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Effects of Hypoxia on the Proliferation and Differentiation of NSCs

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Abstract

Oxygen is vital to nearly all forms of life on Earth via its role in energy homeostasis and other cell functions. Until recently, the effects of oxygen on the proliferation and differentiation of neural stem cells (NSCs) have been largely ignored. Some studies have been carried out on the basis of the fact that NSCs exists within a "physiological hypoxic" environment at 1 to 5% O_2 in both embryonic and adult brains. The results showed that hypoxia could promote the growth of NSCs and maintain its survival in vitro. In vivo studies also showed that ischemia/hypoxia increased the number of endogenous NSCs in the subventricular zone and dentate gyrus. In addition, hypoxia could influence the differentiation of NSCs. More neurons, especially more doparminergic neurons, were produced under hypoxic condition. The effects of hypoxia on the other kind of stem cell were briefly introduced as additional evidence. The mechanism of these responses might be primarily involved in the hypoxic inducible factor-1 (HIF-1) signal pathway. The present review summarizes recent works on the role of hypoxia in the proliferation and differentiation of NSCs both in vitro and in vivo, and the mechanism involved in HIF-1 signaling pathway behind this response was also discussed.

Index Entries: Hypoxia; NSCs; proliferation; differentiation; HIF-1.

Introduction

Oxygen is vital to nearly all forms of life on Earth via its role in energy homeostasis *(1)*. Changes of oxygen concentration (oxidative stress), as an important physiological and pathological regulator, might influence the whole life span, from embryogenesis and development to the maintenance of normal function, dysfunction, and aging.

Neural stem cells (NSCs) exist not only in the developing mammalian nervous system but also in the adult nervous system of all

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mammalian organisms, including humans. Cultured central nervous system (CNS) stem cells have proved useful in defining the pathways that lead to the generation of neurons and glia. The potential therapeutic applications of CNS stem cells in common degenerative and ischemic diseases have become a major focus of research *(2).*

Traditional CNS stem cell culture (as well as virtually all other ex vivo cell culture) is performed in "standard and physiological" conditions to mimic the internal milieu. As a result, the cell culture system generally employs an environmental oxygen (O_2) level of 20%, whereas actual tissue O_2 levels in both developing and adult brains is of a lower order of magnitude, which might result in a distortion of the condition in vivo. In particular, there has been little attention given to the role of local microenvironment hypoxia in stem cell activation and differentiation during the physiology and pathology through the adult.

The present review summarizes recent works on the role of hypoxia in the proliferation and differentiation of NSCs both in vitro and in vivo, and the mechanism involved in the hypoxic inducible factor-1 (HIF-1) signaling pathway behind this response was also discussed. At the end of this review, we discuss the following concerns: (1) whether 1 to 5% hypoxia is real "physiological hypoxia" in vitro; (2) whether there exists a HIF-1 independent pathway involved in the responses of NSCs to hypoxia; (3) suggestions for theoretical and therapeutic considerations of these findings.

Hypoxia Is a Physiological Environment for Embryo Development and Adult Brain Tissue

Hypoxia exists in early embryonic development and adult brain tissue. During the first trimester of pregnancy, there is little endovascular invasion, and as a result, placental development occurs in a hypoxic environment *(3).* In rat embryos, oxygen tensions are low before 9.5 d postcoitum (dpc *[4]*).The human embryo is also located in a low oxygen environment (3% O_2) *(5).* By using the highly sensitive multiparameter, probe the oxygen concentration was determined as 25.6 mmHg in the intervillous space at the first 7 to 10 wk *(6).*

An in vivo study was carried out by means of a hypoxia marker, pimonidazole, and its associated antibody. The results indicated that hypoxia exists in normal developing embryonic tissues, as immunoreactivity of the hypoxia marker was highly detected in developing neural tubes, heart, and intersomitic mesenchyme at an early stage of organogenesis *(7).*

Hypoxia exists not only during the embryos development but also in the adult mammalian brain. The oxygen levels of interstitial tissue are low and range from 1 to 5% *(8–10).* The mean brain tissue oxygen level in the adult rat and fetal sheep was 1.6% *(11,12)* and physiological oxygen levels in some brain regions are even lower.

By using a microelectrode, regional rat brain tissue partial pressures of oxygen were measured as follows. The oxygen level is 2.5 to 5.3% in the gray, cortex 0.8 to 2.1% in the white, cortex 1.4 to 2.1% in the hypothalamus, 2.6 to 3.9% in the hippocampus, and 0.1 to 0.4% in the pons/fornix *(12).* These data suggest that the embryonic, the fetal, and the adult brain tissues were in a hypoxic condition. Some researchers called this phenomenon "physiological hypoxia" *(13–15).*

Effects of Hypoxia on the Proliferation and Differentiation of NSCs

Effects of Hypoxia on the Proliferation and Differentiation of NSCs In Vitro

In 2000, Morrison and Studer reported for the first time that culturing NSCs in a decreased oxygen environment promoted their survival, proliferation, and differentiation. In Morrison's

experiment, neural crest stem cells were derived from sciatic nerves of a E14.5 rat and cultured in a decreased oxygen (5%) condition. They reported that the ability of $P75^{+}PO^{-}$ cells to survive and form colonies was significantly increased after 6 d in hypoxic culture. Although the neural crest cells have the ability to differentiate into dopaminergic neurons with the addition of bone morphogenetic protein 2 (BMP2) plus forskolin, the hypoxic culture promoted the production of greatly expanded dopaminergic neuron numbers in nearly all colonies. Furthermore, these differentiated neurons in low oxygen could also synthesize and release dopamine and norepinephrine *(13).*

Studer et al., who used the CNS precursor from E12 rat mesencephalen, have also carried out parallel experiments. They found that the cells yielded greater members of precursors and showed less apoptosis after being grown in low oxygen (3±2%) for 6 d. The percentage of neurons of dopaminergic phenotype increased to 56% in lowered O_2 compared with 18% in 20% O_2. Together, the increases in total neuron number (proliferation) and percentage of dopaminergic neurons (differentiation) resulted in a ninefold net increase in dopamine neuron yield *(15).*

Storch and his colleagues cultured human mesencephalic neural precursor cells from 9- to 12-wk-old fetal brain in low oxygen (3%) and found long-term proliferation of these cells could grow and survive up to 11 mo. Moreover, these human NSCs with low-oxygen culture could also give rise to dopamine neurons, which exhibited morphological and functional properties as a control *(14,16).*

Consistent with these studies, our study also showed that a hypoxic culture detectably promoted the formation of the primary cultured neural spheres from mesencephalic NSCs of the E13.5 rat. Neural sphere numbers were found to be 1.5 and 2.4 times the "normal condition" (20% O_2) following a 3-d culture with 3% and 10% O_2, respectively. The bromodeoxyuridine (BrdU)-positive cells in the lowered O_2 also significantly increased compared with the "normal condition." The stem cells under 3% O_2 culture also displayed an increase in the absolute number of neurons and a decrease in the absolute number of glia cells *(17).*

The above data indicated the following: (1) Mild hypoxia promotes the proliferation of NSCs from both the peripheral nervous system (PNS) and CNS of rat and human in vitro and (2) lowered oxygen enhances neuronal, especially dopaminergic, differentiation of CNS precursors in vitro. These results suggest that suitable hypoxia can promote proliferation and modify the cell fate of NSCs in vitro, which could be a useful tool for expansion of NSCs for ex vivo cell therapy and for a mechanism study of neural development.

Effects of Hypoxia on the Proliferation and Differentiation of NSCs In Vivo

Recent 10-yr studies have shown that NSCs exist in various regions of the CNS throughout the mammalian life span *(18).* The subventricular zone (SVZ) and subgranular zone of the hippocampus (SGZ) contain a relatively high density of the cells. The division and differentiation of these endogenous NSCs could be regulated by both physiological stimuli and pathological conditions *(19).* Here, we focus on the recent studies on the proliferation and neurogenesis of endogenous NSCs in the adult brain following hypoxia/ischemic insults.

There are two types of ischemic insult of the brain. In animals, global ischemia models replicate the consequences of cardiac arrest or coronary artery occlusion, whereas focal ischemia models replicate the consequences of stroke. However, for the hypoxic animal model in vivo, a high altitude was generally used to mimic this condition.

Global forebrain ischemia gives rise to enhanced cell proliferation in the rodent SGZ *(20–23).* Neurogenesis in the SGZ is also increased by stroke induced by middle cerebral artery occlusion *(19,24).* BrdU-labeled cells increased approximately sevenfold, and polysialylated neural cell adhesion molecule (PSA-NCAM)-positive cells increased approximately

threefold in the subgranular zone, with a peak 10 d after ischemia *(25).* This suggested that the new cells migrate into the granule cell layer and become mature neurons in hippocampus following ischemia.

The neurogenesis from NSCs or progenitor cells located in the SVG lining the lateral ventricle was also triggered by ischemic insults. Jin et al. reported that stroke induced cell proliferation in the rostral SVZ, and the proliferated cells coexpressed markers for immature neurons *(24).* Tonchev et al. investigated the fate of proliferating cells in the adult monkey brain after global ischemia. They found that the number of proliferating cells in the hippocampus and SVZ significantly increased following global cerebral ischemia for 20 min. In the monkeys subjected to ischemia, 20 to 40% of the newly generated cells in the SVZ expressed the neural progenitor cell markers Musashi-1 or Nestin *(26).* Thus, ischemia induces the proliferation and generated new neurons in SVZ.

We treated male Wistar rats (body weight: 160–180 g) with mimicked 3000 and 5000 m high altitude in a sealed container, 4 h/d for 2 consecutive weeks. We found that the number of BrdU-labeled cells in the SVZ and dentate gyrus (DG) increased 62% and 35%, respectively, compared with control groups (unpublished data). This indicated that intermittent hypoxia could increase the proliferation of NSCs in the SVZ and DG.

The molecular mechanisms regulating ischemia/hypoxia-induced neurogenesis are only partly understood. Hypothetically, neurogenesis in vivo following ischemia/hypoxia could proceed as it does during embryonic development, involving the same concerted action of transcription factors, signaling molecules, and growth factors. According to the recent studies, the following factors might be involved in this process *(19):*

1. Stem cell factor (SCF). Jin et al. reported that SCF, which was synthesized in response to hypoxia, triggered neurogenesis in the SVZ and SGZ in vivo, through its receptor c-kit *(24).*
2. Erythropoietin (EPO). Erythropoietin is also produced as part of the ischemic/hypoxic response. Intraventricular infusion of EPO and EPO antibodies leads to increased and decreased production of neurogenesis in the SVZ through its receptors there *(27).*
3. Brain-derived neurotrophic factor (BDNF). Intraventricular infusion of BDNF protein *(28,29)* or overexpression of the BDNF gene *(31)* with intermittent hypoxia increased the number of new pyramidal neurons in hippocampal CA1 and new neurons in the SVZ of adult rat brain.
4. Fibroblast growth factor-2 (FGF-2). Intraventricular infusion of the FGF-2 after global ischemia in rats leads to marked regeneration of hippocampal CA1 pyramidal neurons *(31).*

In addition to these, glutamatergic mechanisms were also involved in the regulation of SGZ neurogenesis after global and focal ischemia/hypoxia *(19).*

Taken together, neurogenesis in vivo is a very complicated process, with many cytokines, growth factors, and local microenvironment involved in it. Hypoxia/ischemia as a physiological/pathological regulated factor might affect NSC development in the entire cellular signal network or through a main route of the HIF signal pathway. The above findings could furnish important signals for developing a new therapeutic intervention to enhance endogenous neurogenesis after brain injury.

Effects of Hypoxia on the Proliferation and Differentiation of Other Stem Cells

In addition to NSCs, the role of hypoxia in the development of the circulation system has been extensively studied, in particular on the stem cell of the hematopoietic and cardiovascular systems *(32).*

Hypoxia (0.9–1% O_2) contributes to the self-renewal of murine and human normal hematopoietic stem cells *(33–35).* Low oxygen (5% O_2) maintains the expansion of the megakaryocyte progenitor, whereas "normal" oxygen (20% O_2) tension favors the terminal differentiation of megakaryocytes into platelets *(36).*

Cytotrophoblasts are specialized placental cells and localize near the uterine surface. Although cultured under the hypoxic conditions (2% O_2) to mimic the inner environment, the cells continued proliferation with poor differentiation, whereas if cultured in 20% O_2, the cells stopped proliferation and differentiated into highly invasive phenotype *(37,38).*

The same effect of hypoxia on the endothelial cells was also reported by Philips et al. Oxygen pressure as low as 5% in the gas phase or 87 mm Hg in the liquid phase induced various neuroendocrine cell (NEC) phenotypes in more than 80% of cells of a cloned fetal Syrian hamster lung epithelial cell line (M3E3/C3), which is of a pluripotent stem cell type. Therefore, hypoxia appeared to possess potency for NEC phenotype induction *(39).*

Skeletal muscle satellite cells are ubiquitous in adult skeletal muscle and are considered stemlike cells because of their great proliferative capacity. Proliferation of satellite cells from adult murine increased in 6% oxygen. Six percent oxygen conditions also accelerated the gene expression of the MyoD family and other multiple myogenic regulatory factors (MRFs) *(40).* Therefore, mild hypoxia (6% oxygen) increased cell proliferation and survival *(41),* whereas hyperoxia (from 20% to 40% oxygen) enhanced fusion of mononucleated myoblasts into myotubes *(41).* Our work with rat myoblasts also indicated that the number of myoblasts cultured in 3% and 10% O_2 conditions increased by 1.5 and 2.5 times compared to control group, respectively *(42).* More recently, Carlo et al. investigated the effect of hypoxia on the ability of myogenic cells to differentiate in culture. Exposure of myoblasts to hypoxia (1%) strongly inhibited the multinucleated myotube formation and the expression of differentiation markers (MyoD, Myf5, and myogenin) *(43).*

During mild hypoxia, myocardial O_2 drops to approx 1 to 3% or lower. Compared with cells cultured in 3% O_2, cardiac fibroblasts that were cultured in 10% or 21% O_2 demonstrated remarkable reversible G2/M arrest and a phenotype indicative of differentiation to myofibroblasts *(44).*

Rat mesenchymal stem cells (rMSCs) represent a small portion of the cells in the stromal compartment of bone marrow and have the multipotential abilities to differentiate into bone, cartilage, fat, and fibrous tissue. While cultured in low oxygen (5% O_2), these mesenchymal progenitor cells gave a greater number of colonies after primary isolation and proliferated more rapidly in vitro. The hypoxia-treated cells even produced more osteogenesis after implantation in vivo *(45).* Our preliminary result also showed an increase in growth of human mesenchymal progenitor cells with 3% O_2 *(46).* Hypoxia also inhibited the differentiation of preadipocyte (3T3-L1) into adipocyte by inhibition of PPARr2 gene expression *(1).*

The P19 cell line (teratoma of mice) can differentiate into many types of neuron induced by retinoic acid (RA). After cultured under a hypoxic condition (3% O_2), the number of tyroxine hydroxylase (TH)-positive cells from P19 cells was over 15% compared with 0.8% of the control *(47).* Oxygen also acts as a potent regulator on various types of other stem cell (*see* Table 1).

In general, environmental hypoxia could have a significant impact on the proliferation and differentiation of many kinds of stem cell rather than on NSCs and the effects of hypoxia on the stem cells are extensive and cell-type-specific.

Role of HIF-1 in the Development of NSCs In Vivo or In Vitro

In general, lack of oxygen leads to cell growth arrest or death and determines tissue pathology, whereas hypoxia promotes its growth and regulates its differentiation in some stem cells. Under physiological hypoxic condition, most stem cells can adapt to the stressful environment and survive. The factors that are involved in this response to hypoxia are poorly understood. However, expression of specific genes within different type of cell appears to be a key.

Until now, few studies have been done to determine the roles of proneural genes and

Table 1
Proliferation and Differentiation of Stem Cells Regulated by Hypoxia

Type of stem cells	Level of oxygen	Proliferation ±	Differentiation ±	Ref.
Neural crest stem cells (rat)	5%	+	DA+	*13*
Mesencephalic precursor (rat)	3±2%	+	DA+	*15*
Rodent mesencephalic progenitor cells	3%	+	DA+	*14*
Neural stem cells (rat)	3%	+	neuron+	*17*
Mmurine skeletal muscle satellite cells	6%	+	Adipogenic cells–	*40*
Rat myoblast	3%	+		*42*
Myoblasts	1%	+	Myotube–	*43*
Rat mesenchymal stem cells (rMSCs)	5%	+	Oseteocytes+	*45*
Human mesenchymal stem cells (hMSCs)	3%	+		*46*
Murine myofibroblasts	3%	+		*44*
Human hematopoietic stem cells	1%		Erythroid progenitor cells+	*48*
Murine hematopoietic precursors	3%	+		*49*
Human hemopoietic precursor cell (hHPCs)	1%	+		*34*
Murine bone marrow cells	1%	+		*35*
Human megakaryocyte progenitor	5%	+	Platelets+	*36*
Cytotrophoblasts	2%	+	Column epithelial cells–	*50*
Human cytotrophoblasts	3%	+		*56*
Human cytotrophoblasts	2%	+		*3*
Fetal Syrian hamster lung line (M3E3/C3)	5%		Neuroendocrine cell+	*39*
Murine embryoid bodies (EBs)	1%	–		*51*
P19 cell line (teratoma of mice)	3%	–	DA+	*47*

neurogenesis genes in the development of NSCs under a hypoxic environment. Among the complicated signal transduction network, primary attention has been paid to the central point of the oxygen-sensitive signal pathway—HIF-1.

HIF-1 and Its Target Genes

Hypoxic inducible factor-1 is a heterodimeric transcription factor consisting of α- and β-subunits. As a master regulator of mammalian oxygen homeostasis, HIF-1 becomes activated by phosphorylation and by the mitogen-activated protein (MAP) kinase ERK in response to hypoxia of most cells. After translocated into the nucleus, HIF-1 could upregulate nearly 50 downstream target genes. Therefore, HIF-1 plays a pivotal role in the process of hypoxia signal transduction in both physiology and pathophysiology. The target genes of activate HIF-1 have the functions of glycolysis, glucose transport, erythropoiesis, and angiogenesis. Many of them are involved in cell proliferation, survival, and differentiation, including erythropointin (EPO), vascular endothelial growth factor (VEGF), transferring and its receptor, platelet-derived growth factor-β (PDGF-β), basic fibroblast growth factor (bFGF), and others *(52).*

Role of HIF in Brain Development

Hypoxie inducible factor-1α is expressed in the developing brain *(53)* and modulates gene activity in response to low oxygen in a hypoxic brain in vivo. Complete deficiency of HIF-1α resulted in developmental arrest and lethality of E10.5 or E11 (HIF-1α –/– embryonic mice), which is accompanied by neural tube defects and marked cell death within the cephalic mesenchyme *(54,55).* Mice with HIF mutations also

developed extensive cardiovascular and neural pathologies *(56).* Cited2 is a cAMP-responsive element-binding protein (CBP)/p300 interacting transcriptional modulator and a proposed negative regulator for HIF-1α through its competitive binding with HIF-1α to CBP/p300. Disruption of the gene encoding Cited2 is embryonic lethal because of defects in the development of the heart and neural tube *(57).*

Erythropoietin, one of the HIF target genes, has been known for its role in erythroid differentiation. The EPO receptor null mouse shows extensive apoptosis in the fetal brain. Lack of EPO receptor affects brain development as early as E10.5, resulting in a reduction in the number of neural progenitor cells and increased apoptosis *(58).* Recently, Tomita et al. generated a conditional knockout mouse with the Cre/LoxP system with the nestin promoter-based neural-precursor-specific Cre recombinase, and the result showed that neural-cell-specific HIF-1α-deficient mice exhibited defective brain development accompanied by a reduction in neural cells and an impairment of spatial memory *(59).* The above indicated that HIF-1 is an essential brain developmental and physiological stimulus.

Hypoxia Affects Proliferation and Differentiation Through HIF Signal Pathway

The role of HIF in cell survival and proliferation has been extensively studied by using gene disruption. By inactivated HIF-1α genes, the proliferation of embryonic stem (ES) cells under hypoxia increased and apoptosis reduced, compared with the wild-type (HIF-1α+/+) ES cells *(60).* The role of HIF-2α in cellular survival was also studied by targeted inactivation of the HIF-2α gene [HIF-2α (–/–)] in murine ES cells. Loss of HIF-2α did not protect ES cells against apoptosis during hypoxia *(61).* In (HIF-1α–/–) ES cells, the level of p53 (a mediator of a genotoxic apoptosis) and p21 (a p53 target gene and effector of cell cycle arrest) were significantly reduced, whereas the amount of the apoptosis inhibitor Bcl-1 was increased *(60).* Mouse gene disruption studies have implicated HIF-2α in embryonic regulation of TH, a hallmark gene of the sympathetic nervous system. These data demonstrate that the transcription factor HIF is essential for cell survival and proliferation in ES cells during hypoxia.

The transcription factor HIF-1 also plays an important role in the proliferation and differentiation of cell exposure to mild hypoxia. Hypoxia-inducible transcription factors HIF-1, HIF-2, and HIF-3 subtypes were all found to be upregulated in the hypoxic fibroblasts. Using the transcription decoy technique, it was found to fully block the capacity of hypoxic fibroblasts to elicit pulmonary artery smooth muscle cell (SMCPA) growth *(62).* Hypoxia could also activate the expression of known hypoxia-induced genes, such as VEGF and TH in neuroblastoma following hypoxia. However, hypoxia decreased the expression of several neuronal/neuroendocrine marker genes but induced genes expressed in neural crest sympathetic progenitors (e.g., c-kit and Notch-1) *(63).* Although the expression of the HIF-1 gene has not changed in CNS precursors cultured in lowered O_2, its targeted genes, FGF8, VEGF, and erythropoietin gene expression increased by reverse transcription–polymerase chain reaction *(15).* Thus, it provided strong evidence that HIF or hypoxia-induced gene expression and the proliferative cell response were causally linked.

The response to hypoxia could also be regulated by changes in HIF expression of proteins. Hypoxia stabilized HIF-1α and HIF-2α proteins in neuroblastoma following hypoxia *(63).* In hamster fibroblasts under low oxygen tension, HIF-1α protein was directly phosphorylated and activated by the MAP kinase ERK *(57).* The transcription factor HIF-1 upregulates EPO following hypoxic stimuli, and then EPO expression was elevated in cultured NSCs. EPO appears to act directly on NSCs, promoting the production of neuronal progenitors at the expense of multipotent progenitors *(15).* The capacity of hypoxic fibroblasts to elicit SMCPA growth were through these soluble factors, which are shown to be controlled or encoded by the expression of genes carrying

a hypoxia-responsive element (DNA-binding domain for HIF transcription factors) in their regulatory regions *(62).*

Cells undergo a variety of biological responses when placed in hypoxic conditions, including alterations in the metabolic state and growth rate. Usually, the regulators for neurogenesis in vivo also played roles in vitro. Many articles have described the importance of growth factors in the proliferation and differentiation of NSCs in vitro (e.g., SCF, BDNF, BMP, FGF, leukemia inhibitory factor [LIF], ciliary neurotrophic factor (CNTF), etc.). Unfortunately, the articles on their roles in the proliferation and differentiation of hypoxic NSCs are rare. Further studies addressing the molecular signature response to hypoxia with the growth factors should be done.

Discussion

Taken together, the above data indicated an important role for hypoxia in the proliferation and differentiation of NSC and other stem cells. These facts should be kept in our mind while we study neurogensis and development of NSC in vivo and in vitro. However, this field has just opened to us and needs more effort to be truly understand. Even for the above works, some questions still remain.

What Is the "Physiological Hypoxia" In Vitro?

As we described earlier, hypoxia exists in the early embryonic development and adult brain tissue. There are reports of 3% O_2 or 25.6 mm Hg during embryonic development and 1 to 5% O_2 in the adult mammalian brain. These data suggest that the embryonic, fetal, and adult brain tissues are normally in a hypoxic condition. As a result, Morrison, Studer, Storch, and others dubbed this phenomenon "physiological hypoxia." They discovered that such a kind of hypoxia could promote the proliferation of NSCs in vitro. This finding is very important for providing not only a new way to study the mechanism of neurogenesis but also a new technique to produce NSCs in quantity for clinic therapy. However, the following questions should be addressed:

1. How should we explain the normal oxygen concentration with 20% O_2? If 1 to 5% O_2 is "physiological," it follows that a higher oxygen content could be an oxidative stress. We have investigated the effects of oxygen levels from 20% to 95% and found out that the proliferation of NSCs was detectably retarded in comparison with that at 3% (physiological hypoxia).
2. As mentioned earlier, the oxygen levels showed substantial differences among different regions of brain. For example, the oxygen level is 2.6 to 3.9% in the hippocampus, but only 0.1 to 0.4% in the pons/fornix. Should we take the difference into serious consideration when we culture NSCs from different regions of the brain?
3. In general, the cell culture condition is just a simulation of a physiological environment in vivo; many differences could exist and many physiological parameters might not be suitable for cell culture. For example, the suitable concentration of glucose in culture is higher than that of the blood level. In addition, we found that the neural sphere number was 1.5 times that of the control at 3% O_2, but 2.4 times with 10% O_2. It indicates that the effect of hypoxia on the proliferation of NSCs is related to the oxygen concentration. Further investigation is called for to determine the suitable oxygen content for different types of stem cell.

Is HIF the Only Hypoxia-Sensitive Pathway in Proliferation?

Consensus does exist about the key role of HIF in the hypoxic signal pathway and in the regulation of the proliferation and differentiation of NSCs. Nevertheless, there are also other findings. Carmeliet suggested that there were at least two different adaptive responses to hypoxia involved in controlling the cell cycle: one is HIF-1α-dependent genes regulation (including the protein p53, p21, Bcl-2), and the other is HIF-1α-independent gene regulation (p27, GADD153) *(64).* As mentioned earlier, some growth factors, which can stimulate neurogensis, are upregulated by hypoxia, such as SCF, BDNF, FGF, and so on. Unfortunately, they are not controlled by the HIF pathway.

Recently, we analyzed profile gene expression of NSCs after a 3-d culture in 3% O_2 condition by means of gene chips, including 12,000 rat genes. Many differentiated genes have not been reported as target genes of HIF (unpublished data). Further studies should be carried out to confirm the real role of HIF in the mechanism underlining the responses of NSC to hypoxia.

Conclusions

Studies reviewed here provide initial experimental evidence that mild hypoxia might promote most adult stem cells and neuronal self-repair after ischemic/hypoxic insults. However, we lack information about the appropriate oxygen concentration for the culturing of different types of stem cell. We know virtually little about the mechanisms that trigger increased cell proliferation, maintain the survival of adult stem cells, and regulate their differentiation into specific neuron types under hypoxia.

Over the past several years, NSCs and other adult stem cells have been the main cells for gene therapy and degenerative diseases. Although the mechanism for division and differentiation under hypoxia is currently incomplete and insufficient, recent data raise the possibility that amplification of stem cells by using a physical factor (low oxygen) might, in the future, be of therapeutic value for patients and that the HIF-1 signaling pathway might also be a target for clinic application of neural stem cells.

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