Journal of Cellular Physiology

Hypoxia or In Situ Normoxia: The Stem Cell Paradigm

ZORAN IVANOVIC*

Aquitaine-Limousin Branch of French Blood Institute, Bordeaux, France

Although O_2 concentrations are considerably lowered in vivo, depending on the tissue and cell population in question (some cells need almost anoxic environment for their maintenance) the cell and tissue cultures are usually performed at atmospheric O_2 concentration (20–21%). As an instructive example, the relationship between stem cells and micro-environmental/culture oxygenation has been recapitulated. The basic principle of stem cell biology, "the generation-age hypothesis," and hypoxic metabolic properties of stem cells are considered in the context of the oxygen-dependent evolution of life and its transposition to ontogenesis and development. A hypothesis relating the self-renewal with the anaerobic and hypoxic metabolic properties of stem cells and the actual O_2 availability is elaborated ("oxygen stem cell paradigm"). Many examples demonstrated that the cellular response is substantially different at atmospheric O_2 concentration when compared to lower O_2 concentrations which better approximate the physiologic situation. These lower O_2 concentrations, traditionally called "hypoxia" represent, in fact, an in situ normoxia, and should be used in experimentation to get an insight of the real cell/cytokine physiology. The revision of our knowledge on cell/cytokine physiology, which has been acquired ex vivo at non physiological atmospheric (20–21%) O_2 concentrations representing a hyperoxic state for most primate cells, has thus become imperious.

J. Cell. Physiol. 219: 271–275, 2009.

Oxygen represents one of the most important factors influencing the evolution of life. Primitive life is considered to have started less than 4 billions years ago, and the first organisms that were capable to convert and store the energy of light in the form of biochemical molecules with higher energetic potential appeared less than 3 billions years ago. These organisms, by doing photosynthesis, started to release oxygen whose concentration in the atmosphere increased during the next billion years. About 2 billions years ago, the concentration of O_2 in atmosphere appeared as a factor influencing life. Oxygen was toxic for living organisms which developed several adaptation mechanisms aimed to detoxify it in parallel with the increase of O_2 concentrations in atmosphere which continued until 500 millions years ago. As a matter of fact, the eukaryots, whose physiology, based on the aerobic metabolism, is built during the evolution in order to detoxify O_2, became dependent on it. However, O_2 concentration should be well adapted to particular cell metabolic type. In the human organisms, O_2 concentration varies significantly between the tissues: in the lung parenchyma and in circulation (McKinley and Butler, 1999; Saltzman et al., 2003; Johnson et al., 2005; Wild et al., 2005), as well as in well irrigated parenchymal organs (liver, kidneys, heart; Wölfle and Jungermann, 1985; Jungermann and Kietzmann, 1997; Roy et al., 2000; Welch et al., 2001; Mik et al., 2004) it is comprised between 14% and 4%. In other tissues, relatively less irrigated, O_2 concentration is even lower: in the brain, it varies from 0.5% to 7% (Whalen et al., 1970; Nwaigwe et al., 2000; Hemphill et al., 2005) in the eye (retina, corpus vitreous), from 1 to 5% (Buerk et al., 1993; reviewed in Yu and Cringle, 2005), in the bone marrow, from 0% to 4% (Tondevold et al., 1979; Chow et al., 2000).

Towards the Concept of "In Situ Normoxia"

In fact, in course of evolution, the cellular metabolism has been adapted to a moderate oxygenation, that is, oxygen concentrations that could be found in tissues (Massabau, 2000, 2003). This adaptation (reviewed by Kenneth and Rocha, 2008) initially concerns the modulation of molecular mechanisms existing in living organisms before the appearance of oxygen in atmosphere. For example, the stabilization of HIF-1α transcripts, which originally initiated the synthesis of molecules acting in an anaerobic metabolism, shifted towards their oxygen-dependent degradation. It is interesting to note that between anoxic condition and 5% of O_2, the degree of stabilization of HIF-1 transcripts is inversely proportional to O_2 concentration (Jiang et al., 1996; Guzy and Schumacher, 2006). Over 5% O_2, the HIF-1 transcripts are degraded. Nearly anoxic conditions result in an anaerobic metabolism, but at relatively higher O_2 concentrations, between 1% and 5% O_2, the synthesis of highly energetic phosphates takes on thanks to a specific isoform of cytochrome *c* oxydase (Fukuda et al., 2007). For many cell populations these oxygen concentrations (1–5% O_2; O_2 concentrations existing in atmosphere approximately 1 billion years ago; Falkowski, 2006; Berner et al., 2007), called by Guzy and Schumacher (2006) "physiological hypoxia" are conditions of the physiological oxygenation, that is, the steady state oxygenation or "in situ normoxia."

The adaptation induced by increased oxygenation of atmosphere conditioned the evolution of a complex circulatory and respiratory system to ensure an adequate O_2 supply for every cell type in organism. The new and very complex mechanisms such as cellular respiration (reviewed in Das, 2006) require O_2 as ultimate electron acceptor. These new mechanisms resulted in energy accumulation in the form of "high-energetic phosphates" that allowed a big progress in evolution enabling to build and maintain the organisms of complex structure and function, such as vertebrates. But the need for O_2 as a metabolic substrate is opposed to the risk of cellular macromolecules oxidative damage (ROS generation). That is why its concentration within cells is maintained within a narrow range that optimally balances supply and demand. A cell could and has to fight against the ROS in excess. This system,

Contract grant sponsor: Etablissement Français du Sang Aquitaine-Limousin.

*Correspondence to: Zoran Ivanovic, Etablissement Français du Sang Aquitaine-Limousin, 5 Place Amélie Raba Léon, BP 24, 33035 Bordeaux Cedex, Bordeaux, France.
E-mail: zoran.ivanovic@efs.sante.fr

Received 8 December 2008; Accepted 9 December 2008

Published online in Wiley InterScience (www.interscience.wiley.com.), 21 January 2009.
DOI: 10.1002/jcp.21690

however, has its limits and oxygen concentration should not go beyond them to ensure the well being of cells (reviewed by Papandreou et al., 2005). Accordingly, it is obvious that the atmospheric O_2 concentration is too elevated for the cells of most tissues. Therefore it is evident that the basic condition to compare all other experimental conditions should represent the "in situ normoxia" for a certain cell type and not "atmospheric normoxia" considered, without any scientific justification, as a "standard tissue culture condition" and being, in fact, hyperoxia.

The "Mental Shortcuts"

Nevertheless, almost all present knowledge related to the action of cytokines and growth factors on cells and on the consequent cellular response (Metcalf, 2008) is based on the experiments at 20–21% O_2, that is, highly hyperoxic conditions. Apart practical reasons related to technical problems in controlling O_2 concentration, it is likely that negligence in the matter of ex vivo culture oxygenation originates from two paradigms: (1) Erythropoietin (Epo)-dependent regulation of Red Blood Cell (RBC) production and (2) Vascular Endothelial Growth Factor (VEGF) and its role in neovascularization. The first one is enabling the amplification of erythroid-responsive progenitor and precursor's pool to increase the RBC production as a response to the tissue hypoxia resulting from a weak oxygenation of blood; the second one was usually assimilated to the neovascularization of hypoxic areas in tumors. Originally, the existence of a factor regulating erythropoiesis was postulated by Carnot and Deflandre (1906) and the Epo discovered (reviewed in Jelkmann, 1986) thanks to the observation related to high altitude-induced polycythemia. This phenomenon appears with decreased O_2 pressure ("high altitude hypoxia" or "hypobaric hypoxia") comparing to sea-level O_2 one, which was then referenced as "normoxia." As a consequence, the term "normoxia" for the atmospheric O_2 concentration was transposed, from classical physiology, to cell biology, that is, to ex vivo culture studies. Also, the term "hypoxia" was regularly associated with the pathology. Of course, these "mental shortcuts" neglect the facts that (a) a "normal" blood oxygenation is much lower than the atmospheric one (20–21% O_2) and it induces, however, a steady-state Epo production, ensuring the erythropoiesis in physiological conditions; (b) physiological tissue O_2 concentrations are even lower than arterial blood ones; (c) Epo acts as a physiological regulator in angiogenesis and brain development, that is, as a non-hematopoietic regulator (Arcasoy, 2008) (c) VEGF is acting as a key regulator of physiological angiogenesis during embryogenesis, endochondrial bone formation, female reproductive functions (reviewed in Ferrara et al., 2003)... In addition, the ex vivo experimentation mainly neglected the knowledge from in vivo physiology on the paradigm for oxygen-regulated gene expression (Stockmann and Fandrey, 2006) usually considering that the cytokines and growth factors other than Epo and VEGF are not concerned. Still, the list of genes coding cytokines, growth factor and their receptors, as well as other molecules exhibiting the "HIF-1-binding sequence"—hypoxia response element (HRE), is long (reviewed in Semenza, 2007) and increasing on a daily basis.

Oxygen Supply, Metabolic Feature, Proliferation and Differentiation: the Stem Cell Paradigme

Taken together, the metabolic/molecular biology studies performed on cell lines (Jiang et al., 1996; Guzy and Schumacher, 2006), show that moderately low O_2 concentrations (1–5% O_2) are permitting cell proliferation since providing the sufficient amount of energy by the adapted mitochondrial respiration. On the contrary, in anoxic conditions, mitochondrial respiration has ceased and the energy is provided by an anaerobic glycolysis, thus insufficient to produce enough energy to support full cell proliferation and, especially, differentiation. An example from hematopoietic cell phenomenology is instructive: the IL-3 activates and stimulates the proliferation of CD34+ cells both at 3 and 20% O_2, but at very low O_2 concentrations approaching anoxia (0.1% O_2), CD34+ cells are inhibited in G0 or, if cycling, returned in G0 (Hermitte et al., 2006) irrespective of IL-3 presence. The action of different cytokine cocktails on stimulation on CD34+ cell proliferation was not prevented at 1–5% O_2 but the maintenance of stem cell activity is better at low O_2 concentrations than in air (Cipolleschi et al., 2000; Ivanovic et al., 2000a,b, 2002, 2004; Danet et al., 2003). The relationship between proliferation and differentiation rates is a parameter highly influenced by O_2 concentration, which remains to be elucidated. Nonetheless, it is evident that low O_2 concentrations (1–5%) decrease the differentiation rate of certain stem cell populations. In fact, it would be more appropriate to say "higher O_2 concentrations increase the differentiation rate" to be in line with the idea on the "negative evolution pressure" transposed to the "generation-age" hypothesis for heterogeneous hematopoietic stem/progenitor cell compartment (Rosendaal et al., 1979): the stem cells are highly sensitive to O_2, in order to be protected, its concentrations should be lower than 1% and approaching zero (Jang and Sharkis, 2007), that is, atmospheric O_2 concentrations existing between 2 and 3 billion years ago. By their metabolic properties (Jang and Sharkis, 2007; Simon and Keith, 2008) and their position in ontogenesis (Powers et al., 2008) stem cells reflect this early evolution stage (Massabau, 2000, 2003). In fact, if exposed to higher O_2 concentrations primitive stem cells "escape" death differentiating into more evolved forms, which are more adapted and resistant to higher O_2 concentrations (altogether is compatible with the general philosophy of Haeckel's concept (Haeckel, 1866)). However this way the stem cells exhaust themselves. This scenario typically takes on at 20–21% O_2, that is, hyperoxic, atmospheric O_2 concentrations (Ivanovic et al., 2004).

We propose that self-renewing divisions of stem cells are conditioned by hypoxic metabolic type. Thus, a limited energy supply would allow only strict replication of DNA unlike activation of the transcriptional differentiation programs. So, the cell proliferates without the differentiation (a phenomenon called "self-renewal"). In fact, the low energy availability probably allows the expression of primary signaling pathways and transcription factors, which are, in general, highly evolutionary conserved (hence dating from very early evolution stages), which control stem cell self-renewal and multipotency ("stemness"), and are activated by HIFs, that is, by conditions typical for hypoxic metabolic cell type (reviewed in Keith and Simon, 2007 and in Lin et al., 2008). A stem cell could afford these "executive extensions" since their expression is compatible with a low oxygen metabolism; they ensure the cellular survival and/or basic cell reproduction functions but not the functions acquired at the ulterior evolutionary stages ("commitment", "differentiation", "specialisation," "maturation"...). Typical examples are Oct 4 (Covello et al., 2006), Notch (Pear and Simon, 2005; Sansone et al., 2007) Wnt-signaling patway (Lim et al., 2008), STATs (Wang et al., 2008), sonic hedgehog (Bijlsma et al., 2008), ATM-p38/MAPK (Bencokova et al., 2008)... This situation is typically understood and considered as an "inhibition" of the differentiation. Of course, the principle is not "all or nothing" and, at different developmental stages and different oxygen availabilities, the stem cells could develop some aspects of aerobic (although still "hypoxic") metabolism, permitting, to a different extent, the commitment and differentiation. Unlike the maintenance of primitive stem cells (usually $CD34^-$ or

$CD34^{low}$), when their quiescent nature and slow proliferation are compatible with their low mitochondrial content (Freyer, 1998; Radley et al., 1999) and the hypoxic metabolic type (Parmar et al., 2007; the low energy demands), the differentiation events are associated with the mitochondria proliferation ("mitochondrial biogenesis") which is paralleled by increase in aerobic metabolic properties of cells. So, the $CD34^{+++}$ and CD133+ cell populations which are, in fact, highly enriched in hematopoietic progenitors (very few of the cells characterized by this phenotype are the stem cells), are the richest in mitochondria (Piccoli et al., 2007; the authors wrongly consider CD34+ and CD133+ cells as "stem cells"). Indeed, the most recent data suggest a coupling between intrinsic metabolic parameters and stem cell fate (Schieke et al., 2008) and clearly show that repressing mitochondrial biogenesis and ROS by a molecular mechanism (TSC-mTOR), maintains quiescence and function ("stemness") of hematopoietic stem cells (Chen et al., 2008).

The differentiation demands the internal and external signals that should be "cached" and "transmitted." For example, to allow an efficient action of a cytokine, a cell should exhibit the receptors and operational corresponding signal transduction pathways. Thus, the effects of cytokines on stem and progenitor cells are depending on their metabolic state, which is crucial for the differentiation-related expression and synthesis (Mostafa et al., 2001b; Brunet de la Grange et al., 2006). Also, the response of cellular molecular machinery depends on the energy supply, thus on its metabolic type and actual O_2 concentration (Bell et al., 2007).

With respect to the stem cell fate, the concept presented above could be applied to two hypotheses: (1) the stem cells exhibit the same metabolic type as other cells, but, residing in highly hypoxic niches (Chow et al., 2000; Nilsson et al., 2001; Wilson and Trumpp, 2006; Arai and Suda, 2007; Parmar et al., 2007) they are quiescent (as proposed by Cipolleschi et al., 1993) or allowed to proliferate slowly without differentiation. This hypothesis is compatible with the concept of slow self-renewing divisions of somatic stem cells (Bradford et al., 1997); (2) stem cells exhibit a particular, peculiar metabolic type, permitting their proliferation without differentiation in highly hypoxic conditions, a property lost in course of differentiation. This concept would be compatible with the rapid self-renewing divisions of embrionnary stem cells and somatic stem cells ensuring maintenance of their pool during tissue regeneration; it is supported by the data on stem cell-specific metabolic features (Ito et al., 2006) as well as the one from thoughtful experiments of Dello Sbarba et al. (1987), showing that the stem cells proliferate in spite of respiratory chain saturation by piruvate that exhibits a cytostatic effect on progenitors. Finally, the stem cell population could be composed of both the stem cells exhibiting peculiar and "ordinary" metabolic type, adding a new dimension to the heterogeneity of the stem cell compartment.

Towards a Revision of Knowledge in Cell Biology

From the late seventies until today, a number of data have been accumulated clearly demonstrating that the cellular response (not only to Epo and VEGF) is depending on the actual O_2 concentration. For example, in the same culture medium and in presence of the same soluble factors, a completely different response of hematopoietic cells has been evidenced at low with respect to the atmospheric O_2 concentration (20–21%; Bradley et al., 1978; Broxmeyer et al., 1985, 1990; Smith and Broxmeyer, 1986; Koller et al., 1992a,b,c). Recently, we have demonstrated a positive effect of IL-6 on stem cell (pre-CFC) maintenance (and hence on the inhibition of stem cell differentiation) revealed only at 1 and not at 20% O_2 (Kovacevic-Filipovic et al., 2007). Basically the same phenomenon was evidenced for VEGF (Brunet de la Grange et al., 2003). The real nature of stem cells, that is, their maintenance in G0 phase, self-renewal, commitment, etc., which are, of course, regulated by certain cytokines and growth factors, should be evaluated only at appropriately low O_2 concentrations characterizing the stem cell niche (Cipolleschi et al., 1993, 2000; Ivanovic et al., 2000a). Thus, here again, the response of stem cells to the cytokine stimulation is completely different at 0.1, 1, 3–5, or at 20–21% O_2, implying a physiological regulatory role of oxygen concentration in early hematopoiesis (Ivanovic et al., 2000a, 2002, 2004; Hermitte et al., 2006). The response of committed progenitors to cytokine stimulation concerns all hematopoietic lineages (Laluppa et al., 1998; Mostafa et al., 2000, 2001a,b). It was pointed for example that "O_2 tension alters the effect of cytokines on the megakaryocyte, erythrocyte, and granulocyte lineage" (Laiuppa et al., 1998). This point is being completely confirmed in the ex vivo model of erythropoiesis: an appropriately low O_2 concentration is required for each developmental stage of erythropoiesis to optimize the ex vivo production of RBC from CD34+ cells (Vlaski et al., 2007). With that respect, the O_2 gradient seems to be a general physiologic regulator, beyond well-known, Epo-related downstream tuning (Cipolleschi et al., 1997; Vlaski et al., 2007; submitted). Recently, a series of papers studying different cell types confirm that the actual O_2 concentration determines the cellular response to cytokines as well as cytokine secretion pattern by the cells (Carswell et al., 2000; Morrison et al., 2000; Csete et al., 2001; Lennon et al., 2001; Desplat et al., 2002; Arnett et al., 2003; Fink et al., 2004; Moussavi-Harami et al., 2004; Scherer et al., 2004; Grinakovskaya et al., 2007; Motohira et al., 2007; Schutyser et al., 2007; Wang and Wang, 2007; Battaglia et al., 2008; Kanichai et al., 2008; Kim et al., 2008; Mancino et al., 2008; Ricciardi et al., 2008). In addition, the DNA repair seems to eperate better at low O_2 concentration (Nijnik et al., 2007).

Concluding Remarks

In order to stress the importance of oxygenation for stem cell fate, the "oxygen stem cell paradigm" was elaborated integrating the "generation-age hypothesis" and hypoxic metabolic properties of stem cells with the oxygen-dependent evolution of life and the position of heterogeneous stem cell populations in ontogenesis, (in line with the general frame of Haeckel's concept). With that respect, stem cells reflect an early evolutionary stage characterized with very limited O_2 availabilities, that is, reminding those unicellular organisms still not completely adapted to oxygen, exhibiting the "hypoxic" metabolic type. So, the self-renewing divisions of stem cells are conditioned by hypoxic metabolic type since a limited energy supply would only allow (i) the strict replication of DNA unlike activation of the transcriptional differentiation programs, and (ii) the expression of highly evolutionary conserved (hence dating from very early evolution stages) signaling pathways and transcription factors involved in control of stem cell self-renewal and multipotency ("stemness"), in other words only enabling the cell survival and proliferation but not commitment and/or differentiation.

The O_2 supply is finely regulated in vivo, with respect to the cell and tissue type. The tissue O_2 concentrations are much lower than the atmospheric ones. Since the cellular response is dependent on O_2 concentration, to get an insight of the real cell physiology, it should be studied only at appropriate O_2 concentrations approximating at the best in situ normoxia for each cell type. With that respect, the atmospheric O_2 concentration could not be considered as "normoxia."

This argumentation urges the revision of our knowledge on cytokine biology, which is acquired ex vivo at non physiological atmospheric (20–21%) O_2 concentrations representing a

hyperoxic state for most primate cells, except mature cells originating from the tissues which are in direct contact with air (such as skin surface, mouth and respiratory epithelium. . .).

The low oxygen, undoubtedly, better approximates the in vivo environment.

Acknowledgments

The author is thankful to Dr. Marija Vlaski for discussions on this article, as well as to Professor Persio Dello Sbarba and Vincent Praloran for a decade long "brain storming" and fruitful collaboration. The English reading by Mrs Elisabeth Volkmann was highly appreciated. This study was supported by Etablissement Français du Sang Aquitaine-Limousin (annual funds for research and development 2008 and 2009).

Literature Cited

Arai F, Suda T. 2007. Maintenance of quiescent hematopoietic stem cells in the osteoblastic niche. Ann NY Acad Sci 1106:41–53.

Arcasoy MO. 2008. The non-haematopoietic biological effects of erythropoietin. Br J Haematol 14:14–31.

Arnett TR, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M, Meghji S. 2003. Hypoxia is a major stimulator of osteoclast formation and bone resorption. J Cell Physiol 196:2–8.

Battaglia F, Delfino S, Merello E, Puppo M, Piva R, Varesio L, Bosco MC. 2008. Hypoxia transcriptionally induces macrophage-inflammatory protein-3alpha/CCL-20 in primary human mononuclear phagocytes through nuclear factor (NF)-kappaB. J Leukoc Biol 83:648–662.

Bell EL, Klimova TA, Eisenbart J, Schumacker PT, Chandel NS. 2007. Mitochondrial reactive oxygen species trigger hypoxia-inducible factor-dependent extension of the replicative life span during hypoxia. Mol Cell Biol 27:5737–5745.

Bencokova Z, Kaufmann MR, Pires IM, Lecane PS, Giaccia AJ, Hammond EM. 2008. ATM activation and signalling under hypoxic conditions. Mol Cell Biol November 3 [Epub ahead of print].

Berner RA, Vanden Brooks JM, Ward PD. 2007. Oxygen and evolution. Science 316:557–558.

Bijlsma MF, Groot AP, Oduro JP, Franken RJ, Schoenmakers SH, Peppelenbosch MP, Spek CA. 2008. Hypoxia induces a hedgehog response mediated by HIF-1alpha. J Cell Mol Med September 4 [Epub ahead of print].

Bradford GB, Williams B, Rossi R, Bertoncello I. 1997. Quiescence, cycling and turnover in the primitive hematopoietic stem cell compartment. Exp Hematol 25:445–453.

Bradley TR, Hodgson GS, Rosendaal M. 1978. The effect of oxygen tension on hematopoietic and fibroblast cell proliferation in vitro. J Cell Physiol 97:517–522.

Broxmeyer HE, Cooper S, Rubin BY, Taylor MW. 1985. The synergistic influence of human interferon-gamma and interferon-alpha on suppression of hematopoietic progenitor cells is additive with the enhanced sensitivity of these cells to inhibition by interferons at low oxygen tension in vitro. J Immunol 135:2502–2506.

Broxmeyer HE, Cooper S, Lu L, Miller ME, Langefeld CD, Ralph P. 1990. Enhanced stimulation of human bone marrow macrophage colony formation in vitro by recombinant human macrophage colony-stimulating factor in agarose medium and at low oxygen tension. Blood 76:323–329.

Brunet de la Grange P, Conrad V, Hermitte F, Ivanovic Z, Praloran V. 2003. Regulation of hematopoietic stem cells functions in hypoxia involvement of VEGF and its receptors. 45 Annual Meeting of ASH. Blood 102:Abstract 3059, 826a.

Brunet De La Grange P, Barthe C, Lippert E, Hermitte F, Belloc F, Lacombe F, Ivanovic Z, Praloran V, 2006. Oxygen concentration influences mRNA processing and expression of the cd34 gene. J Cell Biochem 97:135–144.

Buerk DG, Shonat RD, Riva CE, Cranstoun SD. 1993. O2 gradients and countercurrent exchange in the cat vitreous humor near retinal arterioles and venules. Microvasc Res 45:134–148.

Carnot P, Deflandre C. 1906. Sur L'activite hematopoietique de serum au cours de la regeneration du sang. CR Acad Sci (Paris) 143:384–387.

Carswell KS, Weiss JW, Papoutsakis ET. 2000. Low oxygen tension enhances the stimulation and proliferation of human T lymphocytes in the presence of IL-2. Cytotherapy 2:25–37.

Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, Zheng P. 2008. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med 205:2397–2408.

Chow DC, Wenning LA, Miller WM, Papoutsakis ET. 2000. Modeling pO(2) distributions in the bone marrow hematopoietic compartment. II. Modified Kroghian models. Biophys J 81:685–696.

Cipolleschi MG, Dello Sbarba P, Olivotto M. 1993. The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82:2031–2037.

Cipolleschi MG, D'Ippolito G, Bernabei PA, Caporale R, Nannini R, Mariani M, Fabbiani M, Rossi-Ferrini P, Olivotto M, Dello Sbarba P. 1997. Severe hypoxia enhances the formation of erythroid bursts from human cord blood cells and the maintenance of BFU-E in vitro. Exp Hematol 25:1187–1194.

Cipolleschi MG, Rovida E, Ivanovic Z, Praloran V, Olivotto M, Dello Sbarba P. 2000. The expansion of murine bone marrow cells preincubated in hypoxia as an in vitro indicator of their marrow-repopulating ability. Leukemia 14:735–739.

Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith B. 2006. HIF-2alpha regulates Oct-4: Effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 20:557–570.

Csete M, Walikonis J, Slawny N, Wei Y, Korsnes S, Doyle JC, Wold B. 2001. Oxygen-mediated regulation of skeletal muscle satellite cell proliferation and adipogenesis in culture. J Cell Physiol 189:189–196.

Danet GH, Pan Y, Luongo JL, Bonnet DA, Simon MC. 2003. Expansion of human SCID-repopulating cells under hypoxic conditions. J Clin Invest 112:126–136.

Das J. 2006. The role of mitochondrial respiration in physiological and evolutionary adaptation. Bioessays 28:890–901. Review.

Dello Sbarba P, Cipolleschi MG, Olivotto M. 1987. Hemopoietic progenitor cells are sensitive to the cytostatic effect of pyruvate. Exp Hematol 15:137–142.

Desplat V, Faucher JL, Mahon FX, Dello Sbarba P, Praloran V, Ivanovic Z. 2002. Hypoxia modifies proliferation and differentiation of CD34(+) CML cells. Stem Cells 20:347–354.

Falkowski PG. 2006. Tracing oxygen's imprint on earth's metabolic evolution. Science 311:1724–1725.

Ferrara N, Gerber HP, LeCouter J. 2003. The biology of VEGF and its receptors. Nat Med 9:669–676.

Fink T, Abildtrup L, Fogd K, Abdallah BM, Kassem M, Ebbesen P, Zachar V. 2004. Induction of adipocyte-like phenotype in human mesenchymal stem cells by hypoxia. Stem Cells 22:1346–1355.

Freyer JP. 1998. Decreased mitochondrial function in quiescent cells isolated from multicellular tumor spheroids. J Cell Physiol 176:138–149.

Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. 2007. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 129:111–122.

Grinakovskaya OS, Andreeva ER, Buravkova LB. 2007. Effects of hypoxic gas mixtures on viability, expression of adhesion molecules, migration, and synthesis of interleukins by cultured human endothelial cells. Bull Exp Biol Med 144:130–135.

Guzy RD, Schumacher PT. 2006. Oxygen sensing by mitochondria at complex III: The paradox of increased reactive oxygen species during hypoxia. Exp Physiol 91:807–819.

Haeckel E. 1866 Generelle Morphologie der Organismen. Berlin, G. Reimer.

Hemphill JC III, Smith WS, Sonne DC, Morabito D, Manley GT. 2005. Relationship between brain tissue oxygen tension and CT perfusion: Feasibility and initial results. Am J Neuroradiol 26:1095–1100.

Hermitte F, Brunet de la Grange P, Belloc F, Praloran V, Ivanovic Z. 2006. Very low O2 concentration (0.1%) favors G0 return of dividing CD34+ cells. Stem Cells 24:65–73.

Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, Suda T. 2006. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med 12:446–451.

Ivanovic Z, Dello Sbarba P, Trimoreau F, Faucher JL, Praloran V. 2000a. Primitive human HPCs are better maintained and expanded in vitro at 1 percent oxygen than at 20 percent. Transfusion 40:1482–1488.

Ivanovic Z, Bartolozzi B, Bernabei PA, Cipolleschi MG, Rovida E, Milenkovic P, Praloran V, Dello Sbarba P. 2000b. Incubation of murine bone marrow cells in hypoxia ensures the maintenance of marrow-repopulating ability together with the expansion of committed progenitors. Br J Haematol 108:424–429.

Ivanovic Z, Belloc F, Faucher JL, Cipolleschi MG, Praloran V, Dello Sbarba P. 2002. Hypoxia maintains and interleukin-3 reduces the pre-colony-forming cell potential of dividing CD34(+) murine bone marrow cells. Exp Hematol 30:67–73.

Ivanovic Z, Hermitte F, Brunet de la Grange P, Dazey B, Belloc F, Lacombe F, Vezon G, Praloran V. 2004. Simultaneous maintenance of human cord blood SCID-repopulating cells and expansion of committed progenitors at low O2 concentration (3%). Stem Cells 22:716–724.

Jang Y-Y, Sharkis SJ. 2007. A low level of reactive oxygen species select for primitive hemmatopoietic stem cells that may reside in the low-oxygen niche. Blood 110:3056–3063.

Jelkmann W. 1986. Erythropoietin research, 80 years after the initial studies by Carnot and Deflandre. Respire Physiol 257–266.

Jiang BH, Semenza GL, Bauer C, Marti HH. 1996. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. Am J Physiol 271:C1172–C1180.

Johnson PC, Vandegriff K, Tsai AG, Intaglietta M. 2005. Effect of acute hypoxia on microcirculatory and tissue oxygen levels in rat cremaster muscle. J Appl Physiol 98:1177–1184.

Jungermann K, Kietzmann T. 1997. Role of oxygen in the zonation of carbohydrate metabolism and gene expression in liver. Kidney Int 51:402–412.

Kanichai M, Ferguson D, Prendergast PJ, Campbell VA. 2008. Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: A role for AKT and hypoxia-inducible (HIF)-1alpha. J Cell Physiol 216:708–715.

Keith B, Simon MC. 2007. Hypoxia-inducible factors, stem cells, and cancer. Cell 129:465–472.

Kenneth NS, Rocha S. 2008. Regulation of gene expression by hypoxia. Biochem J 414:19–29. Review.

Kim H, Peng G, Hicks JM, Weiss HL, Van Meir EG, Brenner MK, Yotnda P. 2008. Engineering human tumor-specific cytotoxic T cells to function in a hypoxic environment. Mol Ther 16:599–606.

Koller MR, Bender JG, Miller WM, Papoutsakis ET. 1992a. Reduced oxygen tension increases hematopoiesis in long-term culture of human stem and progenitor cells from cord blood and bone marrow. Exp Hematol 20:264–270.

Koller MR, Bender JG, Papoutsakis ET, Miller WM. 1992b. Effects of synergistic cytokine combinations, low oxygen, and irradiated stroma on the expansion of human cord blood progenitors. Blood 80:403–411.

Koller MR, Bender JG, Papoutsakis ET, Miller WM. 1992c. Beneficial effects of reduced oxygen tension and perfusion in long-term hematopoietic cultures. An NY Acad Sci 665:105–116.

Kovacevic-Filipovic M, Petakov M, Hermitte F, Debeissat C, Krstic A, Jovcic G, Bugarski D, Lafarge X, Milenkovic P, Praloran V, Ivanovic Z. 2007. Interleukin-6 (IL-6) and low O(2) concentration (1%) synergize to improve the maintenance of hematopoietic stem cells (pre-CFC). J Cell Physiol 212:68–75.

Laluppa JA, Papoutsakis ET, Miller WM. 1998. Oxygen tension alters the effects of cytokines on the megakaryocyte, erythrocyte, and granulocyte lineages. Exp Hematol 26:835–846.

Lennon DP, Edmison JM, Caplan AI. 2001. Cultivation of rat marrow-derived mesenchymal stem cells in reduced oxygen tension: Effects on in vitro and in vivo osteochondrogenesis. J Cell Physiol 187:345–355.

Lim JH, Chun YS, Park JW. 2008. Hypoxia-inducible factor-1alpha obstructs a Wnt signaling pathway by inhibiting the hARD1-mediated activation of beta-catenin. Cancer Res 68:5177–5184.

Lin Q, Kim Y, Alarcon RM, Yun Z. 2008. Oxygen and cell fate decisions. Gene Reg Syst Biol 2:43–51.

Mancino A, Schioppa T, Larghi P, Pasqualini F, Nebuloni M, Chen IH, Sozzani S, Austyn JM, Mantovani A, Sica A. 2008. Divergent effects of hypoxia on dendritic cell functions. Blood 112:3723–3734.

Massabau JC. 2000. From low arterial- to low tissue-oxygenation strategy. An evolutionary theory. Respir Physiol 128:249–261.

Massabau JC. 2003. Primitive, and protective, our cellular oxygenation status? Mech Ageing Dev 124:857–863. Review.

McKinley BA, Butler BD. 1999. Comparison of skeletal muscle PO2, PCO2, and pH with gastric tonometric P(CO2) and pH in hemorrhagic shock. Crit Care Med 27:1869–1877.

Metcalf D. 2008. Hematopoietic cytokines. Blood 111:485–491.
Mik EG, van Leeuwen TG, Raat NJ, Ince C. 2004. Quantitative determination of localized tissue oxygen concentration in vivo by two-photon excitation phosphorescence lifetime measurements. J Appl Physiol 97:1962–1969.
Morrison SJ, Csete M, Groves AK, Melega W, Wold B, Anderson DJ. 2000. Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. J Neurosci 20:7370–7376.
Mostafa SS, Miller WM, Papoutsakis ET. 2000. Oxygen tension influences the differentiation, maturation and apoptosis of human megakaryocytes. Br J Haematol 111:879–889.
Mostafa SS, Papoutsakis ET, Miller WM. 2001a. Oxygen tension modulates the expression of cytokine receptors, transcription factors, and lineage-specific markers in cultured human megakaryocytes. Exp Hematol 29:873–883.
Mostafa SS, Papoutsakis ET, Miller WM. 2001b. Oxygen tension modulates the expression of cytokine receptors, transcription factors, and lineage-specific markers in cultured human magakaryocytes. Exp Hematol 29:873–889.
Motohira H, Hayashi J, Tatsumi J, Tajima M, Sakagami H, Shin K. 2007. Hypoxia and reoxygenation augment bone-resorbing factor production from human periodontal ligament cells. J Periodontol 78:1803–1809.
Moussavi-Harami F, Duwayri Y, Martin JA, Moussavi-Harami F, Buckwalter JA. 2004. Oxygen effects on senescence in chondrocytes and mesenchymal stem cells: Consequences for tissue engineering. Iowa Orthop J 24:15–20.
Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, Rodrigues NP, Crockford TL, Cabuy E, Vindigni A, Enver T, Bell JI, Slijepcevic P, Goodnow CC, Jeggo PA, Cornall RJ. 2007. DNA repair is limiting for haematopoietic stem cells during ageing. Nature 447:686–690.
Nilsson SK, Johnston HM, Coverdale JA. 2001. Spatial localization of transplanted hemopoietic stem cells: Influences for the localization of stem cell niches. Blood 97:2293–2299.
Nwaigwe CI, Roche MA, Grinberg O, Dunn JF. 2000. Effect of hyperventilation on brain tissue oxygenation and cerebrovenous PO2 in rats. Brain Res 868:150–156.
Papandreou I, Powell A, Lim AL, Denko N. 2005. Cellular reaction to hypoxia: Sensing and responding to an adverse environment. Mutat Res 569:87–100. Review.
Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. 2007. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci USA 104:5431–5436.
Pear WS, Simon MC. 2005. Lasting longer without oxygen: The influence of hypoxia on Notch signaling. Cancer Cell 8:435–437.
Piccoli C, D'Aprile A, Ripoli M, Scrima R, Boffoli D, Tabilio A, Capitanio N. 2007. The hypoxia-inducible factor is stabilized in circulating hematopoietic stem cells under normoxic conditions. FEBS Lett 581:3111–3119.
Powers DE, Millman JR, Huang RB, Colton CK. 2008. Effects of oxygen on mouse embryonic stem cell growth, phenotype retention, and cellular energetics. Biotechnol Bioeng 101:241–254.
Radley JM, Ellis S, Palatsides M, Williams B, Bertoncello I. 1999. Ultrastructure of primitive hematopoietic stem cells isolated using probes of functional status. Exp Hematol 27:365–369.
Ricciardi A, Elia AR, Cappello P, Puppo M, Vanni C, Fardin P, Eva A, Munroe D, Wu X, Giovarelli M, Varesio L. 2008. Transcriptome of hypoxic immature dendritic cells: Modulation of chemokine/receptor expression. Mol Cancer Res 6:175–185.
Rosendaal M, Hodgson GS, Bradley TR. 1979. Organization of haemopoietic stem cells: The generation-age hypothesis. Cell Tissue Kinet 12:17–29.
Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, Zweier JL, Sen CK. 2000. Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. J Biol Chem 278:47129–47135.
Saltzman DJ, Toth A, Tsai AG, Intaglietta M, Johnson PC. 2003. Oxygen tension distribution in postcapillary venules in resting skeletal muscle. Am J Physiol Heart Circ Physiol 285:H1980–H1985.
Sansone P, Storci G, Giovannini C, Pandolfi S, Pianetti S, Taffurelli M, Santini D, Ceccarelli C, Chieco P, Bonafé M. 2007. p66Shc/Notch-3 interplay controls self-renewal and hypoxia survival in human stem/progenitor cells of the mammary gland expanded in vitro as mammospheres. Stem Cells 25:807–815.
Scherer K, Schünke M, Sellckau R, Hassenpflug J, Kurz B. 2004. The influence of oxygen and hydrostatic pressure on articular chondrocytes and adherent bone marrow cells in vitro. Biorheology 41:323–333.
Schieke SM, Ma M, Cao L, McCoy JP Jr, Liu C, Hensel NF, Barrett AJ, Boehm M, Finkel T. 2008. Mitochondrial metabolism modulates differentiation and teratoma formation capacity in mouse embryonic stem cells. J Biol Chem 283:28506–28512.
Schutyser E, Su Y, Yu Y, Gouwy M, Zaja-Milatovic S, Van Damme J, Richmond A. 2007. Hypoxia enhances CXCR4 expression in human microvascular endothelial cells and human melanoma cells. Eur Cytokine Netw 18:59–70.
Semenza GL. 2007. Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor1. Biochem J 405:1–9 (review).
Simon MC, Keith B. 2008. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 9:285–296. Review.
Smith S, Broxmeyer HE. 1986. The influence of oxygen tension on the long-term growth in vitro of haematopoietic progenitor cells from human cord blood. Br J Haematol 63: 29–34.
Stockmann C, Fandrey J. 2006. Hypoxia-induced erythropoietin production: A paradigm for oxygen-regulated gene expression. Clin Exp Pharmacol Physiol 33:968–979.
Tondevold E, Eriksen J, Jansen E. 1979. Observations on long bone medullary pressures in relation to arterial PO2, PCO2 and pH in the anaesthetized dog. Acta Orthop Scand 50:645–651.
Vlaski M, Chevaleyre J, Duchez P, Lafarge X, Boiron J-M, Ivanovic Z. 2007. L'adaptation de l'oxygenation peut améliorer la production d'érythrocytes ex vivo. 23 national Congress of SFTS. Transf Clin Biol 14 Abstract SPD3-6.
Wang JY, Wang JY. 2007. Hypoxia/Reoxygenation induces nitric oxide and TNF-alpha release from cultured microglia but not astrocytes of the rat. Chin J Physiol 50:127–134.
Wang G, Qian P, Jackson FR, Qian G, Wu G. 2008. Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells. Int J Biochem Cell Biol 40:461–470.
Welch WJ, Baumgärtl H, Lübbers D, Wilcox CS. 2001. Nephron pO2 and renal oxygen usage in the hypertensive rat kidney. Kidney Int 59:230–237.
Whalen WJ, Ganfield R, Nair P. 1970. Effects of breathing O 2 or O 2 +CO 2 and of the injection of neurohumors on the PO 2 of cat cerebral cortex. Stroke 1:194–200.
Wild JM, Fichele S, Woodhouse N, Paley MN, Kasuboski L, van Beek EJ. 2005. 3D volume-localized pO2 measurement in the human lung with 3He MRI. Magn Reson Med 53:1055–1064.
Wilson A, Trumpp A. 2006. Bone marrow haematopoietic stem-cell niches. Nat Rev Immunol 6:93–106.
Wölfle D, Jungermann K. 1985. Long-term effects of physiological oxygen concentrations on glycolysis and gluconeogenesis in hepatocyte cultures. Eur J Biochem 151:299–303.
Yu DY, Cringle SJ. 2005. Retinal degeneration and local oxygen metabolism. Exp Eye Res 80:745–751. Review.