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Role of hypoxia in stem cell development and functioning

The response of stem cells (SC) to hypoxia is one of the main mechanisms of an organism's adaptation to changing terms of external and internal environment. This review describes the role of hypoxia in functioning of various stem cell types – embryonic, hematopoietic, mesenchymal and neural, paying special attention to the very limited data concerning intermittent hypoxia (IH) effects. All stem cells and their precursors exist in microenvironment named stem cell niches. The most crucial factor for their normal functioning is hypoxia, which contributes to maintaining the SC in quiescent state with necessary rate of self-renewal. The key element of these mechanisms is a complex of hypoxia-inducible transcription factors. An additional exogenous hypoxic impact leads to activation of SC system. Very scant information on IH effects on SC that was obtained generally in cell culture models, reveals that intermittent hypoxia at certain duration and intensity is a more potent trigger of transcription activation than constant hypoxia. In the future a method of IH training/treatment could be effectively used for correction of physiological changes and pathological disorders in an organism.

Key words: embryonic stem cells, hematopoietic stem cells, mesenchymal stem cells, neural stem cells, "niches", hypoxia, hypoxia-inducible factors, cytokines.

INTRODUCTION

The last decades were marked by rapid accumulation of knowledge about stem cells, their role in ontogenetic development, physiological regulation of organism functions and their unique importance in regeneration and reparation of injuries. It has become evident that this direction can make a scientific breakthrough and explain not only some obscure vital processes but give an extremely effective tool that provides a further development of biology, biotechnology and medicine. The importance of practical value of stem cell knowledge can scarcely be overestimated because it provides an extreme progress in transplantology, oncology and many others fields of medicine.

Stem cells and progenitor (precursor) cells represent the minor cell populations which are initial elements forming various cell and tissue types during embryogenesis and postnatal ontogenesis [20,82,83]. They are characterized by ability to self-renewal and retention of multipotency in different degree, i.e. the ability to differentiate into various cell types. The term "embryonic stem cells" is used towards the elements derived from inner mass of blastocyst and possessing properties of totipotency, i.e. the ability to originate the whole organism. The term "postembryonic stem cells" (fetal cells and cells at subsequent postembryonic ontogenetic stages) is served for the designation of adult (or regional) stem cells because of their exclusive pluripotency, i.e. the ability to provide a variety of cellular elements mainly within one or two germ layers (such as hematopoietic, mesenchymal, and

neural stem cells). Under certain conditions these cells could undergo the transdifferentiation and generate nonrelevant cell types.

The ability of stem/progenitor cells to migrate and engraft into host tissues is key to their potential use in gene and cell therapy [27]. During last decade stem cells have been extensively studied as individual therapies for treatment of cardiovascular diseases, diabetes, neurological disorders (including Parkinson's and Alzheimer's diseases), bone defects, hemophilia, cancer, etc. [59]. The response of stem cells to hypoxia is one of main mechanisms of the adaptation to changing terms of external and internal environment. It was observed long ago that stem cells are able to respond immediately to hypoxia [45]. Later investigations have shown that hypoxia not only improves the proliferation of various stem cells in vitro but also plays an important role in the differentiation of stem cells, and promotes their survival [14,87,90].

Stem and/or precursor cells exist in a distinct tissue structure called the niche that regulates the self-renewal and differentiation of these cells [38,66]. The stem cell niche is a unique tissue microenvironment. Some recent findings suggest that hypoxia in the stem cell niche is critical for the maintenance of the undifferentiated stem or precursor cell phenotype [38]. Hypoxia is the necessary attribute of bone-marrow niches, which testifies to the fundamental role of hypoxia in recirculation and other mechanisms of stem cells functioning [7]. Different oxygen pressures and time of hypoxic exposure could play important and divergent roles in cell development. Transcription factors and cytokines clearly influence these processes. Recent work has revealed an important link between the factors that are involved in regulating stem and progenitor cell behaviour and the hypoxia-inducible factors, which provides a molecular framework for the hypoxic control of differentiation and cell fate [80].

This chapter reviews the data about the role of hypoxia in functioning of different types of stem cells (embryonic, hematopoietic, mesenchymal, neural) with special attention to the very scant information about intermittent hypoxia effects.

HYPOXIA AND EMBRYONIC STEM CELLS

Hypoxia is by no means a pure pathological condition. Lack of oxygen occurs often under various physiological states, especially when the fast tissue growth leaves behind the development of blood supply. First of all it is concerned to embryogenesis progressing at 1 to 5% oxygen content. However, such a situation is of no harm for normal embryonic development; moreover, it seems to be a necessary and thoroughly "selected" in the process of evolution way of forming mammalian embryos. This may suggest that regular interrelations under hypoxic conditions are common for all the developing tissues. Thus, stem cells as well as embryonic cells and other multipotent precursors exist in the environment named niches [72]. The functioning and differentiation of stem cells are influenced by stromal cellular contacts, extracellular matrix proteins, temperature, and other factors of microenvironment including oxygen supply. A low oxygen level improves the survival of embryonic stem sells (ESC) and hematopoietic stem cells (HSC) [13,17, 44,74]. Hypoxia regulates also the number of cells in internal blastocyst mass and accelerates the development of hematopoiesis and hemangioblast precursors [1,25,63].

Mammalian adaptation to hypoxia at the cellular and systemic levels is primarily provided by transcriptional regulators, hypoxia-inducible factors (HIFs). HIFs are heterodimers consisting of a regulatory α-subunits (HIF-1α, HIF-2α, HIF-3α) and a constitutive β-subunit (HIFβ), which is also known as ARNT (aryl hydrocarbon nuclear translocator) [68,84]. HIFs regulate expression of no less than 180 genes involved in the control

of cellular metabolism, erythropoiesis and angiogenesis [12,29,32] by means of binding hypoxia response elements (HREs) located in enhancers or promoters of these genes [61].

The first revealed HIFα subunit was a HIF-1α structure expressed overall in human and murine tissues, which is considered to be a primary regulator of response to hypoxia. Then HIF-2α also became known as endothelial PAS domain protein 1 (EPAS 1), HIF-1-like factor (HLF) and HIF-1-related factor (HRF) were identified, which put the questions regarding the participation of these factors in adaptation to hypoxia. HIF-1α and HIF-2α have a high degree of homology, which underlines their common capacity to form heterodimers with ARNT, bind with HREs and activate transcription in various in vitro tests [84]. While HIF-1α is widespread, HIF-2α is found in restricted cell types, such as endothelial, derived neural crest cells, pulmonary pneumocytes of II type, hepatic parenchyma and renal interstitial cells [11,16,18,77]. Genetic experiments in mice showed that the loss of HIF-1α or HIF-2α resulted in abrupt distinction of phenotypes; this indicates that each protein has unique physiological functions. It is possible that HIF-1α and HIF-2α are equivalent functionally, but play different roles because of different places of expression. On the other hand, HIF-1α and HIF-2α may regulate partly duplicating, but not identical genes. As to the role of HIF-3α subunit in cell response to hypoxia, this question is still obscure. Some data showed that HIF-3α protein has an opposite activity compared to the other HIF subunits [41].

Low concentration of oxygen (2%) in bovine blastocyst culture enhances cell viability, which is associated with increased expression of glucose 1 transporter (SLC2A1), a complex that facilitates coming of anaphase (ANAPC1) and myotrophin (MTPN), again indicating a role of oxygen in regulation of embryonic development mediated by expression of oxygen-sensing genes. However, a bovine embryo, at least at the stage of blastocyst, has no detectable level of oxygen-sensing HIF-1α gene expression, whereas HIF-2α protein is revealed in a sufficient concentration. The oxygen-regulated expression of lactate dehydrogenase A and SLC2A1 in bovine blastocysts indicate that regulation of these genes might be mediated by HIF-2α. These results confirm the findings that bovine blastocyst cells are unique in relation to oxygen reactivity as compared with somatic cells, and that loss of HIF-1-mediated gene expression reduces a shared response to low physiological content of oxygen in the environment which predetermines the subsequent favourable development [24].

Hypoxia was shown to inhibit stem cells self-renewing and induce their early differentiation in vitro even in the presence of leukemia-inhibitory factor (LIF). These effects are mediated by down-regulation in the system of LIF-STAT3 signaling pathways. Under hypoxic conditions HIF-1α inhibits transcription of a LIF-specific receptor (LIFR) by binding directly with HRE located in LIFR-promoter. Data indicate that in vitro hypoxia-induced stem cell differentiation is triggered, at least partly, by the HIF-1α-mediated suppression of LIF-STAT3 signalling [30].

To differentiate more clearly between unique and duplicating roles for HIF-1α and HIF-2α, the “knock-in” ESC technology was developed. The division, differentiation and function of ESC, adult stem cells and multipotent precursors are regulated by a signal complex of microenvironment including oxygen partial pressure. Using the genetic “knock-in” strategy, it was shown that the target substitution of oxygen-regulated transcription factor HIF-1α for HIF-2α results in enhanced expression the genes specific for HIF-2α, including Oct-4, a transcription factor important for stem cells to preserve their pluripotency. Loss of HIF-2α results in a considerable reduction in the number of embryonic primordial germinal cells, for which transcription factor Oct-4 expression is connected with survival and preservation of

function. These results identify Oct-4 as a HIF-2α-specific target gene and indicate that HIF-2α can regulate both function and differentiation of stem cells through Oct-4 activation. The transcriptional factor Oct-4 plays a significant role in the preservation of ESC, embryonic epiblast and primordial germinal cells in non-differentiated state [47,67]. In addition, it is of critical importance for the regulation of ESC differentiation and preservation of internal blastocyst mass cells pluripotency. The in vitro experiments showed that a twofold reduction in Oct-4 level resulted in loss of both ESC pluripotency and their ability to differentiate in mesodermal, neuroectodermal and ectodermal directions [51,69]; that is why Oct-4 expression is strictly controlled during embryogenesis and postnatal development. For example, a reduction in Oct-4 regulatory influence is required for differentiation of trophoderm and primitive endodermal lines. At the stage of rudimentary cushion, Oct-4 is expressing again in epiblast, and subsequently its activity reduces during gastrulation, although expression is preserved in primordial germinal cells. Moreover, the correlative relationships indicate that, should Oct-4 expression be disrupted, the cloned embryos will not develop following postimplantation stage. In adults, Oct-4 expresses in germinal cells and in populations of regional stem cells, for example HSC [32,75]. At last, ectopic Oct-4 expression is observed in tumor growth and reversible epithelial dysplasia in transgenic mice [8,26]. However, at present the mechanisms of Oct-4 expression regulation are largely unknown.

HYPOXIA AND HEMATOPOIETIC STEM CELLS

In mammalian adults, hematopoiesis is carried out in bone marrow where sparse resting stem cells spring extensive populations of committed progenitors, of which all blood cell lines derive throughout whole life of an individual. The HSC potential is determined substantially by the ability of stem cells to immortalize themselves due to self-renewing. It became clear that the stem cell is seen in association with other cells which determine its behavior. Exogenous signals influencing on the alternative between self-renewing and differentiation are determined by influences originating from discrete domains of microenvironment in bone marrow, so-called “niches” [66].

HSCs are used mostly in hematology due to their ability to maintain hematopoiesis and therefore to protect recipients exposed to lethal irradiation Weissman et al. [82]. Despite the absence of direct evidence about spatial distribution of long-term repopulating HSCs in bone marrow, certain findings indicate that at least a part of HSCs is found in immediate proximity to endosteal bone surface, and that hematopoietic differentiation is oriented in the line of longitudinal axis [39,48,66]. The genetic studies confirmed that endosteal osteoblasts were critical cellular elements of HSC niches [5,88]. Calvi et al. [5] revealed parallel and selective expansion of HSC, when an elevation in osteoblast content was reached using parathyroid hormone. Zhang et al. [88] showed that a decrease in quantity of osteoblast receptors to bony morphogenetic protein results in elevations in the number of both osteoblasts and HSC, suggesting that adhesion of these two types of cells via N-cadherine might play a role in the formation of HSC niches. Arai et al. [2] demonstrated that HSC expressing tyrosine kinase receptor Tie2 were mainly cells of “side population” (SP), which excluded Hoechst (Ho) dye and adhered to osteoblasts. These authors also found that an interaction between Tie2 and its ligand, angiopoetine-1, which could have determined HSC staying in resting state.

Hypoxia is not spread on most normal tissues, but bone marrow represents a unique tissue type having a complicated hierarchical organization, which originates from a small population of resting stem cells whose different compartments may have their own oxy-

gen supply level. Therewith, at present little is known about HSC distribution in bone marrow in relation to blood vessels. Most recently, Simon and Keith [70] noted that although stem cells can be perivascular, the vessels might be associated with venous structures and therefore could be exposed to relatively hypoxic oxygen partial pressure (pO_2). It has been proposed that HSC and their proliferating progenitors are naturally distributed along pO_2 gradient, with the HSC occupying the most hypoxic niches [10,56]. Furthermore, some authors demonstrated that hematopoiesis is improved ex vivo, when the cultured cells are exposed to 1-3% oxygen saturation [10,13,28]. It seems that multiple niches characterized by different O_2 levels might exist in bone marrow. Some stem cells occupy hypoxic niches, whereas others occupy relatively well-oxygenated perivascular microenvironments [70].

Chow et al. [9], applying a mathematical model of oxygen distribution in bone marrow suggested that stem cells sited in the area with very low, almost close to anoxia, oxygen level which protected these extraordinary valuable cells against oxygen radicals generated by damaged cells. Further progress in hypoxic cellular markers technique together with routine methods of blood perfusion measurement enabled to study oxygen gradients in bone marrow [56]. As a result, direct findings have been obtained indicating that HSC in bone marrow are isolated within hypoxic microenvironment, which suggest that hypoxia plays a fundamental role in preservation of stem cells normal functioning.

Methods for detecting hypoxia in tissues and HSC. Several studies on animals used the intravenous administration of Ho dye to visualize the zones of hypoxia by the method of fluorescent microscopy on tissue sections from solid tumors. It was shown that hypoxic cells were localized at a more or less constant and relatively large distance from blood vessels in areas with low Ho dye-staining [3,15,79]. Quantitative determination of Ho dye diffuse gradient is better when using cytometric analysis of disaggregated cells. This technique allows to establish a correlation between the intensity of Ho dye cell staining and their level of oxygenation [52,53]. Similarly Parmar et al. [56] used this method to determine Ho dye inclusion into bone marrow cells. The Ho dye was administrated intravenously, its content was estimated in leukocytes of peripheral blood, thymocytes and bone marrow. Among bone marrow cells, there was a wide distribution of fluorescence intensity, from intensive to light, which ensured the formation of clear gradient. This contrasted with a high level of fluorescence in well-oxygenated blood leukocytes that did not eliminate practically Ho dye and also with a very low level of staining in the thymus, where most cells were in relatively hypoxic state.

Previous studies with the use of oxygen microelectrodes showed that the thymus is a deeply hypoxic organ under normal physiological conditions. The administration of hypoxic marker, pimonidazole, resulted in its accumulation in cortex, medullar substance and corticomedullary junction, which conformed to widespread cell hypoxia in the normal thymus. The hypoxia-associated accumulation of pimonidazole was reduced, but not abrogated by the oxygen administration. In the normal thymus hypoxia-inducible genes are expressed at the basal level, which indicates the existence of physiological adaptation to permanent hypoxia. In contrast to changes in thymus size and cellularity during lifetime, oxygen tension in thymus cells does not change with age [53]. Another combined study of hypoxia and cell death showed that, with hypoxia achieved by a gas mixture application or high density of cultured cells, spontaneous in vitro apoptosis of thymocytes is reduced. These regulatory mechanisms of cell hypoxia preservation may also exist in vivo, since oxygen tension can regulate survival of thymocytes both in vitro and in vivo [23].

The development of mature T-cell repertoire in the thymus depends on the interaction between thymocytes and stromal cells. To facilitate intercellular contacts, epithelium in the thymus differentiates into a unique three-dimensional network. It was established that a three-dimensional configuration of thymic stroma depends upon oxygen tension and permanent physical contact between stromal cells and developing thymocytes [19].

To detect bone marrow-derived hypoxic cells, another methodical approach is used when special chemical marker of hypoxia, the reductive 2-nitroimidazole compound pimonidazole (PIM) is administered [23]. Being administered in vitro, pimonidazole forms stable deposits in hypoxic areas and then can be identified by anti-PIM antibodies. It was confirmed that this marker is really selective to hypoxic bone marrow cells after in vitro treatment by anti-PIM antibodies in the isolated SP-cells. Positive staining by anti-PIM antibodies was only observed under the conditions of exposure to anoxia (95% N2 and 5% CO_2). Since the thymus is a hypoxic organ, it can be used as a positive control in tandem with bone-marrow samples for in vivo detection of PIM-binding cells.

In addition, a study using hypoxic cytotoxin tirapazamine (TPZ) has shown that TPZ reduced selectively the number of HSC in bone marrow [4]. After the treatment with TPZ, a pronounced reduction in the number of cells expressing SP-phenotype occurred (by 95%). Under hypoxic conditions TPZ is reduced to benzotriazinyl radical and other intermediate compounds that eventually result in DNA double helix breakage and cell death. In the presence of oxygen, TPZ-radical is oxidized to non-toxic initial compound. In vivo, TPZ causes reductions in thymus weight and cellularity, thereby confirming that thymic tissue exists under pronounced hypoxic conditions.

It was shown that the formation of PIM deposits depends upon oxygen tension in cells and can serve as an effective marker for the cells with hypoxia less than 10 mmHg [62]. The highest anti-PIM staining was observed in the "tip" fraction of SP-cells, which had the highest HSC concentration [21,40,55]. Parallel measurements in thymic tissue of the same mice were shown to be an important source for comparison and verification of the methods for estimating oxygenation level. In addition, thymus is more accessible for microelectrode pO_2 measurement. According to previous data, thymus pO_2 is about 10 mm Hg [23]. In contrast to most bone marrow cells, thymocytes showed pronounced hypoperfusion and widespread staining with a hypoxic marker PIM. Therefore, only a very small part of bone marrow cells, i.e. HSC fraction and its closely associated cells, exists at a low level of oxygenation, while a larger part of bone marrow tissue containing the committed and differentiating progenitors is oxygenated relatively well.

As it was noticed above, stem cell niches consist of specialized microenvironment that participates in stem cells growing and regulates their pool. Previous studies focused on the endosteal region of bone marrow as a principal place for stem cell niches involved in hematopoiesis [5,88]. It was proved that the interaction between molecules of angiopoietine and osteopontine of adjacent osteoblasts promotes the preservation of HSC dormant state [2,49,73]. Besides that, an association of HSC and sinusoidal endothelium was suggested recently as an alternative place for niches in this tissue [33]. A relatively low level of oxygenation is also a criterion of bone marrow niche of stem cells. Oxygen gradient can realize the position effect determining spatial configuration of the hematopoietic system and connecting the primary physiological situation in maintenance of stem cells homeostasis, since it creates microenvironment, which protects against toxic and mutagenic effects of free oxygen radicals. As far as HSC are substantially non-dividing cells

with a small need for oxygen respiration, they can reside and function sufficiently in hypoxic state.

A quantitative proteomic analysis of the isolated bone marrow populations, which is based on cellular surface markers, has revealed cells expressing a high level of glycolytic and oxidative reducing proteins [78]. Though this special cell population contains also a large number of non-stem cell precursors and is not so highly enriched with HSC as the "tip" SP-cells [6,57], this analysis shows that at least certain bone marrow cells are adapted to anaerobic metabolism. In this respect, the factors that control HSC SP-phenotype are of special importance, since it is already known that active removal of Ho dye is connected with expression of ABC transporter, ABCG2/BCRP1 [92]. The observation showing that SP-cells, especially those capable of the highest removal of Ho dye in region of SP-profile, are positive for a hypoxic marker PIM, which supports the finding of Krishnamurthy et al. (2004), where ABCG2/BCRP1 gives preference of survival under the hypoxic conditions and, moreover, HIF-1α activates expression of BCRP1.

Role of chemokines/cytokines in hypoxia-promoted HSC survival. There are also data about the control of one chemokine - stromal-derived factor-1 (SDF-1) - by HIF-1α, with an account for normal inversed interrelations between oxygen level and SDF-1. This may suggest an interesting possibility of the formation of homing mechanism, by means of which the transplanted HSC find niches of stem cells. Hypoxia-induced transcriptional factors also participate in the control of genes associated with self-renewing of stem cells, including telomerase [50,86], transcription factors Oct4 [12] and Notch [22]. On the whole, HIF proteins due to their stabilization under regionally low oxygen tension can serve as oppositely directed dominant regulators of numerous key genes participating in HSC functioning.

In a liquid culture of murine bone marrow cells, a balance between self-renewing of primitive cell-precursors and clonogenic expansion of precursors is preserved better with 1% oxygen than 20% oxygen [28]. These results are of interest for ex vivo expansion of human cell-precursors, as far as low oxygen tension can restrict an excess proliferative potential of apheresis products. Mobilized colony-stimulating factors and apheretically collected blood cells are a main source of cell-precursors for autologous transplantation. Ivanovic et al. [28] cultivated cells with 1% and 20% oxygen for 7 days in a serum-free medium in the presence of IL-3 and stem cell factor (SCF). The growth of clonogenic precursors (CFU-GM, BFU-E, CFU-Mix) as well as more primitive human HSC, which are able to generate clonogenic precursors in a secondary culture, and the proliferation and differentiation of CD34+-cells were analyzed. The expansion of CD34+-cells and clonogenic precursors was considerably lower in the cultures with 1% oxygen than those with 20%.

Thus, hypoxia is the most important factor of microenvironment, which determines HSC functional activity and the courses of development. To our regret, we could not find any published evidence about intermittent hypoxia effects on HSC.

HYPOXIA AND MESENCHYMAL STEM CELLS

Mounting evidence shows that stem cells are distributed all over the body [76]. Among the most widespread stem cells are mesenchymal multipotent stromal cells (MSC), which have dendritic structure, pronounced adherent properties and non-hematopoietic phenotype including CD105 and CD73 [43]. In bone marrow, there are 10,000 nucleus-containing cells per 1 MSC. MSC can differentiate with the formation of bone, cartilaginous, adipose, muscular and connective tissues. MSC play an important role supporting HSC and ensur-

ing normal hematopoiesis. They are of an exceptional importance in the development of immunological reactions and are notable for pronounced tolerogenic activity. Due to their plasticity, certain capability of transdifferentiation, migration activity and the presence of homing mechanisms, a great number of researchers ascribe MSC to the most important elements, which constitute the basis for regeneration of many tissues both in norm and pathology. It is notable that tissue oxygen tension and hypoxia-induced factors also exert a significant influence on MSC diverse functions.

Embryonic chondrocytic growth plate is a unique avascular and hypoxic mesenchymal tissue [65]. In this microenvironment, chondrocytes not only survive, but also pass through all the cellular processes (proliferation, stasis, differentiation, etc.) necessary for the normal development of enchondral bone. One of the consequences of activated HIF-1α transcription is expression of vascular endothelial growth factor (VEGF), while an excess HIF-1α accumulation is regulated by von Hippel-Lindau (VHL) tumor suppressor, ligase of ubiquitine E3 that realizes its proteolysis. It was shown that each component of this pathway is an important regulator of enchondral bone development. In particular, it was established previously that HIF-1α is a life-supporting factor for hypoxic chondrocytes, which down-regulates cell proliferation. It is also of interest that hypoxia increases accumulation of extracellular matrix by HIF-1α-dependent type, which indicates that HIF-1α might be of critical importance not only for cell survival and proliferation, but also for cell differentiation. It was established that HIF-1б is a factor of differentiation, since it is necessary for MSC for both the early chondrogenesis and the development of joints [60].

To study in depth a role of transcriptional factor HIF-1α in cartilage biology, the investigations were performed with primary epiphyseal chondrocytes, where HIF-1α was removed purposefully. HIF-1α occurred to be necessary for glycolysis regulation under the aerobic and anaerobic conditions. In the absence of HIF-1α, chondrocytes were unable to support the ATP level in hypoxic microenvironment, which indicates a fundamental importance of this factor in regulation of their metabolism. The VEGF synthesis is also hypoxia-induced, however this effect is absent in the HIF-1α-NULL mutant cells. Strongly increased type-II collagen protein levels were detected in wild-type cells after 44 hours of hypoxia. At the same time, type-II collagen mRNA and protein levels were strongly decreased under low oxygen in chondrocytes lacking HIF-1α [58]. These results clearly demonstrate the importance of HIF-1α in maintenance of anaerobic glycolysis, and thereby extracellular matrix synthesis, of epiphyseal chondrocytes.

During the bone formation and reparation the processes of osteogenesis and angiogenesis are tightly coupled. Blood vessels not only delivery oxygen and nutrients to developing bone but also play an active role in bone formation and remodeling by mediating the interaction between osteoblasts, osteocytes, osteoclasts, and vascular cells at a variety of levels. Tissue hypoxia is believed to be a major stimulus for angiogenesis by activating HIF-1α pathway. Such activation is accompanied by certain gene expression, for example VEGF gene, which plays a critical role in angiogenesis, endochondral bone formation, and bone repair following fracture. Recent works have shown that osteoblasts use the HIF-1α pathway to sense reduced oxygen tension and transmit signals that impinge on angiogenic and osteogenic gene programs during bone formation. Using genetic approach, it was demonstrated that overexpression of HIF-1α in mouse osteoblasts via VHL destruction resulted in a considerable elevation of angiogenesis and osteogenesis intensity [80].

In relation to oxygen tension, osteoblasts are located ideally within bone and respond to hypoxia with activation of HIF-1α pathway.

It was shown that mice overexpressing HIF-1α in osteoblasts through selective deletion of VHL gene expressed high levels of VEGF and developed extremely dense, heavily vascularized long bones. By contrast, mice lacking HIF-1α in osteoblasts had the reverse skeletal phenotype of the VHL mutants: long bones were significantly thinner and less vascularized than those of controls. Loss of VHL in osteoblasts increased endothelial sprouting from the embryonic metatarsals in vitro but had little effect on osteoblast function in the absence of blood vessels. Mice lacking both VHL and HIF-1α had a bone phenotype intermediate between those of the single mutants, suggesting overlapping functions of HIFs in bone [81]. These studies suggest that activation of the HIF-1α pathway in developing bone increases bone modeling events through cell-nonautonomous mechanisms to coordinate the timing, direction, and degree of new blood vessel formation in bone.

Role of HIFs in endothelium during blood vessels growth and development was studied by expressing a dominant-negative HIF mutant (HIFdn) that inhibits the transcriptional responses mediated by both HIF-1 and HIF-2, specifically in endothelial cells of transgenic mice [37]. Transgenic HIFdn embryos retarded in growth and died. Primitive vascular networks were established in them, but vascular remodeling in yolk sac and in embryo per se was defective. Vascularization of neuroepithelium was also absent, while cardiac ventricles were thin-walled and trabecula-free. Similar cardiovascular defects were observed in Tie2-deficient mouse embryos. Transgenic HIFdn embryos expressed successively reduced levels of endothelial angiopoetic receptor, Tie-2, whereas other endothelial markers, such as PECAM-1, Tie-1 and VE-cadherin, were not affected. These results show that HIFs in endothelial cells are the key factors for the development of embryonic heart and blood vessels.

More and more findings indicate that adipose tissue-derived stem cells (ASC) can be used in the therapy for ischemic heart disease. However, mechanisms underlying their therapeutic effects are not elucidated. To estimate anti-apoptotic effects of ASC, neonatal rat cardiomyocytes were subjected to hypoxia in a co-cultural system. It was revealed that ASC secrete VEGF and insulin-like growth factor 1 (IGF-1) in significant amount. The apoptosis in cardiomyocytes was largely prevented by ASC, and 62.5% of the effect were mediated by IGF-1, while 34.2% by VEGF. Similarly ASC induced endothelial tubulo-formation by means of VEGF secretion [84].

Taken together, current knowledge has indicated that the MSC have well-developed mechanisms for adaptation to hypoxia, and their realization allows cellular functions to be exercised with sufficient efficacy.

HYPOXIA AND NEURAL STEM CELLS

Recent studies in neuroscience have shown that the adult central nervous system (CNS) contains neural cell precursors and stem cells that are able to generate new neurons, astrocytes and oligodendrocytes. Breaking a previous dogma that any new neurons cannot be developed in CNS of adult mammalians, these findings do create prerequisites for the development of new neural restorative technologies [71]. The possibility of nervous system regeneration appears to be very tempting; so numerous molecular and cellular studies have been performed on the endogenous neural precursors and influencing factors.

Until recently, the effects of oxygen on neural stem sells (NSC) proliferation and differentiation have not been practically studied. Some investigations were performed on the basis of the fact that NSC exist within a «physiological hypoxic» environment at 1% to 5% O2 in both embryonic and adult brains. Subsequently, it was shown that hypoxia in vitro is able to enhance the NSC growth and support their survival. The in vivo studies have

established that the number of endogenous NSC in subventricular zone and dentate gyrus grows up under ischemic/hypoxic conditions. In addition, hypoxia can influence differentiation of NSC, as far as the number of neurons, especially dopaminergic neurons, grows up under these conditions. The mechanism of these responses might be primarily involved with the HIF-1 signaling pathway [93].

Ability of neural precursors to differentiate and a potential role for HIF-1α were examined under the hypoxic conditions. As compared with cultivating under normoxic conditions, NSC cultivated under hypoxia (3% oxygen) showed an increase in the percentage of neurons. In particular, a considerable growth was observed in TH-positive neurons differentiated from NSC; dopamine content in the medium demonstrated an elevation as well. HIF-1α protein expression was also higher from 3 to 72 hours during hypoxia than under normal conditions. These findings indicate that hypoxia facilitates dopaminergic differentiation, and that HIF-1α is involved in regulation of this process [87].

Another in vitro study on NSC isolated from rat embryonic mesencephalon also showed that NSC are able to self-renew and bear a nestine-positive phenotype. Under hypoxic conditions NSC differentiated into a greater number of neurons than under normoxia. The percentage of TH-positive cells differentiated from NSC under normoxia and hypoxia was about 10% and 20%, respectively. In addition, dopamine content in supernatant of culture medium in the hypoxia group was the double of that in the normoxia group [90,91]. These results indicate that hypoxia induces NSC differentiation into neurons, in particular dopaminergic neurons. Therefore, hypoxia might be a potential therapeutic approach to the treatment for Parkinson's disease [35].

To study HIF-1α function in CNS, knockout mice were used with the Cre/LoxP system and a controlled nestine promoter Cre. HIF-1α-deficient mice suffered from hydrocephaly accompanied with a reduced number of nerve cells and impaired spatial memory. These germinal defects were eliminated successfully by means of in vivo HIF-1α gene delivery into embryos. Therefore, these results showed that expression of HIF-1α in nerve cells is of significance for normal brain development. In the studies on knockout mice, it was also established that HIF-1α is a transcriptional factor, which participates in the development and survival of dopaminergic neurons in nigra substance with the involvement of VEGF-dependent pathway [42].

Carotid body is an organ of the peripheral nervous system susceptible to oxygen concentration in blood and responsible for its changes via respiration regulation. Pardal et al. [56] reported about discovery of stem cells in carotid body, which proliferated in response to hypoxia and produced neurons expressing dopamine. Possibly, the found source of adult stem cells might be beneficial in the therapy for Parkinson's disease [34].

Another research group [89] investigated hypoxia (with tissue bath gassed with 95% N_2 + 5% CO_2 vs. 95% O_2 + 5% CO_2 in control) induced changes in neuronal ATP-sensitive potassium (K_{ATP}) channels current in second-order neurons of peripheral chemoreceptors in the nucleus of the solitary tract (NTS). It was shown that 1 week chronic exposure to either sustained or intermittent hypoxia reduces K_{ATP} channel function in NTS neurons. This may represent a neuronal adaptation that preserves neuronal excitability in crucial relay neurons in the peripheral chemoreflex pathways.

Intermittent hypoxia has been found to prevent brain injury and to have a protective role in the CNS. For example, in a study of adult rats exposed to hypobaric hypoxia (corresponding to altitudes of 3,000 m and 5,000 m) for 4 hours per day during 2 weeks, the cells labelled with 5-bromo-2-deoxyuridine-5-monophosphate (BrdU) grew up in subventricular zone (SVZ) and dentate gyrus. The

number of BrdU-labeled cells in the SVZ returned to normal level 4 weeks following the end of intermittent hypoxia. However, the number of BrdU-labelled cells increased two-fold in dentate gyrus in 4 weeks after intermittent hypoxia exposure. [94]. Based on these findings, the authors concluded that intermittent hypoxia facilitates NSC proliferation in situ, and that newly divided cells in SVZ and dentate gyrus respond to hypoxia differently. In other words, proliferation of NSC in SVZ and dentate gyrus, possibly, facilitates adaptation changes following the exposure to intermittent hypoxia.

Other studies on cell culture models showed that, with certain duration and intensity, intermittent hypoxia is a more powerful factor for triggering transcriptional activation than continuous hypoxia. Nanduri and Prabhakar [46] summarized that intermittent hypoxia activates HIF-1, immediate early gene c-fos, activator protein-1, nuclear factor kappa-B, and cAMP-response-element-binding protein. It also induces expression of the proteins associated with neuron survival and apoptosis as well as post-translation modification of proteins, resulting in enhanced biological activity. The comparison between continuous and intermittent hypoxia showed pronounced differences in both the kinetics of protein kinase activation and the types of activated protein kinase. Future studies on both gene and protein levels may provide better understanding of the mechanisms of intermittent hypoxia effects.

CONCLUSION

Hypoxia is an optimal environment for maintenance and functioning of various stem cell types (e.g. embryonic, hematopoietic, mesenchymal and neuronal). It promotes maintaining of stem cells in a quiescent state with a necessary rate of self-renewal. There are certain regulatory mechanisms of the maintenance of hypoxic condition in stem cell microenvironment named niches. This microenvironment influences essentially the stem cell proliferation and differentiation and protects the cells against toxic and mutagenic effects of free oxygen-derived radicals. The key element of these mechanisms is a complex of hypoxia-inducible transcription factors – HIFs. A telomerase gene and also the key cytokine genes indicate the direction of migration and intensiveness of repair processes. An additional exogenous hypoxic impact leads to activation of stem cell system. Very scant information on the intermittent hypoxia effects on stem cells that was obtained generally in cell culture models reveals that intermittent hypoxia at certain duration and intensity is a more potent trigger of transcription activation than constant hypoxia. In the future a method of intermittent hypoxia training/treatment could be effectively used for correction of physiological and pathological disorders in an organism.

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РОЛЬ ГІПОКСІЇ У РОЗВИТКУ І ФУНКЦІОНУВАННІ СТОВБУРОВИХ КЛІТИН

Реакція стовбурових клітин (СК) на гіпоксію є одним з головних механізмів адаптації організму до змін умов зовнішнього та внутрішнього середовища. Мета цього огляду полягає у висвітленні ролі гіпоксії в функціонуванні різних типів СК (ембріональних, гематопоетичних, мезенхімальних та нервових), звертаючи спеціальну увагу на дуже обмежені дані, що стосуються ефектів дії інтервальної гіпоксії (ІГ). Всі типи СК та їх попередники існують в мікрооточенні, що зветься нішами СК. Найбільш критичним фактором для їх нормального функціонування є гіпоксія, яка сприяє підтриманню СК в стані спокою з необхідною швидкістю самовідновлення. Ключовим елементом цього механізму є комплекс гіпоксиіндуцибельних транскрипційних факторів. Додаткове екзогенне гіпоксичне подразненя призводить до активації системи СК. Дуже мізерна інформація щодо дії ІГ на СК, яка

здебільшого була отримана на моделях культури клітин, виявляє, що інтервальна гіпоксія при певній тривалості та інтенсивності є більш могутнім тригером активації транскрипції, ніж постійна гіпоксія. В майбутньому метод тренування/лікування за допомогою ІГ міг би дієво використовуватися для корекції патологічних станів в організмі.
Ключові слова: ембріональні стовбурові клітини, гемопоетичні стовбурові клітини, нейральні стовбурові клітини, "ніші", гіпоксія, гіпоксиіндуцибельні фактори, цитокіни.

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РОЛЬ ГИПОКСИИ В РАЗВИТИИ И ФУНКЦИОНИРОВАНИИ СТВОЛОВЫХ КЛЕТОК

В обзоре рассматриваются результаты изучения роли гипоксии в функционировании различных типов стволовых клеток: эмбриональных, гемопоэтических, мезенхимальных и нейральных. Низкое содержание кислорода играет фундаментальную роль в сохранении нормальной функции стволовых клеток. Их реакция на гипоксию является одним из главных механизмов адаптации к изменяющимся условиям среды. Все типы стволовых клеток и клеток-предшественников функционируют в микроокружении, именуемом нишей стволовых клеток. Наиболее значимым фактором для их нормального функционирования является гипоксия, которая способствует пребыванию стволовых клеток в дремлющем состоянии с необходимым темпом самообновления. Степень гипоксии в клетках и тканях оценивают в основном следующими методами: 1) прямым измерением напряжения кислорода в тканях; 2) по способности гипоксических клеток исключать флуоресцентный краситель Hoechst; 3) иммунофлуоресцентным выявлением проникающего в клетки гипоксического маркера пимонидазола; 4) определением количества клеток, лизирующихся под влиянием специфического гипоксического цитотоксина тирапазамина. Используя эти методы, было установлено, что при определенных обстоятельствах гипоксия существенно влияет на пролиферацию и дифференцировку стволовых клеток, ослабляя или усиливая эти процессы. Ключевым механизмом адаптации клеток к пониженному напряжению кислорода в окружающей среде является синтез индуцированных гипоксией факторов транскрипции HIFs. Эти факторы контролируют большое количество генов, вовлеченных в регуляцию метаболизма клеток, гемопоэза и ангиопоэза, ассоциированных с самообновлением стволовых клеток, включая ген теломеразы, а также генов ключевых цитокинов, определяющих направление миграции и интенсивность репаративных процессов.
Ключевые слова: эмбриональные стволовые клетки, гемопоэтические стволовые клетки, нейральные стволовые клетки, «ниши», гипоксия, гипоксия-индуцибельные факторы, цитокины.

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