Regulation of cell proliferation by hypoxia-inducible factors

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Hubbi ME, Semenza GL. Regulation of cell proliferation by hypoxia-inducible factors. Am J Physiol Cell Physiol 309: C775–C782, 2015; doi:10.1152/ajpcell.00279.2015.—Hypoxia is a physiological cue that impacts diverse physiological processes, including energy metabolism, autophagy, cell motility, angiogenesis, and erythropoiesis. One of the key cell-autonomous effects of hypoxia is as a modulator of cell proliferation. For most cell types, hypoxia induces decreased cell proliferation, since an increased number of cells, with a consequent increase in O2 demand, would only exacerbate hypoxic stress. However, certain cell populations maintain cell proliferation in the face of hypoxia. This is a common pathological hallmark of cancers, but can also serve a physiological function, as in the maintenance of stem cell populations that reside in a hypoxic niche. This review will discuss major molecular mechanisms by which hypoxia regulates cell proliferation in different cell populations, with a particular focus on the role of hypoxia-inducible factors.

Overview of the Cell Cycle

The initiation of DNA replication is a tightly controlled process, the first steps of which are origin recognition, licensing, and activation, which involve formation (during late G1 phase of the cell cycle) of a multiprotein prereplication complex (pre-RC) that marks all potential origins of replication (23). Pre-RC formation begins with binding of the origin recognition complex (ORC), which is composed of six subunits (Orc 1–6), to DNA at replication origins. The ORC subsequently binds Cdc6 and Cdt1, leading to recruitment of the minichromosome maintenance (MCM) DNA helicase (11, 83). The MCM helicase is a hexamer, consisting of the proteins MCM 2 to 7, which functions to unwind DNA during replication (56). However, Cdc6 and Cdt1 inhibit activation of the MCM helicase until the start of S phase (9), when Cdc6 is phosphorylated by S phase cyclin-dependent kinases (CDKs) (45, 77, 83), leading to its nuclear export and degradation. Inactivation of Cdc6 and Cdt1 allows Cdc7 to phosphorylate the MCM helicase at the start of S phase, leading to its activation (58, 90). Cdc45 subsequently binds to the helicase and recruits DNA polymerase-α and the GINS protein complex (Shf5, Psf1, Psf2, Psf3), which initiates DNA replication (42, 74).

The complex events of the cell cycle are coordinated through the action of the CDKs. The activity of the CDKs is regulated through binding to their cyclin partners, whose levels fluctuate according to particular phases of the cell cycle. Cyclin B binds to CDK1, which is activated during M phase of the cell cycle before G1. CDK2 binds to cyclin E at the G1/S phase transition and to cyclin A at S phase, followed by CDK4 and CDK6, which both bind to cyclin D (66). Of note, CDK1 alone is truly essential for the cell cycle and can drive cell division in the absence of the other three CDKs. Knockout mice lacking all other interphase CDKs develop until midgestation (84). Knockout mice lacking individual CDKs or cyclins generally develop relatively mild defects in particular cell populations. Collectively, the results from transgenic mouse models indicate that many proteins traditionally thought of as core components of the cell cycle machinery in fact play major roles only in response to particular stressors or in particular cell types.

In addition to fluctuations in the levels of their cyclin binding partners, CDK activity is regulated through levels of the p21 and p27 CDK inhibitors (32, 80). These proteins directly bind to and inhibit CDK activity in response to growth-arresting stimuli (32, 98). The expression of p21 and p27 is regulated transcriptionally through p53-mediated activation in response to stimuli such as DNA damage (17), whereas expression of p21 and p27 is repressed through the activity of the Myc transcription factor (89, 107). Myc levels and activity are abnormal in a variety of cancers where it drives proliferation (13). This is accomplished through repression of p21 and p27 (89, 107), transcriptional activation of genes that promote growth and proliferation (13), and a direct effect on the DNA replication machinery (15).

Hypoxia-Inducible Factors: Structure, Function, and Regulation

Oxygen-dependent regulation of hypoxia-inducible factor-1 transcriptional activity. Hypoxia-inducible factors (HIFs) were first identified in studies of hypoxia-induced erythropoiesis.
HIF-1 was shown to bind to, and activate transcription of, the human EPO gene encoding erythropoietin, which is a key hormone regulator of red blood cell production (87). Purification by DNA affinity chromatography revealed that HIF-1 is a heterodimer, which is composed of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit (100). Under normal conditions, HIF-1α is subject to oxygen-dependent hydroxylation at proline residues 564 and/or 402 by the prolyl hydroxylase domain proteins PHD1-3, which leads to binding of the von Hippel-Lindau protein (VHL) and an associated ubiquitin-protein ligase complex (21, 43, 44, 108). This leads to ubiquitination and proteasomal degradation of HIF-1α (Table 1), giving it a half-life of only a few minutes when exposed to 20% O2 under standard tissue culture conditions of 95% air and 5% CO2 (81). However, the hydroxylation reaction is inhibited under hypoxic conditions, HIF-1α is stabilized, and the HIF-1 heterodimer activates the transcription of hundreds of target genes to coordinate adaptive responses to hypoxia (81). Of note, since the proline hydroxylase enzymes contain Fe (II) in their catalytic centers and use α-ketoglutarate as a substrate, HIF-1α can also be stabilized pharmacologically through the addition of iron chelators such as desferrioxamine or competitive antagonists of α-ketoglutarate such as dimethylxalylglycine (21). Multiple proteins have been identified that promote oxygen-dependent degradation of HIF-1α through protein-protein (cooperative) interactions (Table 1): O9α and RUNX3 each binds to both HIF-1α and PHD2 to increase the efficiency of hydroxylation (3, 57); MCM7 and SSAT2 each binds to both HIF-1α and VHL to increase the efficiency of the ubiquitination reaction (1, 38); LIMD1 and RHOB1B3 dimerize and form a complex with HIF-1α, PHD2, and VHL to promote both hydroxylation and ubiquitination (109).

Oxygen-independent regulation of HIF-1 activity. Our understanding of the mechanism of HIF-1 regulation has broadened considerably in recent years, with the discovery of multiple protein degradation pathways that are independent of the proline hydroxylation reaction, the VHL ligase complex, or the proteasome altogether (Table 1). RACK1 binds to HIF-1α independent of hydroxylation and mediates ubiquitination and proteasomal degradation of HIF-1α (60). This pathway is regulated by calcineurin (61), which serves to couple HIF-1α activity to calcium signaling. SSAT1 binds to both HIF-1α and RACK1 to promote ubiquitination and degradation of HIF-1α (2). Other oxygen-independent, proteasome-dependent degradation pathways have been described, which involve CHIP and HSP70 (63), hypoxia-associated factor (HAF) (52), JNK1 (110), BHLHE41 (also known as SHARP1) (68), and SIRT7 (36). Recent work has shown that HIF-1α is also subject to chaperone-mediated autophagy (CMA) (22, 35). CMA is a pathway for lysosome-dependent degradation, in which HSC70 binds to particular protein sequences and mediates complex formation with LAMP-2A. Binding of substrates to LAMP-2A monomers stimulates the assembly of multimers at the lysosomal membrane, leading to translocation into the lysosome and degradation of CMA substrates (48). There are additional mechanisms for regulation of HIF-1α stability and regulation of HIF-1α transactivation domain function (81), which are not discussed here.

HIF-2α and HIF-3α. The discovery of HIF-1α was followed by the discovery of HIF-2α, which binds to the common HIF-1β subunit to form the HIF-2 heterodimer (97). In certain contexts, such as hypoxia-induced angiogenesis, HIF-1 and HIF-2 have similar functions. However, in contrast to the ubiquitous expression of HIF-1α, HIF-2α expression is restricted to certain cell populations and can mediate distinct and even opposing functions (81). A third protein, HIF-3α, was originally noted to function as an inhibitor of HIF-1 through sequestration of HIF-1β. HIF-3α is highly expressed in the corneal epithelium of the eye, and this effect is critical in maintaining the avascular phenotype in the cornea (65). However, recent data demonstrate that HIF-3 also functions as a transcriptional activator in certain contexts (112).

Table 1. Regulation of HIF-1α protein stability

<table>
<thead>
<tr>
<th>Site*</th>
<th>O2-Dep*</th>
<th>Effectors(s)</th>
<th>Regulator(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>+</td>
<td>PHD2, VHL</td>
<td>O9α, RUNX3, MCM7, SSAT2, LIMD1, RHOB1B3</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>RACK1</td>
<td>calcineurin, SSAT1</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>CHIP, HSP70</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>HAF</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>BHLHE41</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>p53, MDM2</td>
<td>ND</td>
</tr>
<tr>
<td>L</td>
<td>-</td>
<td>HSC70, LAMP2A</td>
<td>CDK1, CDK2</td>
</tr>
</tbody>
</table>

*Degradation in proteasome (P) or lysosome (L). *Degradation is oxygen dependent (+) or independent (−). ND, not determined.

Regulation of Cell Proliferation by HIFs

Hypoxia has been shown to inhibit cell proliferation in numerous cell types, including embryonic fibroblasts (24, 26), embryonic stem (ES) cells (9), lymphocytes (26), keratinocytes (6), hematopoietic stem cells (18, 19, 96), and a wide variety of cancer cell lines (27, 37, 54). Bromodeoxyuridine incorporation into DNA, a measure of DNA replication, was decreased when ES cells from wild-type mice were exposed to hypoxia, but not when ES cells from HIF-1α-deficient mice were exposed to the same conditions (9). Similarly, fibroblasts or B lymphocytes demonstrated reduced proliferation and DNA incorporation when exposed to hypoxic conditions, but not when the same cell types from HIF-1α-deficient mice were exposed to hypoxia (26). Furthermore, overexpression of HIF-1α alone is sufficient to induce cell cycle arrest (30, 37). The mechanisms by which HIF-1α mediates cell cycle arrest have been a subject of intense study, with both transcriptional mechanisms, centered on the CDK inhibitors p21 and p27, and nontranscriptional mechanisms, centered on direct effects of HIF-1α on the pre-RC, being implicated (Table 2).

HIF-1α-dependent transcriptional mechanisms. Myc, like HIF-1, has pleiotropic effects on metabolism, proliferation, and cell growth. Studies of hypoxia-induced cell cycle arrest have demonstrated an important role for Myc. HIF-1α counteracts the effects of Myc on proliferation by several mechanisms, although the two can have synergistic effects on genes related to angiogenesis and metabolism (13). Koshiji and colleagues first noted an interaction between HIF-1α and Myc (54). HIF-1α overexpression led to displacement of Myc from its DNA binding sites, leading to derepression of the genes encoding p21 and p27. Other studies have confirmed that hypoxia is a stimulus for HIF-1α-dependent induction of p21 and p27 gene.
transcription in other cell types, including lymphocytes (26), fibroblasts (24), and cancer cells (27). Fibrosarcoma cells with targeted deletion of VHL (and therefore constitutive activation of HIFs) have increased levels of p21 and p27 (64). In addition to direct binding to Myc, the consensus HIF-1 binding site partially overlaps with the Myc consensus site, suggesting a second mechanism by which HIF-1α can displace Myc from DNA binding sites. Third, HIF-1 activates expression of the Myc antagonist Mxi-1 (12, 111). Finally, hypoxia is associated with a decreased expression of Myc protein, which may be independent of HIF-1 (12, 104, 111). Thus, hypoxia leads to inhibition of Myc activity by multiple mechanisms.

A second mechanism involves the Jumonji C domain-containing family of histone demethylases. The activity of these proteins is linked to hypoxia through HIF-mediated transcriptional activation (5, 79, 106) and because like the PHD proteins, they use O2 and α-ketoglutarate as cosubstrates and are therefore sensitive to cellular O2 levels. These proteins can regulate HIF activity (62, 101), but may also regulate expression of genes involved in cell proliferation independently of HIFs (99).

HIF-1α-dependent nontranscriptional mechanisms. HIF-1α has a direct effect on the DNA replication machinery as well. Recently, both HIF-1α and HIF-2α were shown to bind directly to multiple components of the MCM DNA helicase (38) and the helicase loading factor Cdc6 (37). HIF-1α strengthened the physical interaction between Cdc6 and the MCM proteins. This was associated with an increase in Cdc6 and MCM chromatin association, but decreased recruitment of the activating kinase Cdc7 and downstream proteins, ultimately leading to decreased DNA replication. By this mechanism, HIF-1α maintained the MCM helicase in an inactive state. Importantly, DNA replication was inhibited by HIF-1α point mutants lacking DNA binding activity and by deletion mutants lacking the transactivation domain, and in cells with stable knockdown of HIF-1β (37). These results demonstrate that HIF-1α, through direct (negative) effects on the DNA replication machinery, inhibits cell cycle progression independent of its established role as a regulator of gene transcription. In this way it plays an analogous role to Myc, which also has a nontranscriptional role as a direct (positive) regulator of the DNA replication machinery (15).

Oxygen-dependent but HIF-independent regulation of proliferation. Although a major role of the PHD proteins is to hydroxylate HIF-1α and mark it for proteasomal degradation, they have other targets relevant to cell proliferation as well. PHD1 regulates cyclin D1 in breast cancer cells (113). When the Phd1 gene is inactivated, cyclin D1 levels and mammary gland proliferation are decreased. In addition, PHD1 hydroxylates CEP192, which is a large protein that serves as a scaffold in human centromeres (70). PHD1-mediated hydroxylation of CEP192 marks it for ubiquitination by SKP2, which is followed by proteasomal degradation. In response to hypoxia, the resultant inhibition of PHD1 activity promotes cell cycle arrest through this HIF-independent mechanism.

Mechanisms to Maintain Cellular Proliferation Under Hypoxia

MCM proteins. The identification of a direct effect of HIF-1α on DNA replication raises the question of how cells proliferate at all under hypoxic conditions. One potential answer lies in the observation that multiple MCM proteins, including MCM2, MCM3, MCM5, and MCM7, were shown to bind to HIF-1α and to inhibit HIF-1 activity (38). MCM7 binds directly to both HIF-1α and to components of the VHL ubiquitin ligase complex, thereby enhancing oxygen-dependent degradation of HIF-1α. It has been known for many years that MCM protein levels were regulated by growth-stimulatory signals. However, even under basal conditions, the MCM proteins are present in vast excess to the number of potential sites of DNA replication, and the majority of MCM proteins do not colocalize with sites of DNA synthesis (40). Prior studies noted that cell proliferation was unaffected under basal conditions by a >90% decrease in levels of various MCM proteins. These observations were known as the MCM paradox (14). However, even a modest decrease in MCM7 levels led to an increase in HIF-1α levels in response to hypoxia with a pronounced effect on cell proliferation (38). Interestingly, other work has shown that, under conditions of replication stress, MCM proteins are essential to license normally latent origins of replication (25, 41, 105). Thus, increased levels of MCM proteins in response to growth signals may serve to promote cell proliferation under hypoxic stress through the degradation of HIF-1α.

Myc. However, even in the presence of elevated HIF-1α levels, many cancer cells maintain proliferation. In general, human cancers express high levels of HIF-1α (115) due not only to the hypoxic tumor microenvironment but also because of dysregulated signaling pathways, which confer a survival advantage through the upregulation of HIF target genes. One potential explanation lies in the relative balance between HIF-1α and HIF-2α. In certain conditions, HIF-2α was postulated to enhance Myc function, whereas HIF-1α inhibited Myc activity (27). Most human clear cell renal carcinomas (ccRCCs) have VHL loss of function, leading to increased HIF signaling. ccRCCs expressing only HIF-2α have elevated Myc activity and increased proliferation in contrast to ccRCCs expressing both HIF-1α and HIF-2α (28). However, forced expression of HIF-2α in other cell lines led to cell cycle arrest (30), indicating that the role of HIF-2α may be cell type specific.

CDKs and CMA. An alternative explanation lies in the regulation of HIF-1α and HIF-2α during specific phases of the cell cycle, which is accomplished by the CDKs (34, 102). CDK1 activity is highest before the G1 phase of the cell cycle, whereas DNA replication proceeds during S phase when CDK2 activity is highest. HIF-1α protein expression is promoted by CDK1 activity (34, 102). In contrast, CDK2 activity, which is regulated by cyclin E at the G1/S phase transition and by cyclin A during S phase, leads to degradation of HIF-1α and HIF-2α (34). The activity of both CDK1 and CDK2 on HIF-1α

Table 2. Oxygen-dependent regulation of cell proliferation

<table>
<thead>
<tr>
<th>HIF-1α*</th>
<th>HIF-1β*</th>
<th>Transcription*</th>
<th>Target(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>KDM3A, KDM4B, KDM4C genes</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>MYC protein</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>MCM, Cdc6 proteins</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PHD1 protein</td>
</tr>
</tbody>
</table>

*Hypoxia-inducible factor (HIF)-1α, HIF-1β, or transcription are required (+) or not required (−).
is blocked by inhibitors of lysosomal function, indicating that CDKs use CMA to selectively degrade HIF-1α before the onset of DNA replication. The data also suggest the existence of a positive feedback loop promoting hypoxic cell arrest, since HIF-1-mediated induction of p21 and p27 expression leads to CDK inhibition and further induction of HIF-1α. p21-deficient cells have impaired induction of HIF-1α protein expression in response to hypoxia, and forced overexpression of either p21 or p27 promotes HIF-1α protein stability in a lysosome-dependent manner.

The importance of CMA-mediated regulation of HIF-1α in hypoxic cell proliferation was clearly demonstrated in Hep3B human hepatocellular carcinoma cells, which express both HIF-1α and HIF-2α, and are resistant to the effects of hypoxia on cell proliferation. However, induction of HIF-1α and HIF-2α by lysosome inhibitors led to cell cycle arrest, which was abrogated by concurrent knockdown of both HIF-1α and HIF-2α, but not either individually (34). Thus, rather than HIF-1α and HIF-2α carrying out opposing functions on cell proliferation, in this context both proteins promote cell cycle arrest, which is overcome by autophagic degradation before DNA replication. It is worth noting that CMA is a pathway that is increased in several human cancers and essential for their growth (53), and that pharmacological inhibition of autophagy leads to a HIF-dependent cell cycle arrest (34, 39, 59). Also of note are data demonstrating that, despite the negative effect of CDK2/cyclin E on HIF-1α protein levels, in certain cell types cyclin E expression was associated with an increase, rather than a decrease, in HIF-1 transcriptional activity (34, 88). Thus, under certain circumstances, cells can dissociate the nontranscriptional and antiproliferative effect of the HIF-1α monomer on DNA replication from the transcriptional effects of the HIF-1 heterodimer, which may indirectly promote growth through adaptive effects on angiogenesis and metabolism.

**HIF-1α vs. Myc.** Finally, one can also speculate that the balance between Myc and HIF-1α plays a role in determining the sensitivity of the cell cycle to hypoxia. Whereas under physiological conditions the balance may be such that HIF-1α can overwhelm the effect of Myc on both gene transcription and DNA replication, deranged expression of oncogenic Myc may overwhelm the effect of HIF-1α on cellular proliferation at multiple levels, including its direct effects on the DNA helicase (15), the repression of CDK inhibitors (54, 89), and the promotion of autophagy (71). Evidence exists that, in cancer cells with high levels of both Myc and HIF-1α, the two factors collaborate to promote transcriptional activation of target genes encoding glycolytic enzymes (49). Thus, by neutralizing the effect of HIF-1α on the cell cycle, while promoting HIF-1α-mediated transcription, Myc enlists HIF-1 to synergistically promote tumor growth.

**Regulation of Stem Cell Maintenance By Hypoxia**

Characterization of the stem cell microenvironment, or niche, is a prominent goal of stem cell research. A better understanding of the stem cell niche might be exploited for therapeutic purposes. In several different contexts, hypoxia has been identified as an important feature of the stem cell microenvironment. Previous work demonstrated that HIF-1α directly binds to the Notch intracellular signaling domain and is recruited to Notch-regulated genes under hypoxic conditions to promote an undifferentiated state of stem-progenitor cells (29). Hypoxia is therefore critical for the maintenance of stem cell populations and in some instances can also promote proliferation. We discuss two illustrative examples, hematopoietic stem cells (HSCs) and neural stem cells (NSCs).

**HSCs.** A variety of experimental approaches have established that HSCs reside in a hypoxic bone marrow niche. These techniques have included direct measurements of O2 concentration in living animals with oxygen electrodes (10) or two-photon fluorescence lifetime microscopy (93) and through staining with surrogate hypoxia markers such as pimidozone (75) or measurement of HIF-1α protein or mRNAs encoded by HIF target genes (92, 96). These studies demonstrated that hypoxia promotes quiescence of HSCs (19, 33).

Several lines of evidence specifically implicate HIF-1α as a regulator of cell cycle progression in HSCs. HIF-1α-deficient HSCs lose their cell cycle quiescence, whereas HSCs with loss of VHL (and therefore increased expression of HIF-1α) have increased cell cycle quiescence and impaired transplantation capacity (96). Deficiency of Cited2, a negative regulator of HIF-1 transcriptional activity, was associated with increased cycling of HSCs, which was rescued by HIF-1α inactivation (16). HIF-1 activity has also been shown to regulate the metabolic properties of HSCs, which use glycolysis rather than oxidative phosphorylation for energy (92, 95). This shift in cellular metabolism is proposed to protect cells from oxidative damage to DNA and other macromolecules.

Interestingly, in situ tissue analysis revealed that HSCs have increased HIF-1α expression, regardless of localization in the bone marrow or adjacency to vascular structures (73). This finding suggests that HIF-1α expression is induced in these cells not only through localization in relatively hypoxic areas of the bone marrow but also through O2-independent mechanisms. Consistent with this hypothesis, various niche growth factors have been shown to induce expression of HIF-1α, including thrombopoietin (51) and stem cell factor (also known as kit ligand) (76).

**NSCs.** NSCs have the ability to proliferate and differentiate into astrocytes, oligodendrocytes, and neurons. The main NSC niches are located in the hippocampus and the subventricular zone. Morrison and colleagues observed an increase in the proliferation of rat NSCs under 5% O2 (69), and similar results were observed by Studer and colleagues using mesencephalic precursors (94). Culturing NSCs in 20% O2 leads to an arrest in proliferation and enhanced glial differentiation, an effect attributed to suppression of BMP-dependent SMAD activation by hypoxia (78). Similar data demonstrating that hypoxia promotes the proliferation of human NSCs have also been published (85).

The mechanisms by which hypoxia promotes, rather than inhibits, NSC proliferation are multifactorial. This effect is partially mediated by the release of growth factors. HIF-1α mediates induction of vascular endothelial growth factor (31), which stimulates neurogenesis (46). Erythropoietin, another HIF-1 target gene product, also promotes proliferation of NSCs (91) through effects on NF-κB signaling. An additional major effect of HIF-1α that was specifically demonstrated in NSCs is an increase in WNT/β-catenin signaling (7, 67). HIF-1α promotes expression of the β-catenin effectors LEF-1 and TCF-1. Consequently, HIF-1α deletion led to impaired Wnt/β-catenin signaling.
activity in the hippocampus with impaired NSC proliferation (67). Interestingly, in HCT116 colorectal cancer cells, the opposite effect of HIF-1α on β-catenin signaling was observed, indicating that the effect of HIF-1α on different signaling pathways is distinct in stem cell populations and differentiated cells or neoplastic cells.

Hypoxia-Induced Proliferation of Cardiomyocytes and Pulmonary Arterial Smooth Muscle Cells

Cancer cells are not the only cells that continue to proliferate in response to hypoxia. In some cell types, hypoxia actually induces increased cell proliferation. Here we describe recent findings regarding regulation of proliferation in cardiomyocytes and pulmonary arterial smooth muscle cells (PASMCs).

Cardiomyocytes. In mammals, cardiomyocytes generally exit the cell cycle soon after birth. Although there is evidence for a preadolescent burst of cardiomyocyte proliferation driven by an increase in thyroid hormone production (72), studies on cardiomyocyte renewal in humans estimate the rate in adulthood to be roughly 1%, with an even greater decrease in old age (4). Several factors are responsible for this loss of proliferative capacity. Matrix rigidity has an important role in regulating differentiation and proliferation of cardiac progenitor cells (55) as in other cell types (20). A physiological increase in mechanical load postnatally is also posited to contribute to cell cycle arrest (8, 103).

Puentes and colleagues hypothesized that the hypoxic state of cardiomyocytes prenatally is a critical factor in promoting proliferation (82). The arterial Po2 rises dramatically after birth along with a shift in cardiomyocyte energy utilization from glycolysis in the embryo to oxidative phosphorylation in adults. They demonstrated that the increase in free radicals resulting from this metabolic shift leads to oxidative damage and a DNA damage response, which leads to cell cycle arrest. Further support for this hypothesis comes from studies of transgenic mice expressing the oxygen-dependent degradation domain of HIF-1α under the control of a cardiomyocyte-specific α-myosin heavy chain promoter or a more ubiquitous promoter, allowing hypoxic cells to be identified on an individual basis (50). With the use of this model, hypoxic cardiomyocytes were shown to have characteristics of proliferating cells.

Our knowledge of species that undergo heart regeneration in adulthood lends some support to the idea of hypoxia as a proliferative signal in the heart. For example, zebrafish are relatively hypoxicemic compared with mammals due to both the aquatic environment and their two-chamber circulatory system, which results in mixing of arterial and venous blood. Recent work has implicated hypoxia as a positive regulator of myocardial regeneration in zebrafish (47). The mechanism by which cardiomyocytes increase proliferation despite induction of HIF-1α during hypoxia is an interesting topic for further study.

PASMCs. Hypoxia is known to induce HIF-1α-dependent changes in gene expression in PASMCs that lead to cell hypertrophy, depolarization, and proliferation, which are critical in the pathogenesis of pulmonary hypertension (86). Most recently, hypoxia was shown to induce HIF-dependent transcriptional activation of the Slc39a12 gene, which encodes the zinc transporter ZIP12, leading to increased levels of intracellular labile zinc, which stimulate PASMC proliferation (114). The Fisher 344 rat strain, which is resistant to the development of hypoxia-induced pulmonary hypertension, was found to harbor a loss-of-function mutation in the Slc39a12 gene (114).

Conclusion

Cell proliferation is coupled to various physiological cues, one of the most important being O2 availability. This review has highlighted the important role of HIFs in mediating cell cycle arrest through both nontranscriptional effects on the MCM helicase during DNA replication and through transcriptional activation of genes encoding the CDK inhibitors p21 and p27. Cancer cells override the effect of HIFs on the cell cycle, through CDK2-dependent autophagic degradation of HIFs at the onset of DNA replication, through MCM-mediated decrease in HIFα levels in response to proliferative signals, and through an increase in DNA damage. Hypoxic cardiomyocytes may promote stem cell self-renewal through secretion of growth factors or through regulation of Notch or β-catenin signaling pathways, and through the avoidance of DNA damage, which can occur due to increased reactive oxygen species at high O2 levels and lead to cell cycle arrest. Finally, hypoxia induces cardiomyocyte and PASMC proliferation in a HIF-dependent manner, underscoring that the adaptive responses to hypoxia that are mediated by HIFs are cell type- and context-dependent.

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