The Expression of Hypoxia Inducible Factor 1-alpha in Lung Cancer and Its Correlation with P53 and VEGF

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Summary: To investigate the expression of hypoxia inducible factor 1-alpha (HIF-1α) and its correlation with P53 and vascular endothelial growth factor (VEGF), immunohistochemical technique was employed to detect the protein expressions of HIF-1α, P53 and VEGF in specimens from 57 patients with lung cancer. The results indicated that the total positive proportion of HIF-1α expression was 63 % and the HIF-1α expression was more frequent in bronchiole-alveolar carcinoma (86 %) than in other lung cancer. There was a strong association of HIF-1α with VEGF and P53 protein expressions. It is concluded that HIF-1α overexpression is a common event in lung cancer, which may be related to the up-regulation of the angiogenic factor VEGF and oncogene mutant P53 protein.

Key words: hypoxia inducible factor-1alpha; P53; vascular endothelial growth factor; lung cancer

Hypoxia is an acknowledged feature of most solid tumors. The ability of tumors to adapt to a hypoxic microenvironment is increasingly recognized as an important mechanism that promotes tumor growth. Hypoxia inducible factor 1 (HIF-1) is a transcriptional factor that is important in regulaton of the expression of various genes that are overexpressed in the presence of cellular hypoxia such as the proangiogenic cytokine-vascular endothelial growth factor (VEGF). Oxygen deprivation within tumor cells results in an overexpression of HIF-1 and a concomitant increase in VEGF expression. Recent investigation has suggested a role for HIF-1 and VEGF in hypoxia-driven angiogenesis in a wide variety of human cancers. Interestingly, little is known regarding the role of HIF-1 and VEGF in lung cancer. HIF-1 is a heterodimer of 2 basic-helix-loop-helix PAS domain proteins, HIF-1α and HIF-1β. HIF-1α is the oxygen-regulated component that determines HIF-1 activity[1-3]. In this study, we evaluated and characterized the expression of HIF-1α, VEGF and oncogene mutant P53 in human lung cancer specimens.

1 MATERIALS AND METHODS

1.1 Patients and Specimens

Fifty-seven specimens from patients with lung cancer who had undergone partial or total pneumonectomy were obtained from the Department of Pathology at the Tongji Hospital. Specimens were selected to represent the various lung cancers including: squamous carcinoma, adenocarcinoma, bronchiole-alveolar carcinoma, mini cell undifferentiated carcinoma and large cell carcinoma. Sections (4 μ min thickness) were cut from paraffin-embedded specimens and mounted onto glass slides. Matched specimens were stained subsequently for HIF-1α, VEGF and P53.

1.2 Reagents

The polyclonal antibody of HIF-1α was procured from NeoMarker Co, USA. The polyclonal antibody of VEGF and P53 were obtained from Jin-Mei Co, China. SP (streptavidin-biotin peroxidase) Kit and DAB (diaminobenzidine) Kit were from Zhongshan Co, China.

1.3 Immunohistochemistry Techniques

Paraffin sections were dewaxed in xylene and hydrated by descending alcohol series (100 %, 95 %, 70 %, and 50 %). Afterwards, the sections were treated as follows: (1) 30 g/L H_2O_2 10 min; (2) normal goat serum, 20 min at room temperature; (3) HIF-1α, VEGF or P53 antibody (1:500), 4 ℃ for 24 h; (4) successive incubation with biotinized goat anti-rabbit IgG and avidin-biotin-complex (ABC) at room temperature for 45 min; (5) treatment with Tris buffer containing 3 mg/L H_2O_2 and 5 mg/L DAB for 8—10 min. The sections were washed with 0.01 mol/L PBS, (pH=7.3) after steps (3) to (5). Then the sections were dehydrated through ascending alcohol series, cleared with xylene and sealed with neutral balsam. As negative control, the dilution fluid PBS was used instead of HIF-1α, VEGF or P53 antibody.

1.4 The Standard of Judgement

A grading system was utilized to evaluate the results of immunohistochemical staining[4]. Tumor were scored on a 4 point scale according to the intensity and extent of staining: 1 represents tumors with absent or weak cytoplasmic reactivity and no nuclear reactivity; 2 tumors with moderate/strong cytoplasmic reactivity in a percentage of cancer cells lower than the mean value and no nuclear re-

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activity; 3 tumors with moderate/strong cytoplasmic reactivity in a percentage of cancer cells higher than the mean value; 4 tumors with a clear nuclear reactivity (with or without cytoplasmic reactivity regardless of the intensity). Tumors with score 1 and 2 were grouped as low HIF reactivity while tumors with score 3 and 4 as high HIF reactivity. The expressions of VEGF and P53 were evaluated and scored on the basis of percentage of cells that expressed VEGF or P53 antigen (negative, 0 to 50 % positive cells; positive, >50 %)[5].

1.5 Statistical Analysis

Statistical analysis was performed using the analysis of variance and *Chi*-squared test, A P value less than 0.05 was taken as significant.

2 RESULTS

2.1 HIF-1α Expression

HIF-1α expression was both cytoplasmic and/or nuclear (fig. 1—5). Total positive HIF-1α expression was 63 % (36/57). HIF-1α expression was more frequent in bronchiole-alveolar carcinoma than in other lung cancer (86 %), while HIF-1α was not related to histology grade, age, sex and clinical stage (table 1).

Table 1 HIF-1α expression in 57 patients with lung cancer

Parameter	n	HIF-1α				P
		1	2	3	4	
Sex	57					
Male	43	6	10	15	12	
Female	14	2	3	6		>0.05
Age phase						
20-40	8	3	1	2	2	
40-60	30	4	7	9	10	
60-80	19	1	5	10	3	>0.05
Histological type						
Squamous carcinoma	25	4	6	8	7	
High-differentiation	12	1	3	5	3	
Middle-differentiation	6	2	2	1	1	
Low-differentiation	7	1	1	2	3	>0.05
Adenocarcinoma	15	2	4	7	2	
High-differentiation	2	0	0	2	0	
Middle-differentiation	7	1	2	3	1	
Low-differentiation	6	1	2	2	1	>0.05
Bronchiole-Alveolar carcinoma	7	0	1	3	3	
Mini cell undifferentiated carcinoma	6	2	1	1	2	
Large cell carcinoma	4	0	1	2	1	>0.05
TNM						
T_{1-2}	33	2	9	15	7	
T_{3-4}	24	6	4	6	8	
N_0	18	1	6	6	5	
N_1	18	1	3	11	3	
N_2	12	4	2	2	4	
N_3	9	2	2	2	3	
M_0	45	5	11	18	11	
M_1	12	3	2	3	4	>0.05
Clinical stage						
Ⅰ, Ⅱ	34	2	9	16	7	
Ⅲ, Ⅳ	23	6	4	5	8	>0.05

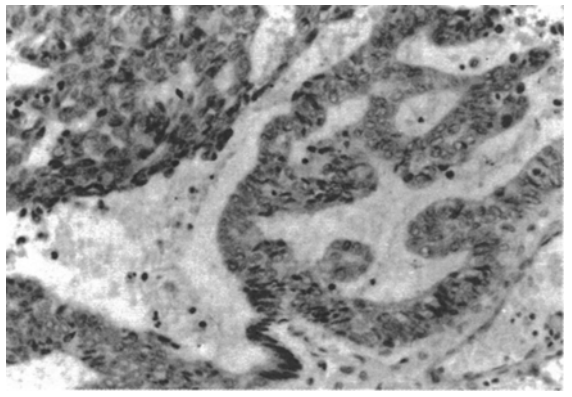

Fig. 1 Expression of HIF-1α in adenocarcinoma, score 1, tumors with weak cytoplasmic reactivity and no nuclear reactivity (IHC×200)

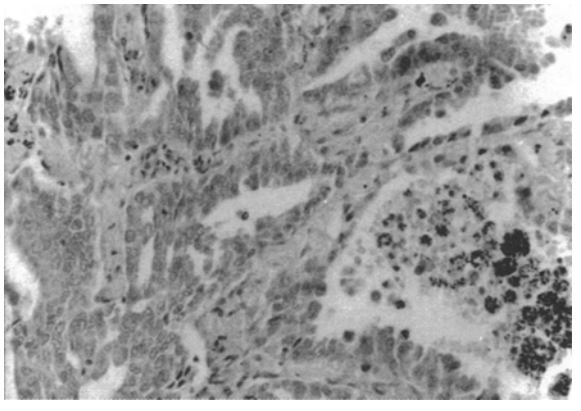

Fig. 2 Expression of HIF-1α in adenocarcinoma, score 2, tumors with moderate cytoplasmic reactivity in a percentage of cancer cells lower than the mean value and no nuclear reactivity (IHC×200)

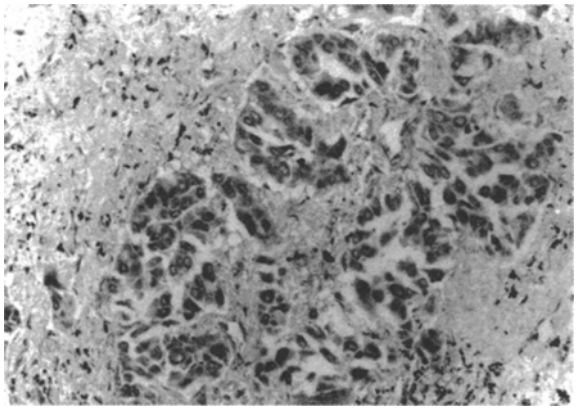

Fig. 3 Expression of HIF-1α in adenocarcinoma, score 3, tumors with strong cytoplasmic reactivity in a percentage of cancer cells higher than the mean value (IHC×200)

2.2 VEGF Expression

VEGF expression was cytoplasmic (fig. 6). Positive proportion of VEGF expression was 70 % (40/57). Correlation analysis of HIF-1α with VEGF expression showed a significant association ($P<0.05$). Out of 36 cases with high HIF-1α expression 29 (81 %) also had high VEGF expres-

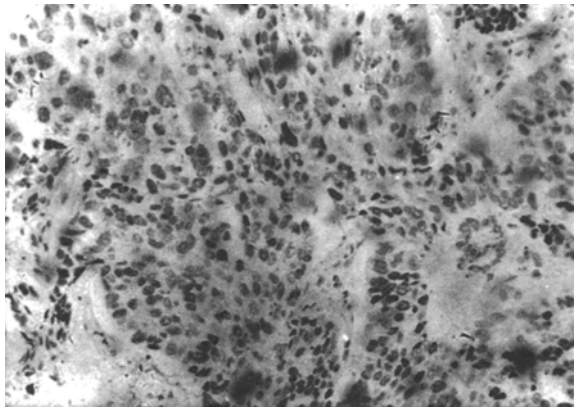

Fig. 4 Expression of HIF-1α in squamous carcinoma, score 4, tumors with a clear nuclear reactivity (IHC×200)

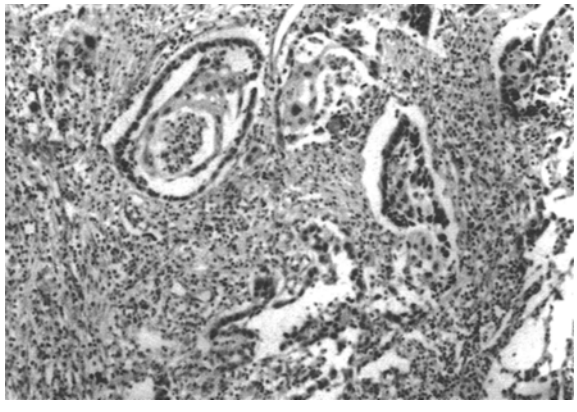

Fig. 5 Expression of HIF-1α in cornified squamous carcinoma(IHC×100)

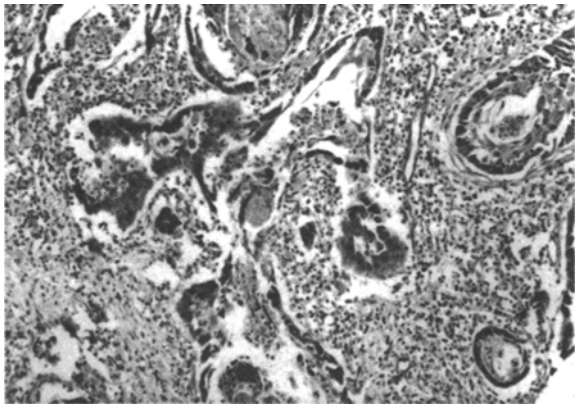

Fig. 6 Expression of VEGF in cornified squamous carcinoma (IHC×100)

sion, while 28 % (11/40) cases with low HIF-1α expression had high VEGF expression.

2.3 P53 Expression

P53 expression was nuclear (fig. 7). Positive proportion of P53 expression was 65 % (37/57). Correlation analysis of HIF-1α with VEGF expression showed a significant association ($P<0.05$). Out of 36 cases with high HIF-1α expression 27 (75 %) had high P53 expression, while 27 % (10/40) cases with low HIF-1α expression had high P53 expression. At the same time, correlation analysis of P53 with VEGF expression showed a significant association ($P<0.05$). Out of 37 cases with high P53 expression 22 (70 %) also had high VEGF expression, while 35 % (14/40) cases with low P53 expression had high VEGF expression.

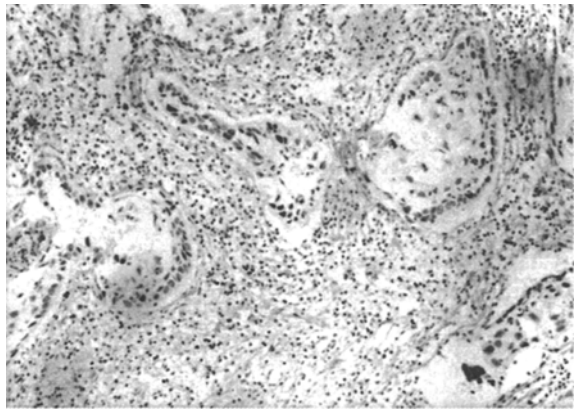

Fig. 7 Expression of P53 in cornified squamous carcinoma. (IHC, ×100)

3 DISCUSSIONS

HIF-1 is from the family of basic helix-loop helix (bHLH) transcription factors that is believed to play an important role in O_2 homeostasis. HIF-1 is composed of 2 distinct subunits: α and β. HIF-1α is a common subunit that is found in all bHLH type proteins. HIF-1α is the unique component of this protein, which is regulated by cellular O_2 levels[1-3]. In response to decreasing levels of oxygen, HIF-1α initiates activation of gene products whose proteins increase the availability of O_2, or allow adaptation to oxygen deprivation. Accordingly, as shown by Zhong *et al*[6], HIF-1α is expressed by various human solid tumors. To date, litter information is available regarding the role of HIF-1α in lung cancer. Our findings showed that HIF-1α expression was both cytoplasmic and/ nuclear. Positive proportion HIF-1α expression was noted in 63 % (36/57). HIF-1α expression was more frequent in bronchiole-alveolar carcinoma than in other lung cancer (86 %). While there was no association of HIF-1α expression with age, sex and clinical stage, its mechanism may be due to the fact that these markers only represent tumor's outside characteristics and don't reflect the degree of tumor hypoxia signals.

Neovascularization has been shown to be important for local tumor growth as well as tumor dissemination and metastasis. In the absence of a neovascular network, tumors cannot grow beyond several millimeters in diameter without outgrowing their blood supply. It is now known that the development of this neovascular network can be in part of a cellular adaptation to hypoxia mediated by the expression of hypoxia-induced gene products. VEGF has been shown to be an important promoter of angiogenesis in various human cancers[7]. HIF-1α serves to regulate transcription of the VEGF gene by binding to a site upstream of the

VEGF gene[8]. The importance of HIF-1α transcriptional regulation of VEGF expression has been confirmed by HIF-1 knockout animal models in which significant reduction of VEGF expression was observed and solid tumor growth was retarded[9]. In this study, positive VEGF expression was noted in 70 % (40/57) of the patients. Moreover, cells expressing HIF-1α were also found to express VEGF, suggesting a relation between HIF-1α and VEGF.

The relationship between HIF-1α and the tumor suppressor P53 is of particular significance. The human P53 tumor suppressor gene encodes a multifunctional transcription factor that mediates cellular responses to diverse stimuli, including DNA damage and hypoxia[10]. In addition to being an integral component of the surveillance mechanisms that arrest cell cycle progression under adverse condition, P53 is also involved in mediating hypoxia-induced apoptosis. Tumor cells subjected to hypoxia undergo P53-mediated apoptosis, which represents a powerful selection for cells that have sustained mutations resulting in P53 loss of function[11]. In unstimulated cells, P53 is bound by MDM2, a ubiquitin-protein ligase that targets P53 for degradation by the proteasome[12,13]. In response to hypoxia, HIF-1α is induced and binds to P53, an interaction that protects P53 from degradation. Instead, MDM2 targets HIF-1α for degradation. Thus, two major consequences of P53 loss-of-function are the prevention of hypoxia-induced apoptosis and increased expression of HIF-1α. The marginal direct association of HIF-1α with P53 nuclear accumulation had been observed by Zhong *et al*[6]. We also found the mutant of P53 enhances HIF-1α expression, the positive proportion P53 expression was noted in 54 % (31/57) of the subjects. The finding supports a relation between P53 and HIF-1α and it may be due to co-induction of both by hypoxia or stabilizing interactions of both proteins.

The genetic alterations responsible for oncogenesis and tumor progression may also underlie the ability of tumor to switch an angiogenic phenotype. Evidence suggest that somatic mutations of the P53 gene positively regulates VEGF exprssion[11]. In this study, we find that out of 37 cases with high P53 expression 22 (70 %) also had high VEGF expression, demonstrating that genetic inactivation of P53 in cancer cells provides a potent stimulus for tumor angiogenesis and providing a novel mechanism by which loss of P53 function contributes to activation of the angiogenic switch in tumors. The mutant of P53 was found to enhance hypoxia-induced HIF-1α expression and augment HIF-1-dependent expression of VEGF in lung cancer, thereby further demonstrating that forced expression of HIF-1α in P53-expressing tumor cells promotes VEGF expression. These findings indicate that inactivation of P53 in tumor cells contributes to activation of the angiogentic switch via amplification of normal HIF-dependent responses to hypoxia.

HIF-1 may play major roles in the development and pathophysiology of lung cancer. Modulation of HIF-1 activity may be of therapeutic utility in patients with lung cancer.

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