Oxygen levels and the regulation of cell adhesion in the nervous system
A control point for morphogenesis in development, disease and evolution?

Kathryn L. Crossin
Department of Neurobiology; The Scripps Research Institute; La Jolla, CA USA

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In this article, I discuss the hallmarks of hypoxia in vitro and in vivo and review work showing that many types of stem cell proliferate more robustly in lowered oxygen. I then discuss recent studies showing that alterations in the levels and the types of cell and substrate adhesion molecules are a notable response to reduced O2 levels in both cultured primary neural stem cells and brain tissues in response to hypoxia in vivo. The ability of O2 levels to regulate adhesion molecule expression is linked to the Wnt signaling pathway, which can control and be controlled by adhesion events. The ability of O2 levels to influence cell adhesion also has far-reaching implications for development, ischemic trauma and neural regeneration, as well as for cancer and other diseases. Finally I discuss the possibility that the fluctuations in O2 levels known to have occurred over evolutionary time could, by influencing adhesion systems, have contributed to early symbiotic events in unicellular organisms and to the emergence of multicellularity. It is not my intention to be exhaustive in these domains, which are far from my own field of study. Rather this article is meant to provoke and stimulate thinking about molecular evolution involving O2 sensing and signaling during eras of geologic and atmospheric change that might inform modern studies on development and disease.

Elevation in Levels of the Hypoxia Inducible Factor, HIF1α, is a Hallmark of Reduced Oxygen Availability in Cell Culture and in Tissues

HIF1α is a constitutively produced protein that is normally hydroxylated by a family of prolyl hydroxylase enzymes, a modification that allows HIF to bind the von Hippel-Lindau tumor suppressor protein, targeting HIF for proteosomal degradation.1-4 When O2 levels are lowered, hydroxylation is reduced and degradation is prevented. The stabilized HIFα can then translocate into the nucleus and directly transactivate a number of genes to respond to hypoxia, among which are those involved in glycolysis, erythropoiesis, and angiogenesis.4,7 HIFs not only have direct transcriptional targets but also influence signaling systems to alter gene expression.1 It is notable that HIF1α levels have been shown to be activated over a wide range of physiological O2 tensions, although the greatest increases were at levels consistent with hypoxia/ischemia in vivo.5 These findings indicate that levels of HIF are functionally involved in O2 homeostasis in cells and organisms to enable the switch from aerobic to anaerobic metabolism when O2 levels are limiting. It is of interest that mice lacking HIF1α or its homolog HIF2α and their binding partner HIF1β all exhibit embryonic lethality, displaying multiple morphogenetic defects, including failure of neural tube closure.6 The importance of O2 concentration on the formation of the neural folds and neural crest development was recognized in early studies of rat neural maturation in cultured embryos.7

Hypoxia vs. In Situ Normoxia

The Merriam-Webster Dictionary defines hypoxia as “a deficiency of oxygen reaching the tissues of the body.” In the scientific literature, however, the word is often used to mean a level of O2 lower than that present in air, particularly in studies in vitro. Air does not reflect the in vivo environment of the majority of cell types and hence the term “in situ normoxia” has been suggested.11 This novel term is meant to give a better indication what normoxia or hypoxia would be for a given cell type based on its exposure to O2 in vivo and allow cell culture to mimic more accurately the physiological state, as is beginning to be understood for neural cells.12,13 It also points to the importance of measurements of the levels of O2 in particular tissues in vivo. In warm-blooded organisms in vivo, such levels are critically dependent on multiple factors including vascularization and angiogenesis, levels of red blood cells and hemoglobin, cardiac capacity, as well as external oxygenation. These considerations are also critical for the interpretation of studies of “hypoxia” in cell lines that were derived in hypoxic conditions, but later exposed to lower O2 levels.
In this regard, it is critical to emphasize two concepts that provide an outline for the sections to follow: (1) few tissues experience atmospheric levels of $O_2$ in vivo and (2) metazoan life originated in an atmosphere considerably lower in levels of $O_2$ than the modern atmosphere.

**Cell Culture Conditions and $O_2$ Levels**

For over a decade it has been recognized that proliferation is enhanced and differentiation is altered in a number of stem cell types when cells are cultured in levels of $O_2$ (1–8% $O_2$) lower than those in air, previously the standard for in vitro studies (reviewed in refs. 12 and 13). Cells from the nervous system cultured under such low $O_2$ conditions include neural progenitor cells from various brain regions,\(^{14-20}\) virally-immortalized human neural progenitor cells,\(^{21}\) and neural crest stem cells.\(^{22}\) Other sensitive cells are iPS cells,\(^{23}\) embryonic stem cells\(^{24}\) and stem cells of different tissues.\(^{32}\) These observations are consistent with what is known about $O_2$ levels in the stem cell niches of the brain and other tissues\(^{12,13,16,32-37}\) and are likely to reflect more closely the native behavior of cells under physiological conditions.

A recent well-received book describes the origins of the now ubiquitous human HeLa cell line.\(^{38}\) The author reviews the early history of cell culture, noting that the recovery of stable human immortalized human lymphocytes was rare, an observation accounting for the extensive use of the surviving line HeLa. Indeed it is possible that many early cell cultures failed because they were grown in air, a hyperoxic environment compared with their in vivo environment. This consideration highlights the importance of taking in vivo physiological $O_2$ tension into account when designing in vitro culture conditions.

**Changes in the Expression of Multiple Types of Adhesion Molecules Accompany the Growth of Neural Stem Cells in Reduced $O_2$ Atmospheres In Vitro**

Cell adhesion between and among cells is a critical component of morphoregulation during development and in disease.\(^{39,40}\) Molecules of the immunoglobulin and cadherin families of cell adhesion molecules (CAMs) link cells together and collectively play major roles in the formation of tissues and organs. In addition, molecules in the extracellular matrix (ECM) surrounding cells, along with integrins and other ECM receptors on the cell surface, are critical for cell migration.

Several recent studies have linked the altered expression of cell and extracellular matrix adhesion molecules and matrix modifying enzymes to growth of neural stem cells in culture in a reduced $O_2$ environment.\(^{23,41}\) Moreover, studies from my laboratory found that the levels of mRNAs for several CAMs, ECM proteins, and ECM modifying enzymes differed in rat embryonic neural stem cells cultured for 72 h in 1% vs. 20% $O_2$.\(^{23}\) Another study evaluated 5704 genes in rat neural stem cells and found that, of all the genes whose expression differed between cells cultured at 3% or 20% $O_2$ for 24 h, a significant fraction were involved in cell adhesion or migration.\(^{41}\)

Adhesion molecule expression in vitro was also altered in several other cell types exposed to reduced $O_2$ levels, including mesenchymal stem cells,\(^{23,44}\) hematopoietic stem cells\(^{45}\) and a melanoma cell line.\(^{44}\)

**Connection between Oxygen Levels, HIF1α, Wnt Signaling and Adhesion Molecule Expression Suggests One Mechanism to Control Neural Stem Cell Proliferation**

A significant finding in two recent papers is the connection between HIF1α stabilization and the activation of the Wnt pathway by lowered $O_2$.\(^{22,23}\) As reported in many systems, activation of HIF-1α occurred in rat embryonic neural stem cells cultured in lowered $O_2$.\(^{22,23}\) As described above, stabilization of the levels of the HIF1α protein is a hallmark of hypoxia,\(^{2,12,13,46,47}\) but HIF1α levels are also known to vary significantly under different physiological levels of $O_2$.\(^{8}\)

Our study\(^{23}\) also showed that a critical downstream target of Wnt was an increased expression of matrix metalloproteinase 9 (MMP9), an ECM modifying enzyme. MMP9 expression had previously been shown to be upregulated by Wnt activation in human lymphocytes.\(^{48}\) More importantly, we showed that the increase in the level and activity of MMP9 was essential for the increased proliferation and migration of embryonic rat neural stem cells at reduced levels of $O_2$.\(^{23}\) Hence modifications to the ECM can control neural stem cell proliferation in vitro and may do so in vivo in the stem cell niche. Evaluating the proliferation and migration of neural stem cells in the MMP9 knockout mouse will be revealing and an assessment of the critical enzyme targets of MMP9 should be facilitated using this model.

Wnt is a critical regulator of cell adhesion in multiple cell types via its ability to alter β-catenin activity, the so-called canonical Wnt signaling pathway.\(^{49-51}\) Canonical Wnt signaling primarily influences cadherin-mediated adhesion, subcellular localization, and effects on cell junctions.\(^{52-56}\) The non-canonical, planar cell polarity Wnt pathway\(^{57}\) involves cell rearrangements and migration during development, particularly those in the nervous system.\(^{58}\) These pathways involve extensive changes in cell adhesion and in the cytoskeleton.\(^{57}\)

Another mechanism by which $O_2$ levels could affect adhesion systems is via the engagement of microRNAs, many of which target various adhesion molecule mRNAs and are known to change dramatically under hypoxic conditions.\(^{59-65}\) Thus $O_2$ influences Wnt activity via HIF1α activation, and Wnt in turn can influence multiple adhesive systems directly or indirectly. Such adhesive interactions are clearly a substrate for the evolution of multicellular organisms over geological time.\(^{57,66,67}\)

**Hypoxia in Early Embryonic Development**

Studies of mammalian placental development were among the first to recognize the importance of $O_2$ tension for proper development, both of the early placenta and later of the first trimester human embryo, prior to the complete establishment of maternal-fetal circulation.\(^{68-70}\) The hypoxic environment in utero...
has been postulated to play a key role in trophoblast proliferation and invasion, in both in vivo and in vitro studies.\(^{55,56}\) In this system, low O\(_2\) promotes the expression of several extracellular matrix adhesion molecules, such as those of the \(\beta1\) integrin family as well as matrix modifying enzymes such as uPA and MMP-2, which play essential roles in trophoblast invasion (reviewed in ref. 68). This process is an essential initial mechanism for formation of the placenta, and aberrations in these early events can have serious consequences for placental function resulting in adverse pregnancy outcomes.\(^{59,60}\)

**Influence of Hypoxia-Ishchemia on Adhesion Molecule Expression in the Fetal and Adult Brain**

Hypoxia in utero is a key factor contributing to impaired neural development. In a mouse model of fetal hypoxia, neuronal migration was increased in the CA1 and dentate gyrus regions of the hippocampus along with biphasic changes in molecules that influence migration, including reelin, disabled and APP.\(^{70}\) Using the hypoxia indicator pimonidazole, it was revealed that hypoxic areas colocalized with HIF1\(\text{\textalpha}\) expression and with the adhesion molecule PE-CAM, a marker of endothelial cells, indicating that hypoxia promotes the proliferation and migration of these cells to stimulate angiogenesis during brain development.\(^{79}\)

Studies of cerebral hypoxia in adult animals induced either by middle cerebral artery occlusion (stroke model) or by hypobaric \(O_2\) exposure (chronic hypoxia) demonstrated changes in multiple adhesion systems. For example, in microvessel-rich regions isolated from stroke-affected human brains by laser-capture microdissection, proangiogenic adhesion molecules were upregulated, including NR-CAM, MMP-2, TIMP-1 and \(\beta\)-catenin.\(^{80}\) In a mouse stroke model, V-CAM, laminin, dystroglycan and \(\beta6\) integrin were upregulated and the number of proliferative endothelial cells were increased suggesting that these molecules contribute to angiogenesis in response to cerebral ischemia.\(^{64}\) In chronic cerebral ischemia, fibronectin was increased as was the expression of integrin \(\alpha5\beta1\) on endothelial cells.\(^{81}\) Subsequently, changes in astrocytes occurred, including increases in \(\alpha6\beta4\) integrin and dystroglycan, presumably contributing to the cell interactions necessary for the formation of astrocytic end feet to support the restoration of a mature brain endothelium.\(^{73}\) It is known that cerebral edema occurs after a hypoxic incident due to a compromised blood-brain barrier and the resultant increase in cerebrovascular permeability. In an in vitro model of the blood-brain barrier subjected to hypoxia, levels of expression of the E-cadherin adhesion molecule were decreased; this molecule is known to be important for blood-brain barrier function.\(^{70}\)

**Adhesion Molecule Expression in Neural Stem Cells Favors Their Incorporation into Regenerating Tissues after Various Lesions to the CNS**

Early studies revealed that the polysialylated form of N-CAM (PSA-N-CAM) was prominently expressed in neural stem cells migrating in the rostral migratory stream from the SVZ to the olfactory bulb.\(^{81}\) Subsequent studies showed that loss of PSA or N-CAM decreased the normal migration of these cells\(^{77,79}\) and allowed their increased migration into demyelinating lesions\(^{64,91}\) or facilitated myelination when transplanted into lesioned spinal cord.\(^{57}\) A recent study indicates that increased expression of reelin helps to facilitate migration of cells from the rostral migratory stream into demyelinating lesions in the corpus callosum.\(^{79}\)

Other studies have indicated that overexpression of the L1 neural cell adhesion molecule in mouse embryonic stem (ES) cells supported the regrowth of corticospinal axons and promoted functional recovery after spinal cord injury.\(^{64,77,78}\) The same L1-ES cells promoted recovery from excitotoxic lesions to the mouse striatum.\(^{64}\) Expression of the extracellular matrix proteins thrombospondin 1 and 2 were correlated with a positive recovery of neural function after transient ischemia.\(^{87}\)

An interesting study relevant to the use of stem cells to promote recovery after stroke used cells selected for adhesion molecule expression.\(^{84}\) Mouse neural stem cells were selected for expression of the integrin CD34/8, which is a receptor for V-CAM in endothelial cells. These selected cells showed enhanced homing toward a hypoxic lesion and enhanced behavioral recovery in a mouse stroke model. The authors postulate that these effects are due to better transmigration through the vascular endothelium.

All of the foregoing perturbations to stem cells and their environments should be of great value for studies aimed at promoting regeneration by mimicking the mechanical forces that bias stem cell fate.\(^{82}\) Alterations of adhesion molecule expression in the endothelial environment, in addition to influencing tumor vascularization and \(O_2\) delivery, can serve to generate signals for neighboring neural stem cells to proliferate and differentiate.\(^{67,77,78}\)

**Hypoxia and the Adhesive Glioma Cell Microenvironment**

Glioma cells bear many striking resemblances to neural stem cells.\(^{1,55}\) These tumors are thought to arise when neural stem cells at specific stages of development either undergo mutation or respond to environmental signals and become immortalized. Like all solid tumors, gliomas show large areas of hypoxia and it has been suggested that hypoxia favors the proliferation and stabilization of cancer stem cells, which can maintain tumor propagation.\(^{94,95,96,97}\) In glioma cells, the effects of "hypoxia," or low \(O_2\) in situ, are similar to that of a low \(O_2\) niche in normal neural stem cells.\(^{13,15}\)

HIFs are commonly expressed in gliomas and HIF1 blockade inhibited migration and invasion of malignant glioma.\(^{78}\) At least in part of the decreased migration was due to the downregulation of MMPs 2 and 9. MMP-13 is expressed specifically in glioma stem cells and its inhibition also results in decreased glioma stem cell migration and invasion.\(^{78}\) It has also been postulated that hypoxia increases the strength of cell-cell adhesion in glioma cells in culture.\(^{78}\)

Glioma stem cells have been shown to express the L1-CAM, which is implicated in increased invasiveness and metastasis.\(^{64}\) The expression of this adhesion molecule is correlated with poor outcome in patients.\(^{78}\) In vitro, decreasing the levels of L1 inhibited cell growth and neurosphere formation and decreased...
apoptosis.\textsuperscript{98} The Wnt signaling pathway, many downstream targets of which are adhesion molecules,\textsuperscript{53-55} has recently been shown to influence glioma tumorigenesis.\textsuperscript{101}

It is well established that the turnover of cell and matrix adhesions is essential to allow for epithelial mesenchymal transformation and support tumor migration and invasion into surrounding tissues.\textsuperscript{95,93,97,102} Targeting of these molecules has provided several avenues for developmental therapeutics,\textsuperscript{93,95,97,98} although the similarities between glioma cells and neural stem cells present difficulties in identifying targeted therapeutics that do not affect endogenous normal cells.\textsuperscript{93,95,97,98}

\textbf{HIFs and Hypoxia at the Organismal Level}

Genetic variations in HIFs have been seen in human and animal populations dwelling at high altitudes,\textsuperscript{103-108} in Drosophila that have been adapted to low $O_2$ levels,\textsuperscript{109,110} and in blind mole rats that live in hypoxic underground burrows.\textsuperscript{111,112} Thus modern organisms have adapted and are capable of adapting to alterations in the $O_2$ environment via HIF1 changes and alterations in genes involved in aerobic metabolism. It is not surprising that HIF mutations and HIF dysregulation are associated with multiple diseases.\textsuperscript{171} As outlined in the final sections, it is tempting to speculate that cell adhesion events influenced by increasing $O_2$ levels in the early earth atmosphere provided the critical control of cell and/or substrate adhesion that favored the emergence of multicellularity during evolutionary time.

\textbf{Organismal Evolution Occurred at Vastly Lower $O_2$ Levels than Those in the Present Atmosphere}

Biologists think about molecular, cellular and organismal evolution, but we seldom consider them in light of geologic and atmospheric change. Integration of this key element in the evolution of life on earth could encourage new ways of thinking about molecular and organismal evolution. Many of us were not trained in earth and atmospheric sciences or even cognizant that such issues might play a key role in the development of life on earth, notwithstanding perhaps metabolic biochemists, who necessarily think about aerobic and anaerobic cellular energy production (e.g., refs. 5, 113 and 114). Indeed this point serves as a reminder that mitochondria, the key players in aerobic metabolism, are central to many aspects of development and evolution (see below). The evolution of metabolic processes\textsuperscript{114,115} played a critical role and continues to do so, especially in high energy-requiring tissues such as the nervous, cardiac, and muscular systems.

When unicellular organisms first appeared on earth, more than three billion years ago,\textsuperscript{116} the Earth’s atmosphere and oceans were anoxic (see Fig. 1).\textsuperscript{117} Around 2.5–3 billion years ago, photosynthesizing cyanobacteria evolved and began producing $O_2$, which initially was absorbed by minerals but, exhausting this sink, eventually accumulated in the atmosphere, resulting in the Great Oxygenation Event.\textsuperscript{117} This event is believed to have caused the extinction of a large number of organisms that were poisoned by toxic $O_2$ levels.\textsuperscript{116} Eukaryotes and archaebacteria appeared about two billion years ago during the Neoproterozoic period as $O_2$ levels were rising, albeit still considerably lower than modern levels.\textsuperscript{117,118} Multicellular life probably arose 1–1.5 billion years ago and bilaterian animals were prevalent 500 million years ago just before the Cambrian explosion.\textsuperscript{116,119}

These topics are complex and have been given short shrift by my exclusive focus on $O_2$ levels. For example, a recent study indicated that as late as the Devonian era, $O_2$ levels were lower than the present and impacted the emergence of larger vertebrates with high metabolic rates, e.g., predatory fish.\textsuperscript{119} Ocean oxygenation appears to have acted (and appears to be doing so still) as an “evolutionary barrier” to the increasing size of predatory fish.\textsuperscript{119,120} In a similar vein, a recent paper postulates that variations in $O_2$ levels acted to select among arthropods during the Cambrian ages, with the most successful species being those that were tolerant of, and had the ability to respond adaptively to, changes in $O_2$ levels in seawater.\textsuperscript{121} Consideration of $O_2$ levels as drivers of evolution is still being intensely debated in studies of both modern evolution and marine ecology, increasingly so as ongoing data emerge from measurements of $O_3$ levels both in geological samples\textsuperscript{117-119,122} and in regions of present-day oceans that have become hypoxic due to the influences of climate change.\textsuperscript{123}

\textbf{Phylogeny of Some Oxygen Binding and Sensing Molecules}

It is of interest that homologs of the globins, molecules that bind $O_2$ via heme coordination, are found in modern prokaroytes.\textsuperscript{124-128} Perhaps such $O_2$-carrying proteins evolved to capture the very small amounts of molecular $O_2$ generated in early cellular life to be used for critical oxidation reactions such as tyrosine synthesis, hydroxylation of amino acids, and, in eukaryotes, sterol biosynthesis\textsuperscript{129} and the hydroxylation of HIF1$\alpha$.\textsuperscript{2} Yet a scan of the current GenBank database indicates that the hypoxia responsive protein, HIF1$\alpha$, is found only in eukaryotes. Homologs of the von Hippel-Lindau protein, which controls HIF degradation,\textsuperscript{3,4,118} are found in Volvox.\textsuperscript{130} Volvox is a spherical, sponge-like member of the green algae with a small number of specialized cell types, including germ cells.\textsuperscript{67,131} Of all extant organisms, Volvox is thought to be the best model system for understanding the emergence of the earliest multicellular organisms as they evolved from unicellular ancestors.\textsuperscript{67,131}

\textbf{Adhesion Molecules, Oxygen Levels, Prokaryotic Symbiosis and Multicellularity}

The last universal common ancestor for all extant life was a prokaryote that had ribosomes and a plethora of redox proteins, but no organelles such as chloroplasts or mitochondria.\textsuperscript{132-136} The appearance of modern cellular organelles, including mitochondria and chloroplasts, and possibly cilia and flagella, almost certainly came about as a result of the symbiotic fusion of individual free-living unicellular organisms early in cellular evolution.\textsuperscript{137-139} Although famously championed by Lynn Margulis,\textsuperscript{137} these ideas were suggested earlier by several authors.\textsuperscript{115} Creation of such symbionts would likely be facilitated by adhesive mechanisms between cells. Support for an adhesive mechanism for symbiosis
has been strengthened by the description of “predatory prokaryotes.” These bacteria adhere to the surfaces of other bacteria allowing their entry into the host cell. Clearly molecular mechanisms for adhesivity, possibly driven by the induction of gene expression by increasing O_2 during evolutionary time, might have facilitated such interactions.

Notably many transcription factors, in addition to HIF1α, are known to be sensitive to cellular redox state (which is influenced by, but not restricted to, alterations in O_2 availability). Targets of these factors would increase the number of molecular changes that might increase in responsiveness to altered O_2 concentrations in addition to the changes in adhesion molecules emphasized here.

The appearance of multicellularity also necessitated a mechanism for cell-to-cell and cell-to-substrate adhesion, which arose by diverse pathways in various lineages. As noted above, the volvocine algae are the extant organisms thought to most closely resemble the earliest multicellular organisms in evolution. Volvox has a cell adhesion molecule homologous to fasciclin I in Drosophila as well as a sulfated glycoprotein that appears at a distinct phase of embryogenesis when it might facilitate cell interactions, such as those that form cytoplasmic bridges in the Volvox embryo. A large number of extracellular matrix molecules in Volvox has been described biochemically and genomically. Several distinct types of ECM zones have been observed ultrastructurally. Moreover, ECM assembly has been shown to be inhibited by sulphydryl reagents, indicating sensitivity to the local redox environment. Thus molecules for cell and extracellular matrix adhesion exist and certainly play a critical role in this primitive multicellular organism.

Other organisms have been used in modern studies to mimic processes that supported the emergence of multicellularity. One is D. discoideum, a single-cell amoeboïd organism that forms aggregates and subsequent fruiting bodies upon starvation. The process of aggregation is guided by the differential distribution of several cell and extracellular adhesion molecules. Indeed, the formation of epithelial structures during morphogenesis requires homologs of both α- and β-catenin, suggesting that these junctional and signaling proteins predated the appearance of metazoans. In the case of multicellular colony formation in the single cell choanoflagellate, S. rosetta, colonies formed through cell division and not by the aggregation of individual cells. Nevertheless, in both model organisms and the evolution of multicellularity, post-division adhesive mechanisms must have been operative to hold cells together, whether by direct cell adhesion or encasement within a common extracellular matrix, similar to what occurs in Volvox. A recent study that selected for aggregates in S. cerevisiae over several generations demonstrated that multicellularity could occur rapidly and provided another example of post-division colony formation. Any of these model systems could be amenable to test whether exposure to O_2 levels comparable to those that occurred during the emergence of multicellular organisms influences the expression of any of the known adhesion molecules in the respective systems. Such experiments would also be a good test of the extent of generality in the evolution of cell adhesion mechanisms.

While it is not novel to suggest that various kinds of adhesion events were necessary for the emergence of multicellularity, the recent studies described here raise the intriguing possibility that the increasing (and at times decreasing) O_2 levels over evolutionary time might have exerted a strong selective influence on the evolution and production of adhesion molecule precursors.

![Figure 1. Key events in biological evolution superimposed on the range of oxygen levels in the earth’s atmosphere. The figure was modified from reference 117 with the author’s permission. More recent data indicate a transient rise in levels (of 0.04–0.1 pO_2) between 2.0 and 2.4 billion years before the present. The biological evolutionary times are compiled from several references and citations within. The geological times have wide variances and change as new data and models accumulate.](image-url)
Summary and Additional Considerations

Low atmospheric O₂ concentrations, at levels that are now commonly referred to as “hypoxia,” were the norm during the evolution of multicellular organisms. It is possible that alterations in O₂ levels drove the emergence and expression of molecules that supported adhesion thus facilitating prokaryotic symbiosis and multicellularity. HIF1 and its hydroxylation (PHD-family) enzymes probably did not evolve to deal with “hypoxia” but more likely acted as O₂ sensors (as has been suggested for PHD2,161) in order to regulate the response of suites of genes to the local and atmospheric O₂ environment over evolutionary time.17,162,163 Consideration of the evolutionary origins of O₂-responsive molecular mechanisms might greatly broaden our understanding of how such molecules function in development, especially in stem cell niches. For example, particular levels of O₂ could select for suites of adhesion molecules that would select among and guide the processes of cell proliferation, migration, and differentiation. For example, altered adhesive mechanisms brought about by differing O₂ levels may occur when stem cells leave the proliferative niche and migrate and differentiate in adjacent tissues. Such an idea is consistent with the observation that neural stem cells proliferate (and possibly switch between activity and quiescence) in the lowest O₂ levels (reviewed in refs. 12 and 13) and that increased O₂ levels support stem cell differentiation into particular lineages.13

In addition to understanding diseases and development of the central nervous system,13,164 these issues are also useful in considering the consequences of hypoxia in tissues and the progression of diseases in which cellular niches low in O₂ are thought to influence cell behavior, particularly cancer.165-168 and other diseases of proliferative misregulation, such as inflammation, fibrosis and sclerosis.169

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