Hypoxia Stimulates the Epithelial-to-Mesenchymal Transition in Lung Cancer Cells Through Accumulation of Nuclear β -Catenin

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Abstract. *Background/Aim:* Recent studies implied a significant role of hypoxia-inducible factor-1 α (HIF1 α) in cell transformation. This study aimed to assess the effects of HIF1 α on the epithelial-to-mesenchymal transition (EMT) and tumorigenesis of lung adenocarcinoma cells. *Materials and Methods:* Invasion, migration and colony formation assays were used to evaluate cell transformation. Expression of EMT-related markers were analyzed by western blot, reverse-transcription polymerase chain reaction or zymography. A luciferase assay was carried out to access the transcriptional activity of β -catenin. *Results:* Hypoxia enhanced migration, invasion and transformation of A549 lung adenocarcinoma cells. Hypoxic stimulation promoted the expression of EMT-related markers in lung cancer cells. The expression of HIF1 α was found to be involved in hypoxia-mediated modulation of expression of snail family transcriptional repressors 1 (SNAI1) and 2 (SLUG). Hypoxia enhanced nuclear accumulation and transcriptional activity of β -catenin. *Conclusion:* β -Catenin promotes expression of EMT-related genes and eventually contributes to the metastatic process.

Lung cancer is a leading cause of cancer deaths and the second most common cancer in the United States (1, 2). The cancer survival rate has improved over the past few decades; however, the improvement is mainly due to early

detection and cancer growth inhibition (3). Cancer metastasis is one of the major causes for poor prognoses of patients with lung cancer. The nervous system, bone, and liver are the most frequent metastatic sites for lung cancer. A previous population-based study indicated that the median survival after diagnosis was 13 months for those with non-metastatic lung cancer and 5 months for those with metastatic lung cancer (4). However, the mechanism of lung cancer metastasis has not been fully elucidated. A lack of understanding of the metastatic mechanisms and process has limited the prevention and inhibition of lung cancer metastasis.

Hypoxia is a feature of most solid tumors (5). One major reason for tumor hypoxia is the formation of insufficient and abnormal vessels in tumor tissues (6). Mechanisms of the tumor cell response to hypoxia are mainly mediated by hypoxia-inducible factor-1 α (HIF1 α), which promotes tumor cell adaptation to a hypoxic environment and potentiates tumor malignancy. Previous studies implied that intratumoral hypoxia and the epithelial-to-mesenchymal transition (EMT) play crucial roles in the progression and aggressiveness of many types of cancer (7-9). For solid tumors, the EMT is known as a critical mechanism in migration and invasion. Recent studies showed that HIF1 α plays a significant role in cell transformation, in which a tumor comprising mostly the sedentary epithelial type of cells changed into a tumor with invasive and metastatic fibroblastic types of cells (7). Several transcriptional repressors (Twist-related protein (TWIST), snail family transcriptional repressors 1 (SNAI1), and 2 (SLUG) are important in regulating the EMT by suppressing several epithelial markers and adhesion molecules, including E-cadherin, in breast and ovarian cancer (10-12).

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Key Words: Lung cancer, hypoxia, epithelial-to-mesenchymal transition, EMT, β -catenin.

Table I. Primers used for the reverse-transcription polymerase chain reaction.

Gene	Encoded protein	Forward primer	Reverse primer
<i>COL1A1</i>	Collagen type I	ACGTCCTGGTGAAGTTGGTC	ACCAGGGAAGCCTCTCTCTC
<i>FN1</i>	Fibronectin	CCGTGGGCAACTCTGTC	TGCGGCAGTTGTACACAG
<i>SLUG</i>	Snail family transcriptional repressor 2	AGTACCCAATGGCCTCTCT	CCAGCCCAGAAAAAGTTGAA
<i>SNAIL</i>	Snail family transcriptional repressor 1	GCCTTCAACTGCAAATACTGC	CTTCTTGACATCTGAGTGGGTC
<i>SIP1</i>	SMAD interacting protein 1	AAAACCATGGCGTGGGTA	CAATAGCCGAGGCATCAAC
<i>TWIST</i>	TWIST	AGTACGCCTTCTCGGTCT	CCTTCTCTGGAAACAATGACATC
<i>ZEB1</i>	Zinc finger E-box binding homeobox 1	ACTGCTGGGAGGATGACAGA	ATCTGCTTCATCTGCCTGA
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC

Targeting hypoxic cells is one pharmacological approach for managing solid tumors (13). Several studies aimed to develop new medications that block hypoxic tumor cells and their underlying progression (13, 14). Based on clarification of the mechanisms of hypoxia-induced EMT, it is possible to develop new therapeutic strategies to manage lung cancer metastasis.

Recently, several studies investigated hypoxia-induced EMT in cancer cells (7, 15, 16); however, details of the underlying mechanism remain unclear. To find new therapeutic medications which target hypoxia-induced lung cancer metastasis, this study investigated mechanisms involved in hypoxia-induced EMT in lung cancer cells.

Materials and Methods

Cell culture. The human alveolar adenocarcinoma A549 cell line (BCRC, Hsinchu, Taiwan, ROC) was cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and with 1% L-glutamine. Cells were incubated in a 37°C humidified culture incubator with 5% CO₂.

Induction of hypoxia. A hypoxic condition was created either by chemical induction or by using a hypoxic environment. For chemical induction, 100 µM cobalt chloride (Sigma, St. Louis, MO, USA) was used as the chemical hypoxia-mimetic agent, which was added to cultured cells for 24, 48, and 72 h. To induce hypoxia, cells were placed in a hypoxic chamber with an oxygen concentration maintained at 1% O₂.

Transfection. The cell line was transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. In brief, 1×10⁶ A549 cells were seeded onto 3.5 cm dishes overnight for cell attachment. Stealth RNA interference (RNAi) for HIF1α (Invitrogen) or the Stealth RNAi negative control (Invitrogen) was mixed with Lipofectamine and then added to cultured cells for 16 h.

RNA isolation and reverse-transcription polymerase chain reaction (RT-PCR) amplification. RNA from the cells was extracted as previously described (17). Aliquots of 5 µg RNA were reverse

transcribed with a first-strand complementary (c)DNA synthesis kit (Fermentas, Waltham, MA, USA). cDNA was then used as a template for PCR amplification. The primers used are listed in Table I.

Western blot analysis. Cell protein extraction and preparation, and the western blot analysis were performed as previously described (18). Primary antibodies to the following proteins were used: α-smooth muscle actin (SMA), connective tissue growth factor (CTGF), and collagen type I were from Santa Cruz (Dallas, Tx USA); β-catenin, γ-catenin, and HIF1α were from Abcam (Cambridge, MA, USA); E-cadherin and N-cadherin were from Millipore (Carlsbad, CA, USA); fibronectin was from Bioworld (Louis Park, MN, USA); and vimentin was from Novus (Littleton, CO, USA). Generic actin (MD Bio, Taipei, Taiwan, ROC) was used as an internal control. Images were analyzed by Image-Pro Plus software (version 4.5; Media Cybernetics, Rockville, MD, USA).

Wound-healing assay. A549 cells were seeded onto 6-well plates overnight, and then a 200-µl pipette tip was used to make a straight scratch through the cell layer. Cells were then treated with normoxia (21% O₂), hypoxia (1% O₂), or 100 µM cobalt chloride. Changes in wound-healing activity were visualized by microscopy 24 h after treatment. The relative distance of the wounded area (µm) was depicted. Cell motility was quantified by measuring the distance between the invading fronts of cells in three randomly selected microscopic fields. The degree of motility is expressed as wound closure compared to that at the zero-time point.

Soft-agar colony-formation assay. For the soft-agar assay, 1 ml of a 1.4% agar solution in DMEM containing 10% FBS was solidified in 6-well plates. Then, 1 ml of 0.7% agar solution, mixed with 1×10⁴ A549 cells, was layered on top of the base agar layer. Cells were next treated with normoxia (21% O₂), hypoxia (1% O₂), or 100 µM cobalt chloride. After incubation for 21 days at 37°C, colony formation was analyzed and counted under light microscopy.

Transwell assay. In total, 5×10⁴ A549 cells in serum-free medium were seeded onto the upper compartment of a transwell chamber (pore size: 8 µm; Corning® Transwell® Costar®, Corning, New York, USA) and allowed to invade into the lower chamber with medium containing 10% FBS under normoxia or hypoxia for 24 h. Invaded cells were fixed by 10% formaldehyde and stained with Giemsa solution (Merck, Carlsbad, CA, USA) and then captured under light microscopy.

Figure 1. Hypoxia enhances cell migration and invasion of A549 human lung adenocarcinoma cells. **A:** A549 cells were subjected to wounding and then incubated under normoxia, hypoxia (1% O₂), or 100 μ M cobalt chloride (CoCl₂) for 24, 48, and 72 h. Changes in the wound-healing activity were visualized by phase-contrast microscopy at the indicated times. **B:** The relative distance of the wounded area (μ m) is depicted. **C:** The invasive ability of A549 cells was evaluated by transwell invasion assays under normoxia, hypoxia, or 100 μ M CoCl₂ treatment for 24 h. Representative images show cells that had invaded through the transwell membrane. **D:** A soft-agar colony-formation assay was performed to evaluate the *in vitro* tumorigenicity of cells. A549 cells were incubated in normoxic or hypoxic conditions for 24, 48, and 72 h. Significantly different at * p <0.05, ** p <0.01, and *** p <0.001 compared to the normoxic group.

Gelatin zymographic assay. Matrix metalloproteinase 2 (MMP2) and MMP9 activities were determined by a gelatin zymographic assay. In brief, the supernatant of A549 culture medium was collected and mixed with sample buffer dye (250 mM Tris-HCl, 10% sodium dodecylsulfate (SDS), 0.5% bromophenol blue, and 50% glycerol) then electrophoresed in a 10% SDS polyacrylamide gel containing 0.1% gelatin. After electrophoresis, the gel was washed three times with 50 mM Tris-HCl containing 2% Triton X-100 and then washed three times with 50 mM Tris-HCl. The gel was then incubated for an additional 18 h at 37°C for the enzymatic reactions of MMPs in reaction buffer (50 mM Tris-HCl, 0.2 M NaCl, 5 mM CaCl₂, 0.02% NaN₃, 2% Triton X-100, and 1 M ZnCl₂). The gel was stained with Coomassie blue R-250 (Sigma) and then destained (methanol/acetic acid/water, 30/10/60). Images were then captured.

Luciferase reporter assay. A549 cells were transfected with the TOPFlash reporter plasmid containing eight copies of optimal (Super 8 $\times$ TOPFlash) or mutant (Super 8 $\times$ FOPFlash) transcription factor/lymphoid enhancer-binding factor (TCF/LEF) binding sites upstream of luciferase. After 12 h of incubation, cells were treated with normoxia (21% O₂), hypoxia (1% O₂), or 100 μ M cobalt chloride for 24 h. All cells were treated with 35 μ l/ml luciferin (Promega, Madison, WI, USA) and then analyzed by luminometer (Sirius luminometer, Berthod, Pforzheim, Germany). The M50 Super 8 $\times$ TOPFlash (Addgene plasmid #12456) and M51 Super 8 $\times$ FOPFlash (TOPFlash mutant) (Addgene plasmid #12457) plasmids were gifts from Randall Moon (19).

Statistical analysis. All experiments were independently performed at least three times. Data are presented as the mean $\pm$ standard error

Figure 2. Effects of hypoxia on the expression of epithelial-to-mesenchymal transition (EMT)-related markers in A549 cells. A: A549 cells were cultured under normoxia (21% O₂, N), hypoxia (1% O₂, H), or 100 μM cobalt chloride (CoCl₂) treatment for 6, 12, and 24 h. Expression of N-cadherin and vimentin were up-regulated by hypoxic stimulation in parallel with down-regulation of the epithelial marker E-cadherin in a time-dependent manner. B: Densitometric analysis of band intensities of each EMT-related marker shown in (A). C: Hypoxia-induced fibronectin and collagen type I mRNA expression was measured by a reverse-transcription polymerase chain reaction assay in A549 cells. D: Quantitative data of (C) are shown as the mean ± SEM. E: Western blot analysis of α-smooth muscle actin (SMA), a precursor form of collagen type I, and connective tissue growth factor (CTGF) expression in A549 cells under hypoxic stimulation. F: Densitometric analysis of band intensities shown in (E). G: Effects of hypoxia on matrix metalloproteinase (MMP) expression and activities in A549 cells. Representative zymography of conditioned media was collected from A549 cells which were cultured under the indicated conditions for 24 h. The protein level and enzyme activity of MMPs were determined by western blot and zymographic analyses, respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and actin were used as the internal control. Significantly different at **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the normoxic group.

of the mean (SEM). Statistical evaluation was performed using Student's *t*-test. *p*-Values of less than 0.05 were considered a statistically significant difference.

Results

Hypoxia enhanced cell migration and invasion of A549 human lung adenocarcinoma cells. To demonstrate that hypoxia is associated with the malignancy of lung cancer cells, cell migration, invasion, and tumorigenicity of hypoxia-treated A549 cells were analyzed through a wound-healing assay, invasion assay, and colony formation assay. After being treated with 1% O₂ hypoxia or cobalt chloride-induced hypoxia, A549 cells showed augmented migratory activity (Figure 1A and B), invasive ability (Figure 1C), and cellular anchorage-independent growth (Figure 1D) within 24, 48, and 72 h. These results suggest that hypoxia enhanced the malignancy of A549 cells.

Hypoxia promoted the EMT of lung cancer cells. Previous studies indicated that the EMT plays an essential role in cancer malignancy. To demonstrate whether the EMT is

associated with enhancement of hypoxia-induced lung adenocarcinoma malignancy, expressions of EMT markers was detected by a western blot assay. As shown in Figure 2A and B, protein expression of HIF1α increased in hypoxia-treated A549 cells, as did that of N-cadherin and vimentin. However, protein expression of E-cadherin and γ-catenin decreased. Switching of cadherin expression implies that the EMT might have occurred under hypoxic circumstances.

To assess the metastatic progression of hypoxia-treated A549 cells, changes in the cytoskeleton and extracellular matrix (ECM) were analyzed. Hypoxia induced fibronectin and collagen type I expression at the messenger (m)RNA level (Figure 2C and D). Increased expression levels of CTGF, collagen type I, and α-SMA were also detected under hypoxic conditions (Figure 2E and F).

Previous studies implicated that up-regulation of MMP2 and MMP9 expression in various types of human cancer might be correlated with advanced stage, invasion, and metastatic properties (20-22). To identify expression and enzymatic activity of MMPs in hypoxia-treated A549 cells, western blot

Figure 3. Hypoxia-inducible factor-1 α (HIF1 α) mediates hypoxia-induced expression of snail family transcriptional repressors 1 (SNAI1) and 2 (SLUG). **A:** Expression of epithelial-to-mesenchymal transition (EMT)-related transcriptional factors SMAD interacting protein 1 (SIP1), SLUG, SNAI1, TWIST, and zinc finger E-box binding homeobox 1 (ZEB1) in normoxic (21% O₂, N) and hypoxic culture conditions (1% O₂, H), and 100 mM cobalt chloride (CoCl₂) mimic hypoxia for 24 h were measured by a reverse-transcription polymerase chain reaction assay (RT-PCR). **B:** Densitometric analysis of band intensities shown in (A). **C:** After transfection with HIF1 α siRNA (siHIF1 α) or scrambled siRNA (Scr), A549 cells were subjected to hypoxia or 100 mM cobalt chloride for 24 h, and an RT-PCR assay was performed to analyze expressions of genes at the mRNA level. Significantly different at * $p < 0.05$ and *** $p < 0.001$ compared to the normoxic group.

assay and gelatin zymographic analysis were carried out. In Figure 2G, both hypoxic and cobalt chloride treatments increased MMP2 and MMP9 expression at the protein level. Hypoxic stimulation also up-regulated the enzymatic activity of MMP2 in A549 cells. These results suggest that hypoxic stimulation promoted the EMT in A549 cells.

HIF1 α is involved in hypoxia-mediated modulation of SLUG and SNAI1 expressions. It was shown that transcriptional repression is one of the critical mechanisms for E-cadherin down-regulation during tumor progression (11, 23). Several transcriptional factors were described as repressors of E-cadherin expression, such as TWIST, SLUG, SNAI1, SMAD interacting protein 1 (SIP1), and zinc finger E-box binding homeobox 1 (ZEB1) (12, 23-26). To further investigate whether transcriptional repressors are involved in E-cadherin suppression under hypoxic stimulation, RT-PCR analysis was performed to detect expression of these repressors. Figure 3A and B show that hypoxia significantly increased mRNA expression of SIP1, SLUG, and SNAI1, but not of TWIST or ZEB1 in A549 cells ($p < 0.001$). To further confirm the role of HIF1 α in SIP1, SLUG, and SNAI1 mRNA expressions under hypoxic conditions, the expression of HIF1 α was knocked-down with HIF1 α -specific small interfering (si)RNA. As shown in Figure 3C, transfection with HIF1 α siRNA dramatically inhibited hypoxia- and cobalt chloride-induced SLUG and SNAI1 expression. These data suggest that HIF1 α mediates the induction of SLUG and SNAI1 mRNA under hypoxic conditions.

Hypoxia enhances nuclear accumulation and transcriptional activity of β -catenin. Previous studies indicated that E-cadherin recruits β -catenin to cell membranes and prevents nuclear translocation and transcriptional activity of β -catenin. Down-regulation of E-cadherin promotes β -catenin release and facilitates the EMT and transformation of cell phenotypes (27-29). Therefore, the next question was whether hypoxia-induced down-regulation of E-cadherin promotes nuclear translocation of β -catenin. Hypoxic stimulation reduced E-cadherin expression in the cytosol, while increased accumulation of β -catenin was found in nuclei (Figure 4A). To further verify whether hypoxia-induced nuclear translocation of β -catenin activates downstream gene expression, a β -catenin/TCF luciferase reporter assay was carried out. We transfected A549 cells with a wild-type β -catenin reporter plasmid and then stimulated them with hypoxia. Luciferase activity was elevated by both 1% O₂ hypoxia and cobalt chloride. On the contrary, luciferase activity did not change in cells transfected with the mutant-type β -catenin reporter plasmid (Figure 4B). Knockdown of β -catenin with siRNA reduced fibronectin, collagen type I, vimentin, MMP2, and MMP9 expression at the mRNA level under hypoxic stimulation (Figure 4C). Taken together, these

Figure 4. Hypoxia increases nuclear accumulation and transcriptional activity of β -catenin. A: Cytoplasmic and nuclear extracts were isolated from A549 cells under normoxia (21% O₂, N), hypoxia (1% O₂, H), or 100 μ M cobalt chloride (CoCl₂) treatment for 24 h. An immunoblot assay confirmed the increase in nuclear translocation of β -catenin in response to hypoxic stimulation. B: A luciferase reporter assay evaluated β -catenin-mediated transcriptional activation using a reporter containing either functional wild-type (WT β -catenin reporter) or mutated (mut β -catenin reporter) transcription factor (TCF) binding sites. A549 cells under hypoxic stimulation showed increased levels of β -catenin activity. C: After transfection with β -catenin siRNA (siCTNB) or control siRNA (siCon), A549 cells were subjected to hypoxic conditions or 100 mM CoCl₂ treatment for 24 h, and a reverse-transcription polymerase chain reaction analysis of epithelial-to-mesenchymal transition-related genes was performed. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HIF1 α : hypoxia-inducible factor-1 α ; MMP: matrix metalloproteinase. Significantly different at * $p < 0.05$ and ** $p < 0.01$ compared to the normoxic group.

findings suggest that hypoxia-induced nuclear translocation of β -catenin and transactivation play crucial roles in the EMT processes of lung cancer.

Discussion

Hypoxia stimulates the EMT in lung cancer cells. Lung cancer is the leading cause of cancer deaths and is the second most commonly diagnosed cancer in both men and women (1, 2). Once lung cancer exhibits the metastatic phenotype, the prognosis and survival rate of patients greatly worsen (4).

Preventing and overcoming lung cancer metastasis are vital issues for lung cancer therapy.

The work presented here shows that hypoxia promoted lung cancer metastasis through nuclear accumulation of β -catenin and thus induced the EMT. In the hypoxic microenvironment of lung cancer cells, HIF1 α induced expression of the transcriptional suppressors, *SNAIL* and *SLUG*, and then down-regulated E-cadherin expression. Lower E-cadherin expression led to β -catenin translocation from cell membranes to nuclei and subsequently enhanced expression of MMP2, MMP9, vimentin, fibronectin, and

Figure 5. A proposed model describing the effects of hypoxia on lung cancer metastasis. In the unfavorable hypoxic microenvironment of the primary tumor (left hand side), hypoxia-inducible factor-1 α (HIF1 α) induces expression of snail family transcriptional repressors 1 (SNAI1) and 2 (SLUG), then inhibits E-cadherin transcription. Cells were more invasive and had reduced cell–cell adhesion. After hypoxia stimulation, lower E-cadherin expression led to nuclear localization of β -catenin (right hand side). These cells exhibited less cell–cell adhesion and greater invasiveness.

collagen type I. Therefore, hypoxic lung cancer cells exhibit less cell–cell adhesion and greater invasiveness (Figure 5).

Hypoxia and cancer progression. Hypoxia is a common condition of the tumor microenvironment. Emerging data suggest that a hypoxic microenvironment might play pivotal roles in progression of solid tumors (6). Due to restricted blood flow, the oxygen consumption rate of cancer cells might override the oxygen supply from the vessels and result in an imbalance between supply and consumption leading to a hypoxic tumor microenvironment (5). Previous studies suggested that hypoxia might induce long-term silencing of breast cancer 1 (*BRCA1*) promoter, and then induce breast cancer genome instability and tumor progression (30). In glioblastoma tissues, hypoxia increased *O*⁶-methylguanine-DNA methyltransferase (*MGMT*) expression and thereby suppressed tumor sensitivity to temozolomide (31). Research has suggested that tumor hypoxia limits patient survival by inducing tumor genetic instability, increasing therapeutic

resistance, and promoting the metastatic ability of tumors (32).

In the hypoxic tumor microenvironment, expression of HIFs increased and that of downstream pathway members, including pluripotency-associated transcription factors [octamer-binding transcription factor 3/4, NANOG, and sex determining region Y-box 2], angiogenic factors (vascular endothelial growth factor), and EMT program-associated molecules (C-X-C chemokine receptor type 4, SNAI1, and TWIST) (33), was activated. These respectively promote tumor self-renewal, tumor vessel proliferation, and tumor-invasive capabilities (33).

HIF1 α induces EMT. Previous studies suggested that hypoxia induces tumor cells to undergo EMT; however, the mechanisms underlying this are still elusive. It was shown that hypoxic induction of dimerization of HIF1 α and HIF1 β in nuclei is important for tumor metastasis. The HIF1 α and HIF1 β heterodimer binds to the hypoxia-response element (HRE) and transactivates EMT-related genes, thereby

promoting the invasive ability of breast cancer and head and neck cancer (34, 35). Furthermore, NOTCH signaling might be essential for hypoxia-induced EMT in tumor tissues. Sahlgren *et al.* (36) and Chen *et al.* (10) suggested that NOTCH signaling mediates HIF1 α recruitment and increases expression of SLUG and SNAIL, thereby increasing the migration and invasion of cancer cells (10, 36). In this study, we demonstrated that hypoxia stabilized HIF1 α and stimulated cell migration, invasion, and anchorage-independent growth of A549 cells.

On the other hand, hypoxia might promote the EMT in pulmonary cells *via* releasing cytokines, such as epithelial growth factor (EGF), insulin-like growth factor-1 (IGF1), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), transforming growth factor β (TGF β), and endothelin-1 (37-42). In 2016, Wang *et al.* suggested that hypoxia enhances TGF β - and IL10-containing exosomes, and thus promotes the migratory ability of lung cancer cells (43). Matsuoka *et al.* indicated that hypoxia might stimulate the EMT *via* autocrine TGF β and TGF β receptor signaling axis in gastric cancer cells (15). Hypoxia-induced cytokine release activates various signaling pathways, such as NOTCH signaling, the EGF receptor pathway, and the WNT/ β -catenin signaling pathway. This might further initiate tumor malignancies including invasion, migration, and metastasis.

β -Catenin crosstalk with HIF1 α and the EMT. β -Catenin plays a vital role in hypoxia-induced EMT. The results of the present study suggest that HIF1 α promotes lung cancer metastasis through accumulation of nuclear β -catenin and thus induces the EMT. Consistent with this, a previous study also reported that crosstalk between β -catenin and HIF1 α promoted colon cancer cell proliferation and adaptation to hypoxia (8). Xi *et al.* also demonstrated that phosphorylated β -catenin, as a critical cofactor, specifically complexed with HIF1 α and promoted transcription of EMT genes (44). On the other hand, the phosphatase and tensin homolog (*PTEN*) might participate in hypoxia-induced accumulation of β -catenin. Recent studies suggested that the hypoxia-induced EMT phenotype was negatively regulated by *PTEN* (7, 45). Kohon *et al.* implied that hypoxia suppressed *PTEN* activity and thus induced β -catenin translocation into the cytoplasm and nuclei (7). Knockdown of *PTEN* resulted in nuclear accumulation of β -catenin and EMT induction in colon cancer cells (45). Overall, β -catenin appears to be an important tumorigenic factor in cancer cells in response to a hypoxic microenvironment. Our findings provide a potential mechanism for the contribution of hypoxia to lung carcinoma metastasis.

Prognostic markers and potential therapeutic interventions. Metastasis in lung cancer is a multifaceted process. It was shown that HIF1 α expression is a significant prognostic

predictor for patients with non-small cell lung cancer (46). Recently, several meta-analytical studies revealed that HIF1 α expression may be a prognostic biomarker and a potential therapeutic target for lung cancer (9, 47). Consistent with clinical and meta-analytical studies, the present study also indicated a potential mechanism of HIF1 α in promoting lung cancer malignancy.

Inhibition of cancer metastasis is still a vital issue in lung cancer therapy. Suppressing hypoxia-induced cancer metastasis might be a potential therapeutic strategy (13, 14). An HIF1 α -targeted therapeutic agent prolonged survival and sensitized to a radiotherapeutic response in preclinical brain tumor research (48). Emodin down-regulated the β -catenin and protein kinase B (AKT) pathways and thus inhibited TWIST1-induced EMT in head and neck squamous cell carcinoma cells (49). While further comprehensive research is still needed, the present study revealed a mechanism underlying hypoxia-induced lung cancer metastasis, that might provide a potential therapeutic target for development of anticancer agents in the future.

Conflicts of Interest

The Authors confirm that there are no known conflicts of interest associated with this publication.

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Received October 8, 2018

Revised October 22, 2018

Accepted October 23, 2018