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Hypoxic Culturing Enhances the Wound-Healing Potential of Adipose-Derived Stem Cells

Won-Serk Kim[1] and Jong-Hyuk Sung[2,3,*]

[1]Department of Dermatology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.
[2]Laboratory of Skin Regeneration, Department of Applied Bioscience, CHA University, Seoul, Korea.
[3]Stem Cell Research Laboratory, CHA Stem Cell Institute, Seoul, Korea.

Jong-Hyuk Sung

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*Correspondence: Laboratory of Skin Regeneration, Department of Applied Bioscience, CHA University, #606-16, Yeoksam-dong, Kangnam-gu, Seoul 135-081, Korea (e-mail: brian99@empal.com).

Abbreviations and Acronyms

Akt = a type of serine/threonine protein kinase
ASC = adipose-derived stem cells
ASC-CM = conditioned medium of ASCs
bFGF = basic fibroblast growth factor
DPI = diphenyleneiodonium
ERK = extracellular signal-related kinase
HIF-1α = hypoxia-inducible factor-1 alpha
mTOR = mammalian targets of the rapamycin
Nox = NADPH oxidase
PDGFR = platelet-derived growth factor receptor
ROS = reactive oxygen species
VEGF = vascular endothelial growth factor

Background: Adipose tissue is one of the richest sources of mesenchymal stem cells that exhibit an outstanding ability to regenerate skin.
The Problem: Although the anatomical sites of adipose-derived stem cells (ASCs) in the body are relatively oxygen-deficient (*i.e.*, 1%–5% oxygen content), ASCs are usually cultured under normoxic conditions, and long-term culturing of ASCs in normoxia may induce their senescence.
Basic/Clinical Science Advances: The review is an overview of the cellular responses of ASCs during hypoxia, which collectively increase the wound-healing potential of ASCs. Furthermore, the mechanism of action for stimulation by hypoxia (*i.e.*, a pivotal role of reactive oxygen species and related signal pathways) will be discussed.
Clinical Care Relevance: Hypoxia is a critical stimulatory factor for ASCs. Therefore, understanding the response and adaptation of ASCs to hypoxia may be invaluable for developing novel cell therapeutic strategies.
Conclusion: Culturing ASCs under hypoxia may uniquely benefit proliferation, stemness, migration, and growth factor secretion. Therefore, the preconditioning of ASCs by hypoxia shows a prominent wound-healing effect in clinical use.

BACKGROUND

Regenerative medicine uses the body's own stem cells and growth factors to repair damaged tissue, such as skin. Adipose-derived stem cells (ASCs) and their soluble factors reportedly have been used to regenerate skin, and they offer a potential solution for skin regeneration.[1–4] For example, ASCs and their soluble factors accelerate wound healing, reduce wrinkling, improve pigmentation, and promote hair growth.[2–5] In addition to differentiation, transplanted ASCs and their soluble factors induce the angiogenesis, increase the proliferation of fibroblasts and keratinocytes, and remodel the extracellular matrix.[6] Recently, we found that culturing in hypoxia enhances the wound-healing function of ASCs by increasing the proliferation and secretion of growth factors for ASCs.[7] Furthermore, the mechanism for the hypoxia-induced stimulation of ASCs was investigated that hypoxia generates reactive oxygen species (ROS), which activate platelet-derived growth factor receptor (PDGFR) pathways.[8] Therefore, this review provides an overview of the cellular responses of ASCs during hypoxia and of the enhanced wound-healing potential under hypoxic culturing conditions. In addition, the mechanism of action for stimulation by hypoxia will be addressed.

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TARGET ARTICLES

1. Kim JH, Park SH, Park SG, Choi JS, Xia Y, and Sung JH: The pivotal role of reactive oxygen species generation in the hypoxia-induced stimulation of adipose-derived stem cells. Stem Cells Dev 2011; **20:** 1753.

2. Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, Park BS, and Sung JH: Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and upregulation of VEGF and bFGF. Wound Repair Regen 2009; **17:** 540.

3. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, and Park JS: Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci 2007; **48:** 15.

CLINICAL PROBLEM ADDRESSED

Vascular complications are commonly associated with problematic wounds, which lead to tissue hypoxia. In general, acute mild hypoxia supports adaptation and survival, whereas chronic severe hypoxia leads to tissue damage.[9] Because the microenvironment near the wound is in an oxygen-deficient state, it is interesting to elucidate the effect of hypoxia on the wound-healing function of ASCs.

Low oxygen tension is reportedly an important characteristic of the stem cell niche, and hypoxia provides signals conducive to the maintenance of definitive stem cell properties.[10,11] Therefore, understanding the response and adaptation of ASCs to hypoxic culturing may be invaluable for developing novel ASC therapies.

RELEVANT BASIC SCIENCE CONTEXT

Most stem cells in the body exist in environments with a low, or very low oxygen supply. Additionally, *in vitro*, oxygen concentration is sensed by stem cells, and low PO_2 modifies their phenotypes; therefore, use of PO_2 matter should be more carefully monitored in stem cell culturing.[12] For example, hypoxia can reduce spontaneous differentiation and maintain clonality of human embryonic stem cells, and it can enhance the generation of induced pluripotent stem cells.[12–14] In addition, when mesenchymal stem cells are cultured under hypoxic conditions *in vitro*, their proliferative and self-renewal capacities are significantly improved.[15] Likewise, ASCs favor hypoxia and their phenotypes are regulated by it.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

The wound-healing effect was investigated in excision wound models of nude mice after topical application of ASCs and conditioned medium of ASCs (ASC-CM).[16] To study the mechanism, primary dermal fibroblasts were cultured, and the effect of ASC-CM on their proliferation, migration, and collagen synthesis was measured. In addition, the effect of hypoxia on the proliferation and growth factor secretion of ASCs was examined using an hypoxia incubator.[7] The ASC-CM was harvested in hypoxia (hypoCM) and the wound-healing potential was compared with normoxia (norCM).[7] ROS generation by hypoxia was directly measured by 2′,7′-dichlorofluorescin diacetate intensity in a fluorescent activated cell sorter. Phosphorylation of PDGFR-β, a type of serine/threonine protein kinase (Akt), and extracellular signal-related kinase (ERK) was measured by Western blot analysis. Study of the inhibition of ROS generation was examined using *N*-acetyl-cysteine (NAC, ROS scavenger) and diphenyleneiodonium (DPI; NADPH oxidase [Nox] inhibitor).[8]

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

ASCs promoted proliferation of dermal fibroblasts not only by cell-to-cell direct contact but also by paracrine factors. ASC-CM enhanced the secretion of type I collagen in dermal fibroblasts by regulating the mRNA levels of extracellular matrix proteins: upregulation of collagen and down-regulation of matrix mataloproteinase-1. Moreover, ASC-CM showed a stimulatory effect on the migration of dermal fibroblasts. In addition to this *in vitro* evidence, ASCs and ASC-CM significantly reduced the wound sizes and accelerated the re-epithelialization of the excision wound model. In addition to our study, other groups have demonstrated the wound-healing effect of ASCs and their mechanism of action, and found that locally transplanted ASCs accelerate wound healing not only by differentiation and vasculogenesis but also by paracrine factors.[6,17] Induced pro-angiogenic potential of ASCs by hypoxia has been reported that the transplantation of ASCs cultured in hypoxia significantly increased the local blood flow and angiogenic gene expression was partially improved by hypoxia preconditioning.[18,19]

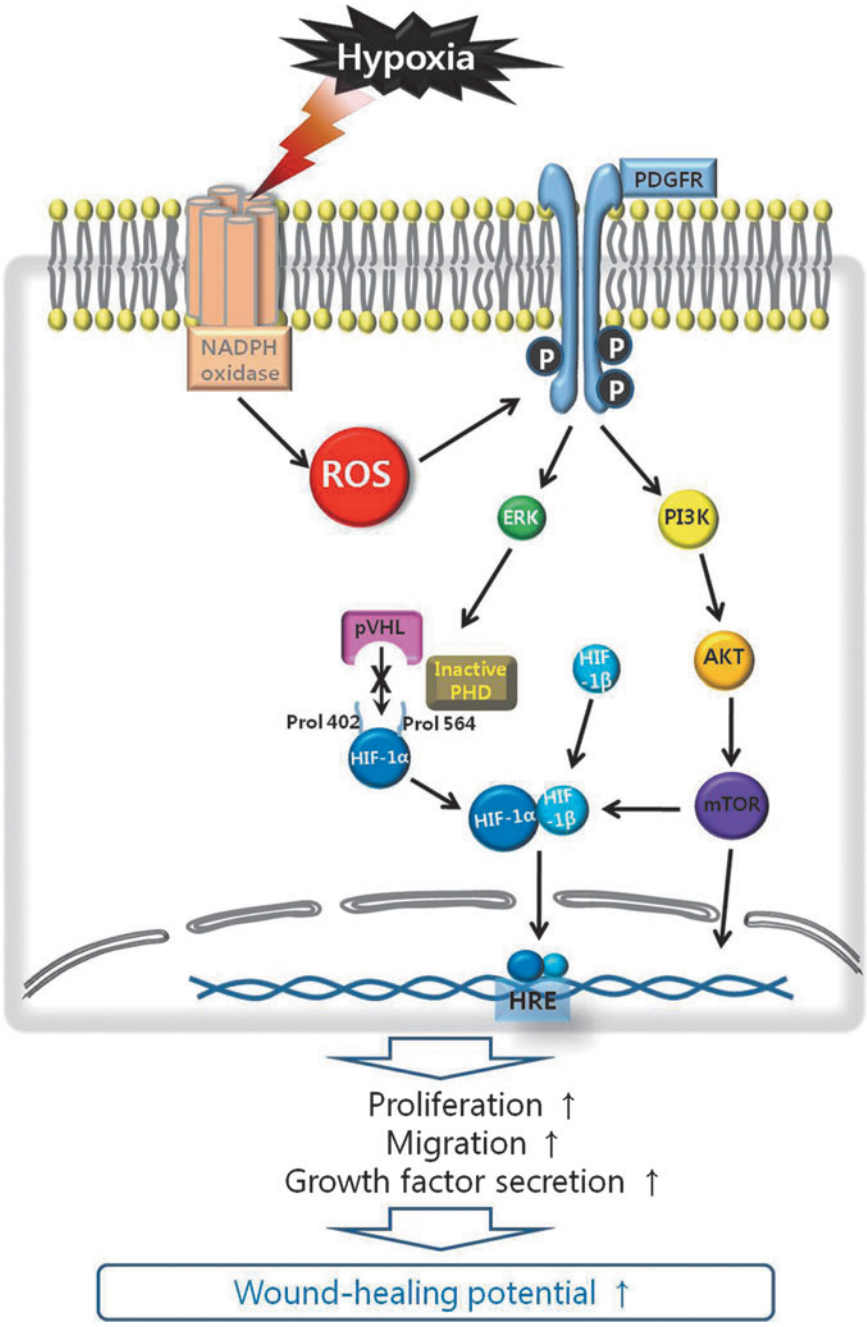
Incubation under hypoxic conditions enhanced the proliferation of ASCs in either the presence or absence of serum. Furthermore, mRNA and protein measurements showed that hypoxia upregulated the secretion of growth factors such as vascular endo-

thelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in ASCs. HypoCM significantly promoted collagen synthesis and the migration of human dermal fibroblasts, compared with norCM. In the animal studies, hypoCM significantly reduced the wound size and accelerated the healing compared with norCM. Inhibition of VEGF and bFGF in conditioned medium using neutralizing antibodies attenuated the wound healing in animal experiment, which indicates that VEGF and bFGF are involved in the enhanced wound-healing function by hypoxia.

Further investigation included a key mediator and a signal pathway involved in the stimulation of ASCs during hypoxia. Hypoxia significantly increased ROS generation, which was greatly reduced by NAC and DPI treatment. Likewise, the hypoxia-induced proliferation and migration of ASCs were reversed by NAC and DPI treatment, suggesting the involvement of ROS generation in ASC stimulation. In Western blot analysis, hypoxia increased the phosphorylation of PDGFR-β, which was followed by an activation of the Akt and ERK pathways. These results suggest a pivotal role for ROS generation in the stimulation of ASCs by hypoxia.

INNOVATION

Hypoxia and the generation of ROS serve as a second messenger in the intracellular signal



transduction pathway.[20,21] However, most studies focus on the negative aspects of hypoxia and ROS generation. Physiological levels of intracellular ROS are required to activate the DNA repair pathway for maintaining genomic stability in stem cells and to increase the proliferation and migration of stem cells. In addition, stem cells efficiently manage oxidative stress and have a high resistance to ROS-induced death.[22,23] Therefore, attention must be paid to the positive effects of hypoxia and ROS generation in ASC physiology.

TAKE-HOME MESSAGE

Basic science advances

- ASCs and their soluble factors accelerate wound healing.
- Hypoxia enhances the wound-healing function of ASCs by increasing proliferation/migration of ASCs and secretion of paracrine factors.
- Hypoxia-enhanced ASC stimulation is mediated by generation of ROS and activation of PDGFR pathways.

Clinical science advances

- Low oxygen tension is an important characteristic of the stem cell niche and hypoxia provides signals conducive to the maintenance of definitive stem cell properties.
- The long-term culturing of ASCs in normoxia drives them to senescence, but hypoxia uniquely contributes to their expansion and wound-healing functions.

Relevance to clinical care

- Before ASC transplantation, adaptation of ASCs to hypoxia may be invaluable for developing a prominent ASC therapy.

SUMMARY ILLUSTRATION

Hypoxia initiates ROS synthesis in ASCs via the Nox family. Increased intracellular ROS levels activate receptor-type or nonreceptor-type tyrosine kinases in ASCs. Of them, PDGFR-β was primarily phosphorylated. Activation of these tyrosine kinases subsequently increases the phosphorylation of PI3K/Akt/mammalian targets of the rapamycin (mTOR) and ERK1/2 signaling pathways. These events inhibit the degradation of hypoxia-inducible factor-1 alpha (HIF-1α) by the propyl-hydroxylation of the von Hippel Lindau tumor suppressor protein and an increase in cytosolic HIF-1α levels. HIF-1α translocates to the nucleus where it modulates the transcription of its target genes after binding to the hypoxia-responsive element in the nucleus. Upregulation of target genes may increase the proliferation, migration, and growth factor secretion of ASCs, which collectively enhances the wound-healing function of ASCs.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Typically, ASCs are cultured under ambient or normoxic conditions. However, the O_2 concentration in the physiological niches occupied by ASCs is much lower. Thus, hypoxia can function as a prophylactic signal, increasing proliferation, adhesion, and migration of ASCs by ROS generation. In addition to oxygen content, diverse culturing conditions (*i.e.*, supplement of growth factors, quality of serum and its concentration, composition of extracellular matrix, and quality of culture wares) collectively influence the proliferation and regeneration potential of ASCs. Therefore, caution should be taken to optimize the expansion conditions for ASC therapies.

FUTURE DEVELOPMENT OF INTEREST

While hypoxia and ROS generation are involved in apoptosis, low and moderate intracellular ROS levels act as a signal transducer that activates ASCs. Because moderate ROS levels increase the proliferation and regenerative potential of ASCs, it is beneficial to expose ASCs to moderate oxidative stress during manipulation. Hypoxia or the addition of an ROS donor may negate the expense of culturing ASCs during expansion, and ROS preconditioning may enhance the wound-healing potential of ASCs in clinical use. Therefore, future study should address the efficiency of ROS donors and the optimal ROS concentrations for an ASC culture.

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AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have no competing interests. The authors were the only writers for this article.

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