

Original Article

Effect of hypoxia inducible factor 1 α on the epithelial-mesenchymal transition of human prostate carcinoma cell and its invasion

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Abstract: Hypoxia inducible factor 1 α (HIF-1 α) is one important factor for transducing signal and inducing hypoxia effects. Previous study has shown the enhanced tumor invasion and metastasis by up-regulating HIF-1 α gene expression. Epithelial-mesenchymal transition (EMT) is known to play a crucial role in tumor invasion and metastasis. This study thus investigated the role of HIF-1 α in EMT and invasion of human prostate carcinoma cell line PC3. PC3 cells were cultured under hypoxia condition, for observing the morphological alternation. Real-time PCR was employed to detect mRNA level of HIF-1 α , while the invasion and migration of PC3 cells were observed by Transwell assay. Protein levels of HIF-1 α , E-cadherin and Vimentin were quantified in Western blotting. Under normal condition, PC3 cells had a polygon shape and regular arrangement of cells. Under hypoxia, cells displayed as oval or spindle shape, with loosely connection and irregular distribution. Real-time PCR showed elevated HIF-1 α mRNA expression in hypoxic PC3 cells ($P < 0.05$). Western blotting confirmed the elevated expression of HIF-1 α and Vimentin, while decreased E-cadherin in those hypoxic PC3 cells, which also had elevated migration/invasion ability. Hypoxia environment may elevate HIF-1 α expression in human prostate carcinoma cells, and enhance their morphological changes. The elevated Vimentin and decreased E-cadherin expression all suggested the facilitation of EMT.

Keywords: Hypoxia inducible factor 1 α , human prostate carcinoma, epithelial-mesenchymal transition, cell migration and invasion

Introduction

Prostate carcinoma is the most common and second deadly cancer in males (only next to pulmonary cancer). For those without metastasis, the 5-year survival rate has been increased to near 100%. Such rate in patients with distal metastasis, however, dropped to less than 30% [1]. Previous study has revealed the correlation between hypoxia and the occurrence of invasion and metastasis [2]. In prostate carcinoma, the hypoxia condition gradually aggravated with the advancing of clinical stage, with the elevated expression of hypoxia-specific markers, which severely compromised the efficacy of surgery and radio-/chemo-therapy [3]. Epithelial-mesenchymal transition (EMT) is widely occurred in epithelial derived carcinoma, and endows the feature of mesenchymal cells

on those epithelial tumors, as shown by the down-regulation of epithelial marker E-cadherin and up-regulation of mesenchymal marker Vimentin, which can enhance the invasion and metastasis of malignant tumors [4]. Hypoxia inducible factor 1 α (HIF-1 α) is one important factor that is up-regulated during hypoxia status. It can bind with hypoxia response elements to activate downstream target genes for facilitating tumor invasion and migration via modulating hypoxia signaling pathway and inducing hypoxia effects, in addition to other pathological processes including angiogenesis [5]. Past study has confirmed the alternation of oxygen pressure in the microenvironment of tumor growth might activate HIF-1 α induced signaling pathway for initiating and modulating EMT, which further facilitated the progression, invasion and migration of malignant tumors [6, 7].

HIF-1 α in prostate cancer

Table 1. Specific primers

Target gene	Sequence	Temperature (°C)	Length (bp)
HIF-1 α	5'-CACCATGAAGCCTACACTGTGTTCC-3'	60	561
	5'-TTAAACCATTCGGCAGCAGCGG-3'		
GAPDH	5'-GCCAAGGTCATCCATGACAA CTTTGG-3'	60	314
	5'-GCCTGCTCACCCACCTTCTTG ATGTC-3'		

This study thus investigated the expression of HIF-1 α , E-cadherin and Vimentin in PC3 cells, along with their invasive ability, in order to analyze the effect of HIF-1 α on EMT and invasion process of human prostate carcinoma cells.

Materials and methods

Cell culture

Human prostate carcinoma cell line PC3 (Basic Medicine Institute, Chinese Medical Academy, China) were incubated in RPMI1640 medium (Gibco, US) in a 37°C humidified chamber with 5% CO₂. The culture condition was adjusted to decrease the O₂ concentration to 1%. After 6 hours of hypoxia incubation, the cell morphology was examined under an inverted microscope.

Real-time PCR

PC3 cells at log-phase normal condition and at hypoxia incubation were collected to extract total RNA using TRIzol kit (Invitrogen, US) following the manual instruction. 200 mg of total RNA were then used to synthesize cDNA, which were used in the following PCR process. Specific primers used in PCR were listed in **Table 1**. PCR conditions were: 95°C denature for 30 sec, 60°C annealing for 30 sec, and 72°C elongation for 30 sec. The reaction was repeated for 40 cycles.

Transwell assay

Cell invasion assay: Using Transwell chamber pre-coated with Matrigel overnight, the basal membrane was incubated in serum-free medium for 1 hour at 37°C. PC3 cells were inoculated in the upper chamber, while the bottom chamber was filled with RPMI1640 medium. At different time points, the chamber was stained by Giemsa dye and the number of invasion cells was counted under an inverted microscope.

Cell migration assay: No artificial basal membrane was used. Other procedures were the same as those in cell invasion assay.

Western blotting

PC3 cells were lysed and extracted for total proteins, which were separated at 8% SDS-PAGE. The proteins were then transferred to PVDF membrane, which was firstly blocked at room temperature for 1 hour, followed by the addition of mouse anti-human HIF-1 α monoclonal antibody (1:200, Santa Cruz, US), mouse anti-human Vimentin monoclonal antibody (1:1 200, Santa Cruz, US), or rabbit anti-human E-cadherin polyclonal antibody (1:1 200, Santa Cruz, US) for overnight incubation. Secondary antibody (goat anti-rabbit, or goat anti-mouse, Santa Cruz, US) with alkaline phosphatase attached was then added for 1-hour incubation, followed by development.

Statistical analysis

SPSS 17.0 software was used to process all collected data, which were presented as mean \pm standard deviation (SD). Measurement data were compared by analysis of variance. Between-group-comparison was performed in student t-test. A statistical significance was defined when P<0.05.

Results

PC3 cell morphology

Under normal condition with sufficient oxygen, PC3 cells displayed epithelial features as polygon shape and regular arrangement. Under artificial hypoxia condition, PC3 cells showed oval or spindle shape, with loosely connection and irregular arrangement (**Figure 1**).

HIF-1 α mRNA level

Using real-time PCR, we detected the mRNA level of HIF-1 α in PC3 cells and found significantly elevated gene expression level in hypoxia cells (P<0.05, **Table 2**).

Protein expression level

We further used Western blotting to detect protein expression level of HIF-1 α , E-cadherin and Vimentin in PC3 cells. Results found elevated

HIF-1 α in prostate cancer

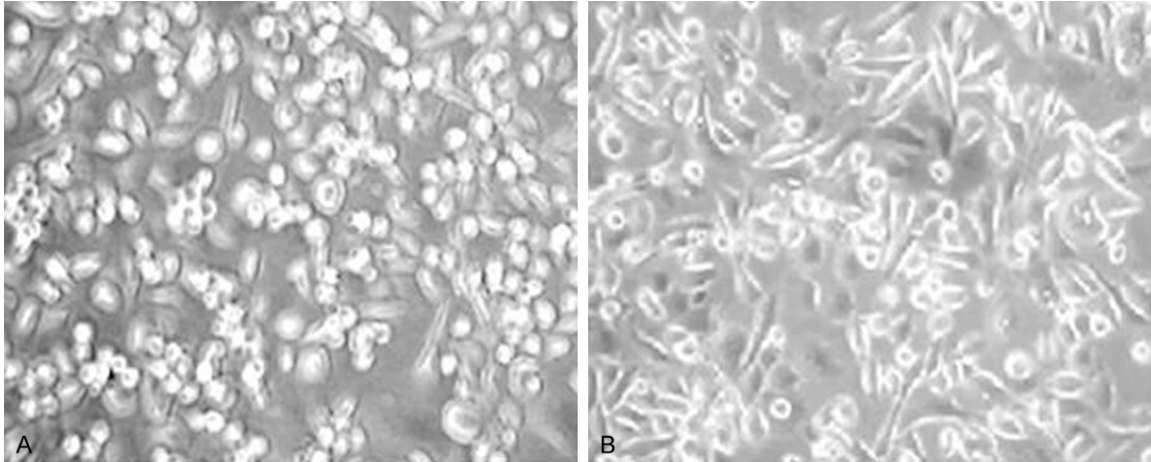


Figure 1. PC3 cell morphology under normal (A) or hypoxia (B) condition ($\times 100$).

Table 2. HIF-1 α mRNA level in PC3 cells

Culture condition	Hypoxia	Normal
HIF-1 α mRNA	6.25 \pm 0.81*	2.79 \pm 0.61

Note: *, P<0.05 compared to PC3 cells under normal condition.

Table 3. EMT related protein expression level

Culture condition	Hypoxia	Normal
HIF-1 α	0.93 \pm 0.12*	0.53 \pm 0.04
E-cadherin	1.06 \pm 0.11*	1.18 \pm 0.13
Vimentin	1.03 \pm 0.15*	0.86 \pm 0.08

Note: *, P<0.05 compared to PC3 cells under normal condition.

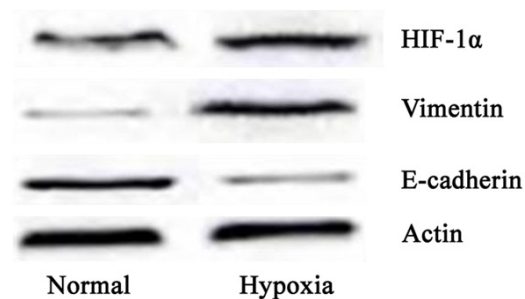


Figure 2. Western blotting of EMT related proteins.

HIF-1 α and Vimentin proteins in hypoxia cells, whose E-cadherin expression level was depressed (P<0.05, **Table 3**; **Figure 2**).

Cell invasion and migration abilities

Using Transwell method to test both invasion and migration abilities of PC3 cells. We found

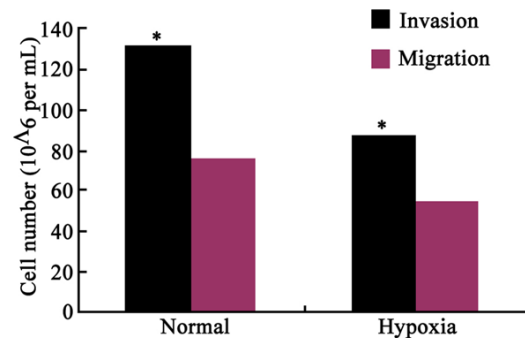


Figure 3. Invasion and migration abilities of PC3 cells. *, P<0.05 compared to PC3 cells under normal condition.

significantly enhanced cell migration and invasion under hypoxia condition (P<0.05, **Figures 3, 4**).

Discussion

Due to the rapid proliferation and high metabolic rate, malignant tumor tissue often suffers from insufficient blood and oxygen supply, making tumor cells under hypoxia status, thus inhibiting cell growth, differentiation and proliferation. Previous study has shown that under hypoxia environment, a series of signaling pathways will be activated to induce the cell apoptosis for inhibiting tumor growth [8]. Meanwhile, normal body tissues under hypoxia condition may facilitate the malignant transformation of tumor cells, especially in inducing EMT for aggravating tumor cells' invasion ability [9]. The response and regulation of body tissue under hypoxia condition is mainly accomplished by

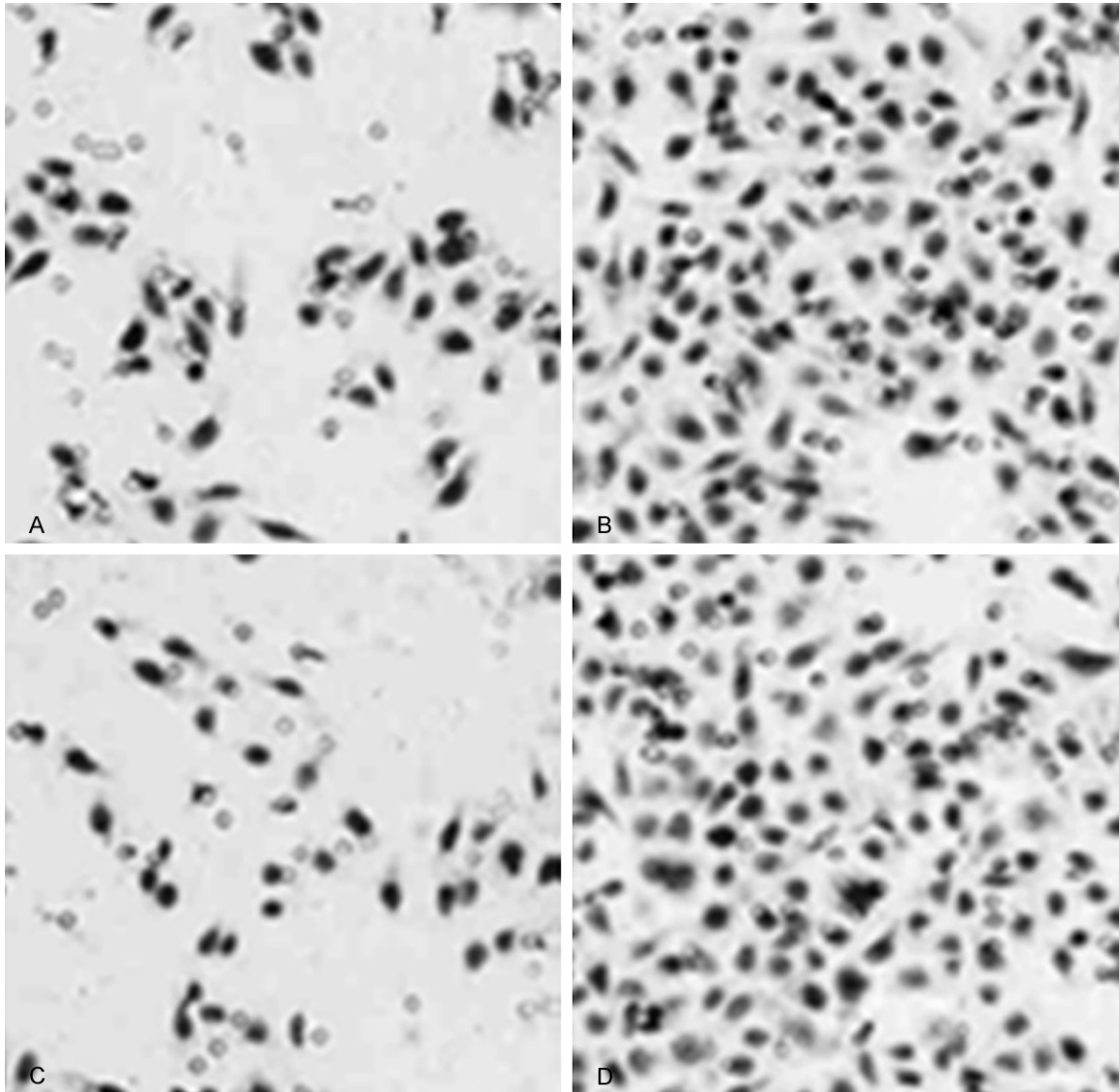


Figure 4. Representative images showing invasion (A and B) and migration (C and D) of PC3 cells. (A and C) Normal culture condition; (B and D) Hypoxia culture.

the dynamic level of HIF-1 α , whose expression level is negatively correlated with oxygen supply in body tissues. Under hypoxia condition, the remarkably increased HIF-1 α may further bind with HIF-1 β for translocation into cell nucleus, where it can regulate downstream target genes for the response to hypoxia condition [10, 11].

EMT is widely occurred in multiple pathological processes of human, as it can facilitate epithelial tumor cells to acquire the ability for focal infiltration and/or distal metastasis. Major pathological features of EMT are the lack of cell polarity, accompanied with acquired markers of mesenchymal cells [12]. In this study we found

remarkable alternation of PC3 cell morphology, as transition from regular polygon tissues under normal condition toward spindle-like cells with irregular distribution after hypoxia stress. The expression of HIF-1 α was significantly elevated under hypoxia, as agreed with previous study showing the induction of EMT by HIF-1 pathway in hypoxia microenvironment [13]. Further evidences showed the transition of epithelial tumor cells from cubic shape to mesenchymal-like spindle shape after EMT [14], as consistent with our observations.

A complete EMT process require three interconnected steps: Firstly, the morphological feature of fibroblasts must be acquired by tumor cells;

Secondly, epithelial marker proteins must be down-regulated while mesenchymal markers were up-regulated; Finally, the degradation of extracellular matrix (ECM) facilitates the migration of tumor cells for focal infiltration or distal metastasis [15]. As one epithelial specific marker, E-cadherin is one Ca²⁺-dependent glycoprotein molecule for transmembrane connection of cells. Vimentin, on the other hand, is one mesenchymal marker and is responsible for cytoskeleton function [16]. This study utilized Western blotting for quantification of E-cadherin and Vimentin, and found elevated Vimentin and depressed E-cadherin proteins in hypoxia treated PC3 cells. EMT is one dynamic process during the migration and metastasis of tumor cells. HIF-1 α may decrease the cell-to-cell adhesion in tumor cells by down-regulating E-cadherin expression, thus removing the intercellular attach and dispatching cells. Meanwhile, the up-regulation of Vimentin can facilitate the velocity of tumor cells for further aggravation of tumor migration. The over-expression of HIF-1 α inside body, on the other hand, can also potentiate tumor invasiveness via inducing EMT of prostate carcinoma cells as previously reported [17].

This study utilized Transwell method to detect migration and invasion abilities of human prostate cancer PC3 cells and found elevated cell invasiveness under hypoxia condition. Previous study has suggested the acquirement of tissue invasion ability across basal membrane and detachment from cellular connections in epithelial tumor cells after EMT [18]. For those tumors with EMT acquirement, repeated radio-/chemotherapy can further aggravate tissue hypoxia, which may further potentiate tumor invasiveness by EMT [19]. EMT can help the entry of tumor cells into blood circulation and lymph tubes for distal metastasis, during which MET, the reverse process of EMT may occur to transform blood-borne tumor cells into epithelial tumors, thus completing the whole metastasis process [20].

In summary, hypoxia microenvironment can up-regulate HIF-1 α expression in human prostate carcinoma PC3 cells, for facilitating morphological transition and enhancement of tissue invasion/migration abilities. The up-regulation of Vimentin and down-regulation of E-cadherin suggested the EMT of tumor cells for further metastasis. The improvement of tumor micro-

environment to block HIF-1 α signaling pathway thus may work as one strategy for inhibiting tumor invasion and metastasis, although detailed mechanisms require further elaborations.

Disclosure of conflict of interest

None.

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HIF-1 α in prostate cancer

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