AMP-activated Protein Kinase Activity Is Critical for Hypoxia-inducible Factor-1 Transcriptional Activity and Its Target Gene Expression under Hypoxic Conditions in DU145 Cells*

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This article has been withdrawn by the authors. In Fig. 1B, lanes 1-2 and lanes 3-4 of the ACC immunoblot on the right were duplicated. The first lane of the pACC immunoblot on the right in Fig. 1B was duplicated in Fig. 2A. The HIF-1α immunoblot on the left in Fig. 1B was reused in Fig. 8A. The pACC and ACC immunoblots in Fig. 2A contained duplicated bands. The VEGF and Glut1 gels in Fig. 3 contained duplicated bands. The HIF-1α and tubulin immunoblots in Fig. 6A had duplicated bands. In Fig. 6B, lanes 3 and 7 of the pACC immunoblot were duplicated. In Fig. 8A, a lighter exposure of the first lane was pasted on top of the pACC immunoblot. Additionally, the pJNK immunoblot contained more than 8 lanes of data. Also in Fig. 8A, lanes 1-4 of the ERK immunoblot were duplicated in lanes 5-8. Lanes 1-3 of the pp38 immunoblot were duplicated in lanes 4-6 in Fig. 8A. In Fig. 8C, the last two lanes of the HIF-1β immunoblot were duplicated. The Journal also raised questions regarding the pERK immunoblot in Fig. 8A, which the authors were unable to address. Because the original data are no longer available, the authors state that they repeated the above-mentioned experiments and obtained essentially identical results. The authors state that they have full confidence in the conclusions of this paper. The authors apologize to the readers.

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The abbreviations used are: HIF-1, hypoxia-inducible factor; GLUT1, glucose transporter 1; ERK, extracellular signal-regulated kinase; AICAR, 5-aminimidazole-4-carboxamide-1-β-d-ribofuranoside; ELISA, enzyme-linked immunosorbent assay; MAP, mitogen-activated protein; MAPK, MAP kinase.
AMPK-mediated Regulation of HIF-1 Transcriptional Activity

Acute hypoxia leads to the activation of AMPK because of failure to generate sufficient ATP required for cellular functions (13–15). Thus, under the hypoxic conditions, AMPK and HIF-1 initiate various adaptive responses in response to two different cellular parameters, namely the decreased ATP level and the reduced oxygen level, respectively. Although the relationship between AMPK and HIF-1 had never been examined, there seems to be a possibility that part of the signals transduced by the lowered energy levels may be interlinked at the molecular level; AMPK and HIF-1 exert similar effects on anaerobic glucose metabolism as recent studies demonstrated that hypoxia-induced glucose uptake (26) and hypoxia-stimulated glycolysis (14) are also mediated by AMPK. Thus, in the present study, we explored the possibility that AMPK is involved in the regulation of HIF-1. Although most of the currently identified substrates of AMPK are metabolic enzymes, a growing body of evidence demonstrated that AMPK is also implicated in the regulation of gene expression, and there are indeed several examples of transcription factors and cofactors that are directly phosphorylated and regulated by AMPK (27). Here, by using a pharmacological and molecular approach, we demonstrate that AMPK activity is critical for the HIF-1 transcriptional activity and its target gene expression in several cancer cell lines, implying a novel role of AMPK in cancer pathogenesis as well as in oxygen-regulated cellular physiology. Moreover, we also show that AMPK transmits a positive signal to HIF-1 via a signaling pathway that is independent of PI 3-kinase/AKT and MAP kinase pathways. To our knowledge, this is the first report demonstrating that AMPK is involved in the regulation of oxygen-regulated gene expression.

EXPERIMENTAL

Materials—RPMI medium was purchased from Invitrogen, USA. 2-deoxy-D-[3H]glucose (6.0 Ci/mmol) was purchased from PerkinElmer Life Sciences. The anti-phospho-specific antibodies that recognize a phosphoamino acid are capzymes, a growing body of evidence demonstrated that AMPK is also implicated in the regulation of gene expression, and there are indeed several examples of transcription factors and cofactors that are directly phosphorylated and regulated by AMPK (27). Here, by using a pharmacological and molecular approach, we demonstrate that AMPK activity is critical for the HIF-1 transcriptional activity and its target gene expression in several cancer cell lines, implying a novel role of AMPK in cancer pathogenesis as well as in oxygen-regulated cellular physiology. Moreover, we also show that AMPK transmits a positive signal to HIF-1 via a signaling pathway that is independent of PI 3-kinase/AKT and MAP kinase pathways. To our knowledge, this is the first report demonstrating that AMPK is involved in the regulation of oxygen-regulated gene expression.

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AMPK-mediated Regulation of HIF-1 Transcriptional Activity

**AMPK Is Rapidly Activated in Response to Hypoxia or CoCl₂**—We first determined the kinetics of AMPK activation in DU145 human prostate carcinoma cells. DU145 cells were exposed to hypoxia (1% O₂) or CoCl₂ (100 μM) for the indicated period. AMPK was immunoprecipitated with AMPK-α antibodies, and in vitro activity assay was performed using SAMS peptide as a substrate. The results represent the means ± S.E. for two independent assays in duplicate. B, total extracts were prepared under the identical conditions and subjected to Western blot assay using anti-phosphospecific ACC-Ser79 (P-ACC), anti-ACC (ACC), anti-HIF-1α (HIF-1α), and anti-c-Myc antibodies, respectively. (A) AMPK is rapidly activated in response to hypoxia or CoCl₂. DU145 cells were incubated under the hypoxic conditions or treated with CoCl₂ at the normoxic conditions for the indicated times, and AMPK activity was monitored by Western blot analysis using c-Myc antibody, respectively (A). The AMPK activity was measured under these identical conditions, and the results represent the means ± S.E. for two independent assays in duplicate (B).

**AMPK Is Required for HIF-1 Transcriptional Activity**—The temporal profiles of AMPK activity and HIF-1α expression under the hypoxic condition prompted us to investigate whether AMPK is required for HIF-1α and its target gene expression. To this end, we have taken a pharmacological and molecular approach to inhibit the AMPK activity (Fig. 2), and then we examined its subsequent effect on the HIF-1-dependent gene expression. Recently, a potent and selective small molecule AMPK inhibitor was identified and named compound C by Zhou et al. (33). Consistent with the reported concentration, pretreatment of DU145 cells with 20 μM compound C for 30 min almost completely prevented the hypoxia- or CoCl₂-induced AMPK activation as indicated either by ACC- or CoCl₂-mediated AMPK activation as indicated either by ACC-α Ser79 phosphorylation level (Fig. 2A, upper panel, 1st to 5th lanes) or by a direct enzyme activity assay (Fig. 2B), suggesting that this inhibitor could be used as a powerful tool to study the role of AMPK. However, compound C has not been intensively characterized yet, so we also attempted to confirm our observations by using molecular approaches to rule out any nonspecific effects of compound C. To this end, we generated the inactive form of AMPK α1 subunit (α1-DN) by replacing Asp175 with alanine because this mutant was reported to exert a dominant negative effect over the endogenous AMPK (29). To ensure a high level expression, we further developed the recombinant adenovirus, which delivers c-Myc-tagged AMPK α1 wild type (Ad-α1WT) and AMPK α1 dominant negative DNA (Ad-α1DN). Following infection with Ad-α1WT or Ad-α1DN, the expression of each form was monitored by Western blotting using c-Myc
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Fig. 3. AMPK inhibition blocks the hypoxia-induced VEGF<sub>165</sub> and GLUT1 mRNA expression. DU145 cells were identically treated as described in Fig. 2 legend either by compound C (left panels) or by Ad-α1WT and Ad-α1DN infection (right panels). After exposure to hypoxia (1% O<sub>2</sub>) or CoCl<sub>2</sub> (100 μM) for 8 h, total RNA was extracted from these cells, reverse-transcribed, and subjected to semi-quantitative RT-PCR using specific primers for VEGF<sub>165</sub>, GLUT1, GAPDH, and β-actin genes. The amplified cDNA was analyzed on 1% agarose gel. Normoxia, adenosine with no exogenous gene; WT, Ad-α1WT; DN, Ad-α1-DN.

To examine the role of AMPK in adaptive responses to hypoxia, we first examined the effect of AMPK inhibition on HIF-1 target gene expression such as VEGF<sub>165</sub> and GLUT1. DU145 cells were pretreated with 20 μM compound C (Fig. 3, left panels) or CoCl<sub>2</sub> for 8 h, then exposed to hypoxia or CoCl<sub>2</sub> for 24 h (Fig. 3, right panels). Total amount of ACC was essentially the same under each condition (Fig. 3, middle panel). Because the formation of a trimeric subunit complex is necessary for an optimal AMPK activity (34), it is known that overexpression of wild type α subunit does not exert any positive effect on an endogenous AMPK activity. Consistent with this report (34), Ad-α1WT had little effect on the hypoxia-induced AMPK activity (Fig. 2).

In accordance with the transcript level (Fig. 3), the secreted VEGF protein amount, as measured by a commercial ELISA kit, increased ~3–4-fold in culture media of DU145 cells that were exposed to hypoxia or CoCl<sub>2</sub> for 24 h (Fig. 4A). Under these conditions, pretreatment with 20 μM compound C significantly abrogated the hypoxia- or CoCl<sub>2</sub>-induced VEGF secretion as shown in Fig. 4A. Likewise, the hypoxia- or CoCl<sub>2</sub>-induced glucose uptake was also attenuated by compound C pretreatment (Fig. 4B). In DU145 cells, GLUT1 is a major isoform of glucose transporters, so it seems reasonable to consider that this result also reflects the GLUT1 mRNA level shown in Fig. 3. Taken together, our results (Figs. 3 and 4) indicate that AMPK activity is necessary for the hypoxia-induced VEGF<sub>165</sub> and GLUT1 gene expression. Under these conditions (Fig. 4), cell viability was not significantly affected by compound C (data not shown).

To determine whether AMPK modulates VEGF or GLUT1 transcription by HIF-1-dependent mechanism, we transfected DU145 cells with a luciferase reporter (pEpoEm-luc) driven by the human erythropoietin HIF-1-binding site (5′-TAGGGTGT-3′) and SV40 promoter (35), and we investigated the effect of AMPK inhibition on HIF-1-dependent luciferase expression (Fig. 5). Hypoxia or CoCl<sub>2</sub> induced a HIF-1-dependent luciferase activity ~7–11-fold, whereas the cells transfected with pEpoEm-luc with a mutated site (5′-TAAAAGCT-3′) showed no response to these stimuli (Fig. 5B). Hypoxia- or CoCl<sub>2</sub>-induced luciferase activity was significantly diminished by pretreatment of compound C (Fig. 5A) or by cotransfection of pcDNA3 expression vector containing AMPK-α1DN cDNA (Fig. 5B), indicating that AMPK activity is indeed required for the HIF-1 transcriptional activity and thereby expression of its target genes. We further examined the role of AMPK in several different human cancer cell lines including HepG2 hepatocellular carcinoma, HeLa cervix carcinoma, and MCF7 breast adenocarcinoma (Fig. 5C). Hypoxia rapidly activated AMPK as well in these cells (data not shown), and the HIF-1-dependent luciferase expressions induced by hypoxia were also significantly attenuated by cotransfection of AMPK-α1DN expression vector in these cells (Fig. 5C). Therefore, AMPK activity is likely to be necessary for the HIF-1 transcriptional activity in a broad range of cancer types. Although we have used the adenovirus-mediated gene transfer throughout the present study, we performed the cotransfection assay in this particular experiment (Fig. 5, B and C) because infection of DU145 cells with a null adenovirus containing no exogenous gene caused an aberrantly high expression of the luciferase gene even in the absence of any stimuli.
AMPK Is Not Involved in Modulation of HIF-1α Protein Expression, Stabilization, or Nuclear Translocation—As an initial attempt to understand the underlying mechanisms how AMPK regulates the HIF-1 transcriptional activity and its target gene expression, we examined the effects of AMPK inhibition on HIF-1α protein level because the functional activity of HIF-1 is primarily regulated by accumulation of HIF-1α protein (16–18). DU145 cells were infected with Ad-α1WT or Ad-α1DN and exposed to hypoxia or CoCl2 for 4 h, and then total cellular protein extracts were subjected to Western blot analysis to determine HIF-1α protein level. The results showed that AMPK inhibition did not affect total protein level of HIF-1α (Fig. 6A, 1st 7 lanes). Under these conditions, the mRNA level of HIF-1α or the protein level of HIF-1β was not affected by AMPK inhibition either (data not shown). Moreover, pharmacological activation of AMPK under normoxic conditions by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), which becomes a potent AMPK activator after its intracellular phosphorylation to AMP-mimetic AICA-ribotide (1–3), did not induce HIF-1α protein expression, either (Fig. 6B). Therefore, these results exclude the involvement of AMPK in modulation of HIF-1α mRNA expression or stabilization. Next, we investigated whether HIF-1α nuclear translocation would be altered by AMPK because Snf1, the yeast homologue of AMPK, is known to modulate the subcellular localization of transcription factors such as the yeast homologues of HIF-1α or a transcriptional activator of stress response element-regulated genes, Msn2 (36). Therefore, these results exclude the involvement of AMPK. As a result, these data (Fig. 6) suggest that AMPK action is likely to be at the level of some other post-translational modification of HIF-1 than HIF-1α protein induction, stabilization, or nuclear translocation.

AMPK Activation Alone Is Not Sufficient to Stimulate the HIF-1α Transcriptional Activity—Accumulating evidence indicates that phosphorylation of the HIF-1α subunit is required for the full activation of HIF-1 (38–40). Therefore, as an attempt to test the possibility that HIF-1α could be a direct phosphorylation target by AMPK, we next investigated whether AMPK activation alone could lead to stimulation of HIF-1α activity. To this end, DU145 cells were cotransfected with pEpoE-luc and pcDNA3 expression vector containing HIF-1α cDNA, and then the effect of AMPK activation by AICAR on HIF-1α-dependent luciferase expression was examined under normoxic conditions (Fig. 7). Introduction of exogenous HIF-1α resulted in ~11-fold induction of HIF-1α-dependent luciferase expression under normoxic conditions. AICAR treatment of these cotransfected cells stimulated AMPK activity in a dose- and time-dependent manner as demonstrated by the phosphorylation level of ACC-α Ser79, and 4-fold induction of AMPK activity, which is a similar degree of activation observed under hypoxic condition, was achieved by 0.25 mM AICAR treatment for 1 h (Fig. 6B, upper panel). During 12 h of exposure, 0.25 mM AICAR did not further stimulate HIF-1-dependent luciferase activity under normoxic conditions (Fig. 7A), suggesting that AMPK activation alone is not sufficient to stimulate HIF-1 activity. Exposure of these cells to 0.25 mM AICAR (Fig. 7A) or 0.5 mM AICAR for longer than 8 h (Fig. 7B) led to a slight decrease in HIF-1-dependent luciferase activity, and this may be due to the nonspecific effect of AICAR because
A prolonged exposure to AICAR was reported to lead to an AMPK-independent cell death (41).

**The AMPK Signaling Pathway Leading to HIF-1 Is Independent of PI 3-Kinase/AKT and MAPKs in DU145 Cells**

Hypoxia influences various lipid kinase or protein kinase signaling transduction pathways such as PI 3-kinase/AKT, ERK, p38, and JNK (38–40). These kinases were previously implicated in stabilization or transcriptional activation of HIF-1α protein, although the actual roles of these kinases are highly cell type- and stimuli-dependent. Because AMPK activation alone was not sufficient to stimulate HIF-1 activity (Fig. 7), thereby being likely to require additional signal-relaying intermediates, we next attempted to elucidate the AMPK signaling pathway by checking a cross-talk with PI 3-kinase/AKT, ERK, JNK, or p38 MAP kinase under hypoxic conditions. To this end, we first examined the changes in the level of a phosphoactivated form of each kinase in DU145 cells that were exposed to hypoxia for the indicated times (Fig. 8A). Immunoblot analyses with phosphospecific antibodies against ERK, p38, JNK, and AKT kinase revealed that only ERK1 and ERK2 were mildly and progressively activated in response to hypoxia. Compared with the kinetics of AMPK activity, ERK activation was quite slow, being detected in 2–4 h after hypoxic exposure. A phosphoactivated form of p38, JNK, and AKT was not detected at all during 24 h of exposure to hypoxia. As a positive control for potency of each antibody, protein extracts of DU145 cells exposed to 1 mM H2O2 for 30 min were used, and a distinctively phosphoactivated form of each kinase was detected. To evaluate further the functional role of ERK for HIF-1 regulation, DU145 cells were transiently transfected with pEpoE-luc plasmid, pretreated with PD98059 (MEK1 inhibitor), and exposed to hypoxia for 24 h. However, the hypoxia-induced luciferase expression was not attenuated by PD98059 but rather slightly increased (Fig. 8B). However, this increase was not statistically significant, indicating that the ERK activity induced by hypoxia is not involved in the HIF-1 regulation in DU145 cells. Moreover, inhibition of ERK activity by PD98059 also did not affect the phosphorylation state of ACC-α Ser79 (P-ACC), and the protein level of ACC (ACC). Therefore, AMPK is likely to modulate the HIF-1 transcriptional activity via its own signaling pathway that is independent of PI 3-kinase/AKT and these MAP kinases.

**DISCUSSION**

Cellular oxygen concentration in all higher organisms is precisely regulated because it serves as a substrate for oxidative phosphorylation and other metabolic reactions. Even a slight decrease in normal oxygen concentration can impair ATP generation, hence affecting cell viability, and disruption of oxygen homeostasis is implicated in the etiology of many disease processes including cancer, heart disease, cerebrovascular disease, and chronic lung disease (42). For this reason, cells possess highly sophisticated protective mechanisms in response to hypoxia. In the present study, we explored a couple of such protective mechanisms, mainly focusing on the relationship between AMPK and HIF-1, which represent a cellular energy sensor/effectors (1–3) and oxygen sensor/effectors (16–18), re-
spectively. Our results clearly showed that AMPK is a novel and essential component of HIF-1 regulatory machinery; hypoxia-induced responses such as HIF-1 target gene expression (VEGF and GLUT1), VEGF secretion, glucose uptake, and HIF-1-dependent reporter gene expression were significantly attenuated by inhibition of AMPK activity via pharmacological or molecular approach. Identical results were also obtained from cells that were exposed to cobaltous ions, which mimic hypoxia. These results thus indicate that a part of energy-sensing signals and oxygen-sensing signals are tightly linked at the molecular level, converging into HIF-1 molecule under hypoxic stress. As a result, an energy-sensing signal appears to be one of the critical components for the oxygen-regulated gene expression.

So far, more than 40 HIF-1 target genes have been reported, and their protein products play important roles in angiogenesis, vascular reactivity and remodeling, energy metabolism, erythropoiesis, cell proliferation, and survival (16–18). Although it is unknown at this point whether AMPK activity is required for every HIF-1 target gene expression, the current implication of AMPK in the modulation of HIF-1 activity as well as VEGF gene expression may extend a role of AMPK to the mechanisms of angiogenesis, a process leading to growth of new blood vessels. VEGF is a specific mitogen for vascular endothelial cells, and the binding on its receptor on these cells promotes their proliferation, leading to vessel formation. The

**FIG. 7.** AMPK activation alone is not sufficient to stimulate HIF-1 transcriptional activity under normoxic condition. A, DU145 cells were cotransfected with pEpoE-luc and pcDNA3 containing HIF-1α cDNA (pcDNA3/HIF-1α) with a 1:1 ratio. After 24 h post-transfection, cells were treated under normoxic conditions with the indicated concentrations of AICAR for 12 h (A) or with 0.5 mM AICAR for the indicated times (B). Then protein extracts were prepared and subjected to the luciferase assay to determine activity. Data are presented as mean ± S.E. for six determinations.

**FIG. 8.** The AMPK signal pathway leading to HIF-1 is independent of MAPKs and PI 3-kinase/AKT in DU145 cells during hypoxia. A, DU145 cells were exposed to hypoxia (1% O2) for the indicated times, and the protein level of HIF-1α (HIF-1α), phosphorylation level of ACC-α Ser79 (P-ACC), and protein level of ACC (ACC) were monitored during the period by Western blot analyses. In addition, activities of ERK, p38 MAP kinase, JNK, and AKT were indirectly measured by Western blot analysis using antibodies that specifically recognize the phosphoactivated form of each kinase (p-ERK, p-p38, p-JNK, and p-AKT). The total amount of each kinase was also compared by using antibodies recognizing each kinase regardless of phosphorylation (ERK, p38, JNK, and AKT). Protein extracts of DU145 cells exposed to 1 mM H2O2 for 30 min were used as a control to support the potency of each antibody (H2O2).

B, DU145 cells were transfected with pEpoE-luc plasmid. After 24 h of transfection, cells were pretreated with 20 μM compound C or 25 μM PD98059 and exposed to hypoxia for an additional 24 h, and then the luciferase activity was measured. C, the effect of 20 μM compound C or 25 μM PD98059 on hypoxia-induced ACC-α Ser79 phosphorylation (P-ACC), HIF-1α protein level (HIF-1α), and HIF-1β protein level (HIF-1β) was examined by immunoblot analysis using specific antibodies.
effective vascular remodeling after ischemic injury in heart or brain is positively affected in HIF-1-dependent mechanisms (42). In the case of myocardial ischemia, the role of AMPK as a critical mediator in controlling fatty acid and glucose metabolism has been demonstrated by several research groups (3, 15, 43). Therefore, in addition to this role, our results suggest that AMPK may be involved in the regulation of the vascular system during ischemic injury. Furthermore, a potential role of AMPK during embryonic development deserves further investigation because the significance of VEGF and HIF-1 during embryonic and vascular development was also well demonstrated (44, 45).

More relevant to our study, HIF-1 and VEGF also contribute to tumor pathogenesis by facilitating angiogenesis, by which tumors continue to maintain a blood supply during development. HIF-1 is known to be critical for cancer growth and progression (46). The elevated levels of HIF-1 and VEGF proteins are observed in a variety of primary malignant tumors, and their overexpression in many types of cancer cell correlated well with treatment failure and mortality (46). In addition to secretion of proangiogenic factors such as VEGF, an increased rate of anaerobic glycolysis is another hallmark feature of hypoxic adaptation of cancer cells. In fact, we demonstrated that hypoxia-induced VEGF secretion or glucose uptake was significantly blocked by AMPK inhibition (Fig. 4). Considering the functional requirement of AMPK for such critical features of hypoxic tumor cell adaptation, it is tempting to speculate that AMPK may play an important role in cancer pathogenesis under hypoxic conditions. Moreover, our study may have potential implications regarding an anti-cancer therapy. One advantage of the hypoxic environments of tumors for anti-cancer therapy such as development of selective anti-cancer strategies, HIF-1 has been considered as a putative target, and such an anti-cancer therapy because its activity is not likely to be required for the HIF-1α protein induction or stabilization under hypoxic conditions, and overexpression, nuclear translocation of HIF-1α seems to be independent of AMPK activity (Fig. 6), implying that AMPK-mediated HIF-1 regulation occurs at some other post-translational level of HIF-1. Because AMPK activation alone was not able to stimulate HIF-1 activity (Fig. 7), we further examined a cross-talk with PI 3-kinase/akt, ERK, JNK, or p38 MAP kinase to elucidate the AMPK signaling pathway. However, we were not able to demonstrate any significant functional role of these kinases for HIF-1 activity in DU145 cells (Fig. 8). In fact, the actual roles of PI 3-kinase/akt or MAP kinases in the regulation of HIF-1 are highly cell type- and stimuli-dependent. For example, the PI 3-kinase/akt pathway has been known to be required for growth factor-dependent HIF-1 induction (49–51). However, hypoxic induction of this pathway is not only cell type-dependent, but its requirement for the hypoxia-induced HIF-1 activity was also recently challenged (52, 53). Consistent with our result (Fig. 8), a previous report showed that hypoxia did not activate PI 3-kinase/akt pathway in prostate cancer cells (54). Similarly, hypoxia is able to activate ERK in some cell lines, but their functional requirement for HIF-1 activity depends on cell type as well (39). Likewise, ERK was activated in DU145 cells, but its activity seems to be dispensable for the HIF-1 activity (Fig. 8). In contrast to these protein or lipid kinases, AMPK may be more generally required for HIF-1 activity under hypoxic stress in a variety of cancer cells because the nature of AMPK is to be activated by the reduced cellular energy level, which is an inevitably occurring phenomenon under hypoxic stress. Our demonstration of the significance of AMPK for HIF-1 activity in at least four different cancer cell lines may support this possibility (Fig. 5C).

Consequently, independent of PI 3-kinase/akt and MAPK pathway, AMPK is likely to possess its own signaling pathway leading to the post-translational modification of HIF-1a, and we assume that there are at least three different scenarios for this event. First, it is conceivable that AMPK activation could lead to indirect phosphorylation of HIF-1α via unknown protein kinase cascades because AMPK activation alone did not stimulate the HIF-1 activity induced by introduction of exogenous HIF-1α under normoxic conditions (Fig. 7). However, we still cannot exclude the possibility that AMPK could directly phosphorylate HIF-1α, thereby contributing to its transcriptional activity in vivo. In this case, other post-translational modifications, such as NO may mediate the effect of AMPK-mediated HIF-1α phosphorylation. It has been shown to increase nitric oxide (55). Furthermore, the transcriptional ability of HIF-1α may be associated with various cofactors such as Histone H3, H4, and Trans-activation domains, which allow the HIF-1α to interact with the target genes by associating with various transcriptional factors such as CREB/p300, and CBP/p300. It was recently reported that AMPK could phosphorylate CBP/p300, thus modulating a subset of p300 activity. Although a physiological role of this phosphorylation still remains to be elucidated (59). Thus, p300 could modulate HIF-1α transcriptional activity in an AMPK-dependent manner. We are currently investigating these possibilities to further reveal a detailed mechanism of how AMPK activity transmits the positive signal for HIF-1 activity.

Most of the currently identified substrates of AMPK are metabolic enzymes, and its role thus has been focused on the regulation of metabolic pathways, which leads to maintenance of ATP homeostasis. However, our data presented here imply that AMPK may play critical role(s) in far more various cellular physiologies than ever speculated because HIF-1 is essential for embryonic vascularization and development, tumor angiogenesis, and tissue ischemia.

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