymal stem cell properties such as proliferation, differentiation, senescence, metabolic balance and other physiological features should be performed under hypoxic conditions, similar to the natural microenvironment of these cells.[50] For this goal, 5% O_2 pressure is similar to the physiological condition for MSCs. MSCs can live and adjust to changes in their microenvironment: human mesenchymal stem cells isolated from the umbilical cord when compared to MSCs derived from other tissues exhibited metabolic changes through adaptation during hypoxia.[57] In relation to surface marker expression in MSCs, we can tell that these cells are positive for CD73 (ecto-5'-nucleotidase), CD54 (intercellular adhesion molecule-1), CD44 (homing-associate cell adhesion molecule) and CD90 (Thy-1), in cultured adherent PBN- and PBH-derived, but negative for CD31 (Platelet-endothelial cell adhesion molecule-1), CD45 (leukocyte common antigen), CD18 (β2 integrin), CD49d (α4 integrin chain) and CD49f (α6 integrin chain).[58] Total cell numbers were reduced in hypoxia versus normoxia at primary cultivation while they were increased at the next passage.[103] In some reports, a steady phenotype was observed over time and no important phenotypic changes among hypoxic and normoxic conditions were detected.[104] However, in other experiments under hypoxias STRO-1 was transiently expressed and reduced in the next passage. Typical surface markers of MSCs expressed by bone marrow-derived human MSCs are able to differentiate into adipogenic, osteogenic and chondrogenic lineages. Cultivation of UC-derived human MSCs at 1.5% O_2 shows a slight rise in apoptosis. Similar information was gained in bone marrow-derived MSCs.[50,60] Furthermore, the level of cell injury or necrosis under 1.5% O_2 hypoxia was, importantly, less than in the normoxic control cultures.[82,83] Finally, one report showed that hypoxia led to the circulation of a slight number of MSCs constantly in the PB under inactive circumstances. Then the circulating pool increases, and this increase is moderately definite for MSCs, while HPCs exhibit a limited or no increase under hypoxic conditions.

Conclusion

Mesenchymal stem cells display several biological responses to oxygen depletion in different contexts. Hypoxia markedly influences major MSCs features including cell viability, proliferation capacity, differentiation, migration pattern and metabolism. The reported conflicts in about the role of hypoxia on MSC biological properties, elucidate the importance of more dedicate research in stem cell biology. While hypoxia intensity is not the same in most of studies, the diversity of reported results should be cautiously evaluated as a variable when the literatures are reviewed. However, promising reports of hypoxia preconditioning supporting effects on cell survival and genetic instability of MSC, suggest a new hope to overcome poor engraftment after transplantation in bed side. Although before totally successful cell based regenerative therapies many of covert points should be clarified.

Acknowledgments

Authors would like to thank Tabriz Blood Transfusion Research Center for supporting this project.

Ethical Issues

There is none to be declared.

Conflict of Interest

The authors declare no conflict of interests.

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