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The Role of Hypoxia in Stem Cell Differentiation and Therapeutics

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Abstract

Stem cells differentiate into a variety of cell lines, making them attractive for tissue engineering and regenerative medicine. Specific micro-environmental cues regulate self-renewal and differentiation capabilities. Oxygen is an important component of the cellular micro-environment, serving as both metabolic substrate and signaling molecule. Oxygen has been shown to have a variety of effects on embryonic and adult stem cells. This review examines the role of hypoxia in regulating stem cell biology, specifically focusing on growth, maintenance of pluripotency, differentiation, and production of growth factors. Particular attention is paid to hypoxia and stem cells in relation to therapeutic angiogenesis. We conclude that further study is needed to optimize the use of hypoxia as a stimulus for various stem cell functions including its potential role in therapeutic angiogenesis.

Keywords

Hypoxia; stem cells; differentiation; therapeutic angiogenesis

INTRODUCTION

Stem cells either self-renew or differentiate depending upon various micro-environmental cues including soluble growth factors, extra-cellular matrix, and mechanical forces. Our laboratory has used these various stimuli in vitro to differentiate adult stem cells derived from adipose tissue (ASC) into endothelial cells for the purpose of creating autologous blood vessels/vascular grafts.^{1–3} While ASC can acquire several important characteristics of endothelial cells, differentiation appears incomplete as evidenced by minimal expression of eNOS. This led us to evaluate the effect of another key micro-environmental component, oxygen tension, on the differentiation and function of these cells. To our surprise, hypoxia appears to suppress the commitment of ASC towards endothelial cells; instead, it stimulates the expression of vascular endothelial growth factor (VEGF) via up-regulation of hypoxia-induced factor (unpublished observations). Given the peri-vascular location of these stem

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cells, this finding suggests that ASC play a paracrine role in stimulating angiogenesis, and that hypoxia may be a useful stimulus in manipulating these cells for therapeutic purposes.

Molecular oxygen serves as both a metabolic substrate and signaling molecule for cells both in vitro and in vivo. Its effect on stem cell self-renewal, differentiation and ultimate function in vitro is incompletely understood, largely because the differential effects of this cue depend on oxygen concentration and cell type. In certain types of adult stem cells, low oxygen concentration in vitro promotes proliferation and maintenance of a multipotent state.^{4,5} Conversely, other investigators have demonstrated hypoxia to be a potent stimulus for differentiation into specific cell lines.^{6,7} Alternatively, hypoxia can stimulate cytokine production, thereby potentially playing a role in therapeutic angiogenesis.⁸⁻¹⁰ Participation in angiogenesis by stem cells may occur directly via differentiation of cells that participate in angiogenesis, or indirectly via cytokine production stimulated by hypoxia.^{11, 12}

This review focuses on the known effects of hypoxia on stem cells. We explore the currently known roles of low oxygen concentration on maintenance of self-renewing properties, differentiation, and production of angiogenic growth factors. Lastly, we expound upon the potential clinical benefits of hypoxia on stem cells in promoting therapeutic angiogenesis.

OXYGEN CONCENTRATIONS IN THE STEM CELL MICRO-ENVIRONMENT

Most tissue cultures are maintained in vitro at oxygen levels of approximately 20%. Ironically, natural cell micro-environments appear to contain much lower oxygen tensions: the mean oxygen concentration of arterial blood approximates 12%, and that of tissue is 3%, with considerable variation based on location.⁵ Optimization and understanding stem cell growth and function requires knowledge of the specific micro-environmental conditions in vivo. Similarly, one needs to be mindful of oxygen tension when interpreting experiments involving stem cells in vitro.

Embryonic stem cells, in particular, live at low oxygen concentrations beginning at implantation and continuing through fetal development. During implantation of the embryo, the lack of access to maternal circulation results in a hypoxic environment.¹³ The uterine surface typically has oxygen concentrations of 2% during early pregnancy. Even after the embryo establishes connection to the maternal vasculature, placental oxygen levels only increase to approximately 8%.^{14,15} Hence, the normal physiologic environment of embryonic stem cells is one of relative hypoxia compared to traditional in vitro culture conditions.

Adult stem cells similarly live in hypoxic conditions in vivo. The most direct evidence arises from research on hematopoietic stem cells which share an environment with bone marrow-derived mesenchymal stem cells (BM-MSC).¹⁶ In a study evaluating bone marrow aspirates of volunteers, specimens were found to be consistently hypoxic, with some levels as low as 1-2%.^{17,18} Similarly, oxygen tension in the bone marrow of mice is significantly lower than other tissues.¹⁹

While the exact anatomical location of ASC within fat is not precisely known,²⁰ it is postulated that they surround the capillaries where they interact with endothelial cells and provide structural vascular stability.²¹ Given that lower oxygen concentrations are observed in adipose tissue compared to other tissues, ASC likely live in a hypoxic environment despite their presumed proximity to the vasculature.²²

EFFECT OF HYPOXIA ON STEM CELL GROWTH AND SELF-RENEWAL

Given hypoxic conditions are the physiological norms for a variety of stem cell niches, more research has incorporated hypoxia into tissue culture technique. A variety of studies demonstrate significant benefit in terms of cell proliferation using low oxygen tensions.^{23,24} Embryonic stem cells, in particular, appear to grow more efficiently under low oxygen concentrations when compared to room air. For example, bovine blastocysts demonstrate significantly more inner cell mass when cultured in hypoxia.^{25,26} Similar benefits occur in a variety of other species, including human embryonic cells.^{16,27}

Further support to the benefits of hypoxia on cell growth and expansion arises from research on adult stem cells, mainly human BM-MSC. In a study by Grayson et al. low oxygen culture resulted in a 30-fold increase in the expansion of cells compared to normoxic conditions.⁴ Studies involving ASC, however, have yielded conflicting results regarding the benefits of low oxygen concentration in tissue culture.^{8,28} Further study is needed to delineate the effects of hypoxia on ASC proliferation.

In addition to the improvement of cell growth and expansion on various stem cells, some investigators note that hypoxic culture conditions allow maintenance of potency. Embryonic stem cells remain undifferentiated in hypoxia for up to four weeks, with one study showing prolonged pluripotency up to 18 months duration.^{29,30} This finding is corroborated by normal morphological appearance and preservation of OCT-4. In adult BM-MSC, culture in hypoxic conditions (2% O₂) for up to six weeks increases the expression of embryonic markers such as OCT-4.^{4,31} Some investigators observe that hypoxia halts differentiation of ASC, allowing prolongation of the dedifferentiated state.³²

Although these studies suggest that hypoxia results in maintenance of potency, often small shifts in oxygen tension stimulate differentiation. Furthermore, this response to hypoxia may depend on various culture conditions. More information is required to understand the optimal oxygen tensions for promoting self-renewal and maintaining potency.

HYPOXIA AS A STIMULUS FOR STEM CELL DIFFERENTIATION

While research demonstrates a beneficial role of hypoxia in maintaining an undifferentiated stem cell, some researchers have explored the possibility of using hypoxia to stimulate differentiation. Manipulation of oxygen tension shows promising results in driving stem cells towards specific cell lines, particularly chondrocytes and cardiomyocytes.⁶ The optimal oxygen concentration to stimulate differentiation versus maintenance of stemness is unknown, however, and is likely affected by other culture conditions.

The majority of evidence for hypoxia's effect on differentiation involves formation of cartilage. As a tissue, cartilage is avascular and receives nutrients and oxygen mainly from the surrounding synovial fluid.⁶ Oxygen concentration within cartilage is reported between 1-8%.³³ In a study by Kaoy et al, human embryonic stem cells cultured in 2% oxygen concentration significantly increase production of cartilage matrix proteins, most notably collagen II.⁶ This finding has been confirmed in other stem cell lines, including bone marrow derived MSC and ASC.^{7, 34,35}

Similarly, osteocytes live at low oxygen tensions (4-7%) in vivo.^{18,36,37} Unlike chondrogenesis, however, the results for stimulation of osteogenesis appear mixed. Research demonstrates both a beneficial and deleterious response to hypoxia for stimulating osteogenesis. In a study of rat bone marrow-derived MSC, culture in 5% oxygen produces significant increases in bone production markers and proliferation of the differentiated cells.³⁸ Conversely, Malladi et al demonstrate that 2% oxygen inhibits osteogenic differentiation

in ASC.³⁹ It appears that the ultimate effect of hypoxia depends upon several factors, including stem cell line, degree and duration of hypoxia, as well other culture conditions.

Other studies, although few in number, examine the role of hypoxia adipogenic, cardiogenic and endothelial and differentiation. Fink et al demonstrate that culture in 1% oxygen induced an adipose phenotype in bone marrow-derived MSC but no increase in adipocyte-specific genes.⁴⁰ Conversely, Lee et al note that culture at 2% oxygen inhibits adipogenesis in ASC.⁴¹ Again, general findings remain inconclusive and mandate further research. In a study using embryonic stem cells, investigators reveal an increase in the number of cardiomyocytes in cultures at 4% oxygen tension compared to those grown in normoxic conditions.⁴² Cao et al demonstrate significant neovascularization in an in vivo mouse hind limb ischemia model after treatment with ASC; given evidence of stem cell incorporation into new vessels, they surmise that the stem cells differentiate into endothelial cells, presumably in these hypoxic conditions.¹¹ Another study suggests that hypoxia stimulates an endothelial phenotype in ASC, but specific endothelial cell markers are not present.⁸ In culture conditions previously shown to induce endothelial differentiation in ASC,¹ we found that hypoxia (2-5%) over a three week period inhibits the expression of both von Willebrand Factor and CD31 (unpublished data).

EFFECT OF HYPOXIA ON THERAPEUTIC ANGIOGENESIS

Oxygen is an important signaling molecule which impacts cellular activity. In stem cells, like others, hypoxia is known to increase expression of specific genes involving glycolysis, erythropoiesis and angiogenesis (glut-1, Epo, and VEGF, respectively).⁴³ Using hypoxia to stimulate angiogenesis via stem cells may therefore hold promise for treating vascular occlusions in the coronary and peripheral circulations.

Independent of hypoxia, stem cells may promote angiogenesis via several mechanisms, including differentiation into cells which participate in new blood vessel formation (e.g., endothelial cells) and production of growth factors. Although evidence exists for the former mechanism, mentioned above,¹¹ several studies suggest stem cells may influence angiogenesis via a paracrine mechanism. Both bone marrow-derived MSC and ASC produce a variety of angiogenic cytokines, including VEGF.^{9, 10, 44} Additionally, when stimulated by hypoxia, ASC express a 5-fold increase in VEGF production.^{8,10}

In a hypoxic environment, VEGF produced by stem cells directly impacts surrounding cells. When co-cultured with endothelial cells in hypoxic conditions, ASC produce VEGF which stimulates increased capillary formation by the endothelial cells in vitro.⁸ Similarly, other studies show that conditioned media from ASC or BM-MSC cultured in hypoxia increases endothelial cell growth and prevented apoptosis.^{10,45,46} The latter protective benefit is also seen in other cells; Sadat et al notes that co-culture with ASC decreases hypoxia-induced apoptosis in rat cardiomyocytes.⁹

In vivo models provide further support for the use of stem cells in promoting angiogenesis. Numerous studies show significant improvement in revascularization using ASC and BM-MSC in a mouse hind limb ischemia model.^{10,36,46} In this model, improvements are demonstrated both histologically, where an increase in vascular density in the treated group is seen, and by laser doppler testing. In a clinical trial of patients with claudication, there is a 3.7 fold increase in pain-free walking distance and improvement in ankle-brachial indices of patients receiving intra-arterial and intramuscular transplantation of autologous bone marrow-derived MSC.⁴⁷ Improvement in ejection fraction is noted in rats with ischemic cardiomyopathy after treatment with ASC.⁴⁸ In this study, no significant cardiomyocyte differentiation is demonstrated leading the authors to conclude that the stem cell effect is derived from increase in growth factors. In studying the influence of BM-MSC on new

blood vessel formation, Ziegelhoffer et al did not observe direct incorporation of the BM- MSC into new blood vessels; rather, they notice strong localization of these cells around the nascent collateral arteries, suggesting an indirect mechanism of incorporation.⁴⁹

CONCLUSION

Oxygen concentration is an important factor in the maintenance, differentiation and function of stem cells. The use of low oxygen concentration as a means to simulate the physiological norm of the stem cell micro-environment can be useful in maintaining and expanding a population of cells that may be limited supply or difficult to cultivate. Under other conditions, hypoxia may be used as a stimulus to promote differentiation into various cell lines. Finally, hypoxia has a significant influence on the production of growth factors involved in promoting angiogenesis. Given the effect of stem cells on promoting angiogenesis in vitro and in vivo, including in clinical trials, it is possible that pre-conditioning of the cells in hypoxic culture, prior to clinical use, may further their efficacy in clinical use.

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Figure.

Algorithm of treatment for patient presenting with coronary artery or peripheral vascular disease. If the patient is not a candidate for standard revascularization options, cell-based therapeutic angiogenesis may be an option. Areas of future research should define which tissue source is most optimal for promoting angiogenesis, the ideal pre-conditioning strategy using hypoxia prior to use, as well as the most efficacious route of administration. Hypoxia may prove to be a useful stimulus for both culture expansion and stimulating cytokine expression.

Table

Key references investigating the effect of hypoxia on stem cell differentiation and function.

Author	Ref	Stem cell source	Oxygen concentration	Major findings
Prasad	30	Human Embryonic Stem Cells	5%	Promotion of stemness
Grayson	31	Human Bone Marrow derived Mesenchymal Stem Cells	2%	Increase in expansion and prolonged maintenance of stemness
Lin	32	Mouse Adipose derived Stem Cells	1%	Promotion of stemness
Koay	6	Human Embryonic	2%	Increase in chondrogenesis
Khan	7	Human Adipose derived Stem Cells	5%	Decrease in proliferation with an increase in chondrogenesis
Lennon	38	Rat Bone Marrow derived Mesenchymal Stem Cells	5%	Increase in proliferation and osteogenesis
Malladi	39	Mouse Adipose derived Stem Cells	2%	Increase in proliferation with decrease in osteogenesis
Rehman	10	Human Adipose derived Stem Cells	1%	Increase in vascular endothelial growth factor (VEGF) production and survival of endothelial cells exposed to hypoxic conditioned media
Thangarajah	8	Mouse Adipose derived Stem Cells	1%	Increase in proliferation and VEGF production.