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Review

Molecular mechanisms underlying hypoxia-induced inhibition of cell differentiation in myogenic cells

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ABSTRACT

Oxygen plays an important role in regulating cell differentiation in a variety of cell types. Satellite cells, which are the primary stem cells in adult skeletal muscle, are wedged between the plasma membrane of the myofiber and the basement membrane, and they contribute to muscle growth, maintenance, repair, and regeneration. Several lines of evidence suggest that satellite cells can adapt to changes in O_2 availability via molecular signaling mechanisms that convert this information into appropriate physiological responses. Understanding how cells adapt to altered physiological conditions will provide a valuable insight into the underlying mechanisms that regulate cellular homeostasis. Here we will summarize the current knowledge about the molecular mechanisms responsible for myogenic differentiation under hypoxia. We will focus especially on how hypoxia inhibits myogenic differentiation.

KEYWORDS: hypoxia-inducible factors, MAPKs, myogenic regulatory factors, Notch, PI3K/Akt

INTRODUCTION

Hypoxia is a relative term, and is most usefully defined as a reduction in the amount of O_2 available to a cell, tissue, or organism [1]. Tissue O_2 levels are usually much lower than those in the ambient air. For instance, O_2 levels in ambient air, arterial blood, and normal tissues are approximately 20.9%, 12%, and 0.65-6.5%, respectively [2]. The mean tissue level of O_2 is about 3%, with considerable local and regional variation [3]. In skeletal muscle, the mean O_2 levels is 1.7%-2.4% with the heterogeneity in O_2 concentration within skeletal muscle (1.8%-10.5%) [4]. Although hypoxia is widely linked to pathologies, such as cancer [5], it also may act as a regulator of physiological process, such as differentiation in satellite cells [6].

Satellite cells are defined anatomically, by their position beneath the basal lamina and adhered to myofibers [7]. Satellite cells, traditionally considered as a population of skeletal muscle-specific committed progenitors, play a crucial role in the postnatal maintenance, growth, repair and regeneration [8]. Under normal physiological conditions, these cells remain in a quiescent and undifferentiated state [8, 9]. However, when skeletal muscle is damaged by unaccustomed exercise or mechanical trauma, quiescent satellite cells are activated to proliferate, differentiate, and fuse with the existing myofibers or fuse to form new myofibers to regenerate skeletal muscle tissues [8, 9]. A connection between O_2 levels and satellite cells was first reported in the 2000s, when Chakravarthy *et al.* [10] demonstrated that hypoxia enhanced their proliferation and differentiation ability *in vitro*. Since this report, molecular mechanisms by which O_2 levels modulate the ability of satellite cells to differentiate into myofibers have been elucidated by focusing on multiple signaling pathways, which regulate myogenic differentiation. In this review, we will summarize the current knowledge about the molecular mechanisms responsible for myogenic differentiation under hypoxia. We will focus especially on how hypoxia inhibits myogenic differentiation.

Oxygen level in cell culture

Skeletal muscle cell culture as a research tool has been extensively utilized to facilitate understanding the mechanisms regulating myoblast proliferation and differentiation. Although O_2 concentration is an important signal for virtually all cellular processes [3], the atmospheric conditions used in traditional myogenic cells culture *in vitro* have been largely disregarded [11]. Since the 1930s, cell culture experiments have used a combination of 5% carbon dioxide and 95% air [12] to help maintain physiological pH. Thus, cells have been exposed to O_2 tensions close to 20.9%. Recently, it has been shown that maintaining cells under more physiological atmospheric conditions have many potential effects on myogenic cells [11]. Since the 2000s, researchers have investigated the effects of hypoxia on cell differentiation using myogenic cells. The O_2 levels used in cell culture experiment have been reported to range from 0.01 to 10% (Table 1) [6, 10, 13-26].

The difference between satellite cells and immortalized myogenic cells in response to hypoxia

The establishment of permanent myogenic cell lines including rat L6 and L8, mouse C2 and C2C12, MM14 have permitted extensive biochemical and molecular analyses, although these models do not always fully mimic the biology of satellite cells [27]. Indeed, hypoxia (3%) enhanced differentiation in satellite cells [10] but repressed it in immortalized myogenic cells [23]. Obviously, there are substantial differences in response to hypoxia between satellite cells and immortalized myogenic cells. However, as mentioned below, it appears that immortalized cells also had adaptability to chronic hypoxia using C2C12 cells [14].

Effects of hypoxia on cell differentiation

The effect of hypoxia on differentiation in satellite cells and immortalized myogenic cells is summarized

Table 1. Effects of hypoxia on myogenic differentiation.

Model	Hypoxia	Differentiation	Reference
Rat satellite cells	3%	↑	Chakravarthy *et al.*, 2001
C2C12, L6E9	1%	↓	Di Carlo *et al.*, 2004
C2C12, Mouse satellite cells	0.01-2%	↓	Yun *et al.*, 2005
C2C12, Mouse satellite cells	1%	↓	Gustafsson *et al.*, 2005
C2C12	6%	↑	Hansen *et al.*, 2007
Bovine satellite cells	1%	↑	Kook *et al.*, 2008
Mouse satellite cells	1%	no change	Ciavarra *et al.*, 2010
Human satelllite cells, L6	1%	↓	Launay *et al.*, 2010
C2C12	1%	↓	Ren *et al.*, 2010
C2C12, G8	1%	↓	Itoigawa *et al.*, 2010
C2C12	1%	↓	Augustin *et al.*, 2010
Human satelllite cells	2%	↑	Koning *et al.*, 2011
C2C12	3%	↓	Li *et al.*, 2011
C2C12	2-10%	↓(2%)	Sato *et al.*, 2011
C2C12, Mouse satellite cells	1%	↓	Majmundar *et al.*, 2012
Mouse satellite cells	1%	↓	Liu *et al.*, 2012

Promote (↑); Inhibit (↓)

in Table 1. Hypoxia (2-3%) promoted myotube formation in rat and human satellite cells [10, 22]. In contrast, hypoxia (0.5-1%) repressed myotube generation in mouse and human satellite cells [6, 20, 24, 26], although some studies showed hypoxia (1%) promoted myotube formation in bovine satellite cells [16] or failed to affect myogenic differentiation in mouse satellite cells [18]. In immortalized myogenic cells, hypoxia (6%) promoted myotube formation, whereas, hypoxia (0.01-3%) repressed myotube generation, indicating that a threshold for inhibition of myogenic differentiation may exist between 3% and 6% O_2. Yun *et al.* [14] examined that the effects of three different levels of hypoxia: physiological hypoxia at 2% O_2, pathological hypoxia at 0.5% O_2, and extreme pathological hypoxia at 0.01% O_2. Myogenic differentiation was considerably inhibited at less than 2% O_2 and completely suppressed at 0.01% O_2. Intriguingly, this inhibition could be reversible [14]. C2C12 cells were differentiated at different O_2 levels (2, 0.5, or 0.01%) for 1 to 3 days, followed by 3 days at 21% O_2. Myogenic differentiation could recover from physiological (2% O_2) and pathological (0.5% O_2) hypoxia, but only to some extent from extreme pathological (0.01% O_2) hypoxia. These results suggest that myogenic differentiation is not irreversibly inhibited by hypoxia, as myoblasts retain their capacity to differentiate when oxygen levels are restored. Furthermore, myogenic differentiation could occur when chronically exposed to hypoxia [14]. When C2C12 cells cultured at 0.5% O_2 were forced to differentiate, myogenic differentiation was suppressed at 0.5% O_2 on day 3 but extensive myogenesis occurred by day 6 and progressed further by day 12. These results indicate that immortalized myogenic cells also have adaptability to chronic hypoxia.

Hypoxia-inducible factor-1α (HIF-1α)

HIF-1 plays a central role in the transcriptional response to changes in O_2 availability [28]. The hypoxia-inducible factor-1 (HIF-1) is a heterodimer that consists of a constitutively expressed HIF-1β/ARNT (aryl hydrocarbon receptor nuclear translocator) subunit and an O_2-regulated HIF-1α subunit [29]. Under normoxia, HIF-1α is hydroxylated by prolyl hydroxylase domain-containing proteins 1, 2, and 3 (PHD1, PHD2, and PHD3) [30]. Once hydroxylated, von Hippel-Lindau protein (pVHL) binds to HIF-1α in a complex with multi-component ubiquitin ligase (pVHL-Elongin BC-Cul2-Rbx), thereby marking HIF-1α for degradation by the 26S proteasome [31]. Under hypoxia, hydroxylation of HIF-1α becomes reduced, allowing HIF-1α to escape recognition by the pVHL ubiquitin-ligase complex, and leading to HIF-1α stabilization [31]. Accordingly, HIF-1α accumulates, dimerizes with an HIF-1β/ARNT, translocates to the nucleus, and transcriptionally activates many target genes involved in erythropoiesis, iron metabolism, angiogenesis, glucose metabolism, cell proliferation/ survival, and apoptosis [32].

There remains some controversy as to whether HIF-1α plays a role in regulating myogenic differentiation. Yun *et al.* [14] examined the role of HIF-1 in the myogenic differentiation using an O_2-insensitive and constitutively active form of HIF-1α: ΔODD (deletion of the oxygen-dependent degradation domain) and the double proline mutant (P402A/P564G). O_2-insensitive and constitutively active HIF-1α could not accelerate myogenic differentiation at 21% and 0.5% O_2. Recently, shRNA-mediated gene silencing of HIF-1α promoted myotube formation at 21% O_2 and could moderately restore hypoxia-induced differentiation deficiency [24]. These results suggest that excess activity of HIF-1α may not be required for myogenic differentiation; HIF-1α may play only a relatively modest role in regulating cell differentiation in myogenic cells.

Myogenic regulatory factors (MRFs)

The MRFs, a group of basic helix-loop-helix (bHLH) transcription factors consisting of MyoD, myogenin, Myf-5 and MRF4, play important regulatory functions in the skeletal-muscle differentiation program [33]. MRF4 and Myf5 act in a genetic pathway upstream of MyoD to direct embryonic cells into the myogenic lineage [34]. Myogenin, which acts in a genetic pathway downstream of MyoD and Myf-5 [35], directly controls the differentiation process, including the formation of myotubes [36, 37]. MRFs transcriptionally and epigenetically determine the myogenic capacity of satellite/stem cells [38].

Liu *et al.* [26] examined whether hypoxia (1%) affects the proportion of myoblasts in the quiescent state using the satellite cell self-renewal marker, Pax7 (paired box protein 7) and satellite cell activation and myogenic differentiation, MyoD. It has already been established that $Pax7^{+}MyoD^{-}$, $Pax7^{+}MyoD^{+}$ and $Pax7^{-}MyoD^{+}$ expression profiles mark subpopulations of self-renewed (quiescent), proliferating, and differentiating myoblasts, respectively [39, 40, 41]. $Pax7^{+}MyoD^{-}$ quiescent (self-renewed) cells were dramatically increased by hypoxia, whereas $Pax7^{-}MyoD^{+}$ differentiating cells were decreased by hypoxia. $Pax7^{+}MyoD^{+}$ proliferating cells were marginally decreased by hypoxia. At the same time, the expression of myogenin was also repressed by hypoxia. This result suggests that Pax7 plays an important role in allowing activated satellite cells to reacquire a quiescent, undifferentiated state and inhibiting myogenic differentiation under hypoxia. Indeed, overexpression of Pax7 downregulated MyoD, promoted cell cycle exit, prevented myogenin induction, and blocked differentiation [40].

Immortalized cell lines, hypoxia (less than 3%) downregulated MyoD and myogenin [13, 14, 19, 20, 23-25]. The expression of both the MyoD and myogenin appeared to be repressed in an O_2-dependent manner [14]. Physiological (2%) and pathological (0.5%) hypoxia considerably repressed and extreme pathological (0.01%) hypoxia completely suppressed the expression of both the MyoD and myogenin [14]. Several possibilities can be envisaged. Yun *et al.* [14] examined the stability of MyoD mRNA at 0.5% O_2, using actinomycin D. Hypoxia failed to change the stabilities of MyoD mRNA. Instead, the transcription activity of *MyoD* promoter decreased probably through decreased histone acetylation of the *MyoD* promoter [14]. This suggests that hypoxia may induce chromatin modifications and regulate transcription of *MyoD* gene. Di Carlo *et al.* [13] examined whether hypoxia affects MyoD protein stability by increasing its turnover, using cycloheximide. Hypoxia (1%) decreased MyoD protein by accelerating its turnover. They examined whether hypoxia inhibits MyoD protein accumulation by promoting its proteasomal degradation. C3H10T1/2 cells stably transfected with MyoD were grown in hypoxic conditions and then treated with two 26S proteasome-specific protease inhibitors, MG132 or lactacystin. Both lactacystin and MG132 treatment increased MyoD protein in C3H10T/2 cells cultured under hypoxia, indicating that hypoxia-induced loss of MyoD protein is mediated by the ubiquitin-proteasome pathway. Supporting this, hypoxia (1%) induced expression of atrogin-1/MAFbx (muscle atrophy F-box protein), muscle-specific ubiquitin ligase [42], that targets MyoD for degradation [43]. In contrast to MyoD, the regulation of myogenin under hypoxia remains to be elucidated. Hypoxia slightly decreased myogenin mRNA stability [14]. Like MyoD, myogenin shows a short half-life [44]. Myogenin was polyubiquitinated by SCF (Skp1/Cullin 1/F-box protein) followed by proteasomal degradation [45] and MAFbx functioned as an F-box protein for ubiquitination of myogenin [46]. In addition, PHD3 regulated myogenic differentiation by interacting with, stabilizing myogenin protein, preventing it from pVHL-mediated degradation [47]. It can be therefore supposed that myogenin loss under hypoxia also may be mediated by the ubiquitin-proteasome pathway.

Notch signaling

Notch signaling defines an evolutionarily conserved cell-to-cell communication mechanism, which acts in many cell types and at various stages during development [48]. In canonical Notch signaling, Notch signaling is activated when the transmembrane Notch receptor interacts with one of its cell-membrane-anchored DSL ligands (Delta/Jagged, Serrate, or Lag2) [49]. When activated by ligand binding, Notch receptors undergo a series of cleavages from metalloproteases and γ-secretases to liberate the Notch intracellular domain (Notch ICD), which translocates to the nucleus and interacts with a CBF1/Suppressor of Hairless/LAG-1 (CSL) family DNA-binding protein [C promoter-binding factor (CBF1) is also known as recombination signal binding protein for immunoglobulin kappa J region (RBPJ-κ) or kappa-binding factor 2 (KBF2) in mammals, as Suppressor of Hairless [Su(H)] in flies and Longevity-assurance gene-1 (LAG-1) in C. elegans] and initiates the

transcription of Notch target genes including Hairy/enhancer of split (Hes), Hes related with YRPW motif (Hey), and Notch-regulated ankyrin repeat protein (Nrarp) [48, 49].

Notch signaling inhibits myogenic differentiation [50]. The possible mechanisms of Notch signaling prevents myoblast differentiation have been proposed. For instance, Notch signaling activated Hes1, which then inhibited MyoD expression [51]. Notch signaling inhibited the ability of MEF2C, a myocyte enhancer binding factor-2 (MEF2) family of MADS (MCM1, agamous, deficiens, and serum response factor) box transcription factors, in cooperation with MyoD and myogenin [52]. Hes1 induction was mediated by RBP-J and blocked myogenic differentiation by subsequent inhibition of MyoD expression [53]. Hey1 and Hey2 functioned as repressors of the myogenin promoter, by preventing the binding of the MyoD/E47 heterodimer to the E-box and by forming an inactive heterodimer with MyoD [54]. Hey1 didn't interact with MyoD and instead it was shown that Hey1 prevented MyoD to bind to Myogenin or MEF2C promoters by inhibiting their transcription and repressing myogenic differentiation [55].

It remains inconclusive whether Notch signaling may be involved in repression of myogenic differentiation under hypoxia, although it is difficult to compare data because of differences in experimental conditions. Gustafsson *et al.* [6] showed that Notch modulated myogenic differentiation in concert with HIF-1α under hypoxia. Hypoxic-inhibition of differentiation of C2C12 cells and satellite cells was to a considerable extent reverted after incubation with a γ-secretase inhibitor, L-685,458. Supporting this, hypoxia stimulated the expression of Hey2 after hypoxic treatment of C2C12 cells. They elucidated the potential molecular mechanism of hypoxic-inhibition of cell differentiation. Hypoxia increased the activity of Notch ICD and stabilized the Notch ICD, leading to the expression of Notch target genes. HIF-1α was physically recruited to a DNA-binding complex containing the Notch ICD. HIF-1α was recruited to a Notch-responsive promoter in response to hypoxia and activated Notch signaling under hypoxia. Based on the experimental data, they suggest a new mode of action of HIF-1α under hypoxia that differs from the canonical response, in which it needs to dimerize with HIF-1β to activate the transcription of hypoxia-responsive genes. They hypothesized a model in which HIF-1α, once stabilized by hypoxia, interacts with the Notch ICD and is an active part of the Notch 1 ICD/CSL transcriptional complex. There, HIF-1α would contribute to stabilize Notch 1 ICD and would enhance the transcriptional activity of the complex through the recruitment of coactivators such as CBP/p300. In this context, they showed a possible role of FIH-1 (factor inhibiting HIF-1) in cross-coupling between the Notch and hypoxia signaling pathways [56]. FIH-1 hydroxylated Notch ICD at two residues (N^{1945} and N^{2012}) that were critical for the function of Notch ICD as a transactivator. FIH-1 negatively regulated Notch signaling activity and accelerated myogenic differentiation. In its modulation of the hypoxic response, Notch ICD enhanced recruitment of HIF-1α to its target promoters and derepressed HIF-1α function. Addition of FIH-1, which had a higher affinity for Notch ICD than for HIF-1α, abrogated the derepression, suggesting that Notch ICD sequesters FIH-1 away from HIF-1α. It seems therefore possible that FIH-1-mediated hydroxylation of Notch ICD and HIF-1α may also modulate myogenic differentiation under hypoxia.

Yun *et al.* [14] examined the role of the Notch signaling in myogenic differentiation under hypoxia. C2C12 cells were differentiated under hypoxia in the presence of a γ-secretase inhibitor, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-(S)-phenylglycine t-butyl ester (DAPT). Notch signaling molecules including Notch1-3, Notch ICD, Hey1 were not induced under hypoxia. Pharmacological inhibition of Notch signaling had no significant effects on myogenic differentiation either at 21% or 0.5% O_2. Majmundar *et al.* [24] showed that hypoxia (0.5%) induced Hey2, but not Hey1, HeyL, or Hes1 in C2C12 cells. They assessed whether hypoxic induction of Hey2 requires Notch signaling by employing the Notch ligand JAG1 to activate signaling as well as DAPT. DAPT treatment did not significantly abrogate the hypoxic activation of Hey2, suggesting this is mediated through the Notch signaling-independent mechanism. They also showed that hypoxia (0.5 and 1%)

repressed myogenic differentiation irrespective of DAPT.

These intriguing results raise new question. What are the key Notch target genes under hypoxia? Hey1 and HeyL were highly expressed but Hey2 could not detect in quiescent satellite cells [57]. Furthermore, by analyzing Hey1/HeyL double-knockout mice, Hey1 and HeyL were essential to generate undifferentiated quiescent satellite cells and to maintain satellite cell numbers [57]. Notch signaling was active in quiescent satellite cells, which highly expressed Hes1, Hes5, Hey1, Hey2, and HeyL and their expression was downregulated in activated satellite cells [58]. Neither Hes1 nor HeyL, but only Hey1 overexpression could block myogenesis by directly suppressing the *Myogenin* promoter [50]. These data indicate that Hey1 would be a good candidate to mediate, at least in part, Notch regulation of satellite cell maintenance by preventing their differentiation. However, expression Hey1 failed to change or downregulated in myogenic cells cultured in hypoxic conditions [14, 24]. Therefore, it remains uncertain whether Hey1 acts as a repressor of myogenic differentiation under hypoxia. Recently, Liu *et al.* [26] showed that hypoxia (1%) promoted quiescence in satellite cells by upregulating Pax7, and downregulating MyoD and myogenin. Hypoxia activated the Notch signaling, which subsequently repressed the expression of miR-1 and miR-206 through Hes1 and Hey1 proteins, leading to upregulation of Pax7. In addition, it should be considered whether Hey2 may be involved in hypoxic-inhibition of myogenic differentiation. It was shown that Hey2 could be regulated through Notch signaling-independent mechanisms. Hey2 was activated by fibroblast growth factor (FGF) and blocked hair cell differentiation [59]. It was shown that FGF-2 expression was upregulated in rat cortical neurons [60] and 911 human embryonic retinoblast cells [61] under hypoxia. Accordingly, further detailed research is needed to elucidate Notch signaling in myogenic differentiation under hypoxia.

Phosphatidylinositol 3-kinases (PI3Ks)/Akt signaling

The PI3K/Akt signaling has been shown to promote myogenic differentiation *in vitro* [62-65]. PI3K is a lipid kinase and generates phosphatidylinositol-3,4,5-trisphosphate (PI(3, 4, 5)P3). PI(3, 4, 5)P3 is a second messenger essential for the translocation of Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1) and mammalian target of rapamycin complex 2 (mTORC2) [66]. Akt is phosphorylated at two key regulatory sites, Thr308 and Ser473, by PDK1 and by mTORC2, respectively [66]. Since Thr308 phosphorylation is necessary for activation of Akt and Ser473 phosphorylation is only required for maximal activity [66]. Hypoxia has shown to decrease the phosphorylation of Akt in myogenic cells [21, 24, 42, 67]. Majmundar *et al.* [24] examined whether oxygen regulates myogenic differentiation through PI3K/Akt signaling. Hypoxia (0.5%) repressed the phosphorylation of Akt^{Thr308} and Akt^{Ser473} both in C2C12 and satellite cells. Intriguingly, incubating C2C12 myoblasts at 5% or 1.5% O_2 had modest effects on phosphorylated AKT^{S473} levels, indicating a threshold for Akt inactivation may exist between 1.5% and 1% O_2. Multiple direct substrates of Akt including GSK-3α^{Ser21} (glycogen synthase kinase-3α), GSK-3β^{Ser9}, $FOXO1^{Thr24}$ (forkhead box class O1), and $FOXO3a^{Thr32}$ exhibited decreased phosphorylation under hypoxia. They evaluated whether insulin-like growth factor-I (IGF-I) signaling activity is regulated by O_2 availability, as IGF-regulated PI3K/Akt pathway controls muscle differentiation [68]. Hypoxia reduced the phosphorylation of IGF-I receptor $\beta^{Tyr1135}$ in despite of IGF-I treatment. Taken together, these data suggest that hypoxia broadly affects Akt activity probably through the decreased sensitivity of the IGF-I receptor to growth factors, resulting in repressing myogenic differentiation.

Akt indirectly promotes mTORC1 activity [69]. The substrates of mTORC1, p70 S6 $kinase^{Thr389}$ and S6-ribosomal $protein^{Ser240/244}$ were less phosphorylated under hypoxia [24], indicating that mTORC1 activity was repressed. The regulation of mTORC1 by hypoxia has shown to required the hypoxia-inducible gene, REDD1 (regulated in development and DNA damage response 1) and TSC1/2 (tuberous sclerosis complex 1/2) [70]. They function as negative regulators of mTORC1 [70]. REDD1 regulates

mTORC1 activity through its ability to bind directly to 14-3-3, leading to the release of TSC2 from 14-3-3 [71]. Hypoxia induced REDD1 expression in differentiating myoblasts [21]. When rats exposed chronic hypoxia, a strong increase in REDD1/14-3-3 association was induced [72]. Therefore, hypoxia-induced REDD1 may inhibit mTORC1 activity.

It remains inconclusive whether the downregulation of Akt activity by hypoxia is regulated through HIF-1α-dependent or -independent mechanisms. Ren *et al.* [21] showed that the levels of IGF-I-induced phosphorylated Akt in HIF-1α knockdown cells were higher than those of the control cells under hypoxia (1%), suggesting that hypoxia repressed IGF-I-induced Akt signaling through HIF-1-dependent mechanism. Majmundar *et al.* [24] showed that C2C12 cells expressing either empty vector or *HIF-1α* shRNA cultured in 21% or 0.5% O_2 exhibited similar reductions in Akt activity, suggesting that hypoxia inhibits PI3K/Akt activity in myoblasts through HIF-1-independent mechanism. This may reflect difference in experimental condition. Majmundar *et al.* [24] measured phosphorylated Akt ^{Ser473} in control and HIF-1α knockdown cells at 21% or 0.5% O_2, which makes it possible to evaluate how O_2 affects Akt activity in the presence or absence of HIF-1α. It cannot be completely excluded, however, that Akt activity could be independent on HIF-1α activity, since HIF-1α loss resulted in a modest induction of Akt activity at 20.9% O_2 [24].

Mitogen-activated protein kinase (MAPK) signaling

In mammalian cells, three MAPK families have been clearly characterized: namely classical MAPK [also known as extracellular signal-regulated kinase (ERK)], c-Jun NH_2-Terminal Kinase/stress-activated protein kinase (JNK/SAPK), and p38 kinase [73]. MAPK signaling pathways have shown to regulate myogenic differentiation [74-78]. Conflicting data exist on whether hypoxia modulates ERK signaling. Activation of ERK1/2 increased during myogenic differentiation at 20.9% O_2 but was repressed at 1% O_2 [23]. Forced expression of mitogen-activated protein kinase kinase 1 (MEK1), which can phosphorylate and activate ERK1/2, could only slightly reverse the inhibition of myogenic differentiation by hypoxia [23]. In contrast, hypoxia (1%) did not repress ERK1/2 activity during myogenic differentiation [21]. Similarly, phospholylation of MEK1/2$^{Ser217/221}$ and ERK1/2$^{Thr202/Tyr204}$ failed to change in response to hypoxia (0.5%) [24]. Furthermore, phospholylation of ERK1/2 $^{Thr202/Tyr204}$ slightly increased during hypoxia [67]. It should be, however, noted that ERK pathway has dual roles during mygenic differentiation: being inhibitory at early stages and stimulatory at late stages [77]. It has been shown that ERK signaling is involved in repression of myogenic differentiation by cytokines and growth factors. For instance, treatment of myoblasts with LIF (leukemia inhibitory factor) induced phosphorylation of ERK, and the LIF-induced inhibitory effect on myogenic differentiation was blocked by U0126, a specific MEK inhibitor and dominant negative-MEK1 [79]. The myostatin-repressed myotube formation and expression of skeletal muscle differentiation marker genes were attenuated by blockade of ERK pathway with MEK1 inhibitor, PD98059 [80]. Intriguingly, myostatin expression was induced in rat skeletal muscle when chronically exposed to hypoxia and in the patient with chronic obstructive pulmonary disease, which is characterized by airflow limitation [81]. Therefore, changes in O_2 availability may contribute to the upregulation of myostatin. Hypoxia prolonged IGF-I-induced ERK1/2 activation through HIF-1-dependent mechanisms [21]. Pharmacological inhibition of ERK1/2 by U0126 increased myogenic differentiation in response to IGF-II under hypoxia [21]. IGF-I activated ERK1/2 via the formation of the Gab1 (growth-factor receptor-bound protein 2-associated binder-1) and SHP2 (SH2 domain containing protein tyrosine phosphatase 2) complex in C2C12 myoblasts [82]. The Gab1-SHP2-ERK1/2-signaling pathway inhibited IGF-I-dependent myogenic differentiation [82]. Therefore, further studies are required to elucidate the possible role of ERK pathway in regulating myogenic differentiation under hypoxia.

The activity of p38 MAPK activity was also repressed by hypoxia [21]. p38 MAPK pathway regulates the activity of MRFs and MEF2 family. For instance, p38 MAPK stimulated the

transcriptional activity of MyoD through an increased association of MyoD with E47, which could be directly phosphorylated by p38 MAPK [77, 83]. p38 MAPK enhanced the transcriptional activities of MEF2A and MEF2C by direct phosphorylation [77] and regulated myogenin expression by requiring MyoD and MEF2 directly binding to the myogenin promoter [84]. p38 MAPK facilitated MyoD and MEF2D binding the specific sites in the promoter to induce skeletal muscle-specific genes [85]. In addition, p38 MAPK pathway may contribute to hypoxic inhibition of myogenic differentiation by modulating Akt signaling. Activation of p38 MAPK preceded Akt phosphorylation during myogenic differentiation [86]. Inhibition or activation of p38 MAPK with p38 MAPK specific inhibitor, SB203580, dominant-negative p38, or MKK6EE (constitutively active MKK6 mutant) inhibits or stimulated Akt kinase activity [86]. IGFs/PI3K/Akt-mediated pathway increased myogenin transcription by activating both MyoD and MEF2 proteins, which bind the specific sites in the myogenin promoter [87, 88]. Taken together, p38 MAPK may directly or indirectly regulate the activities of MRFs and MEF2 under hypoxia.

CONCLUSION

In this review, we have attempted to summarize the current knowledge about the molecular mechanisms responsible for myogenic differentiation under hypoxia. The satellite cells can adapt to changes in O_2 availability through molecular signaling mechanisms that convert this information into appropriate physiological responses. While it is clear that Notch signaling functions at multiple steps of morphogenesis, including an early critical role in cell fate determination and later roles in the regulation of cell proliferation and differentiation, several questions remain to be elucidated. For instance, how can cells activate Notch signaling in response to hypoxia? What are the key Notch target genes under hypoxia? Does the cross-talk occur between Notch and other signaling pathways under hypoxia? In addition to Notch pathway, PI3K/Akt and p38 MAPK pathways are likely to be involved in regulating myogenic differentiation under hypoxia. Do these pathways act separately or cooperatively in regulating myogenic differentiation under hypoxia? Much still needs to be elucidated with regard to the role of hypoxia in regulating myogenic differentiation. Given the substantial role of hypoxia in myogenic differentiation, understanding how multiple signaling pathways adapt to changes in O_2 availability will provide a valuable insight into the underlying mechanisms that regulate cellular homeostasis.

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