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Original article

Overexpression of hypoxia-inducible factor-1 alpha in gastric adenocarcinoma

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Abstract

Background. **The transcriptional factor hypoxia-inducible factor 1α (HIF-1α) controls angiogenesis and metabolism by upregulating hypoxia-induced genes, such as the vascular endothelial growth factor (*VEGF*) gene and the glucose transporter (*GLUT-1*) gene. In addition to its regulation by oncogenes or tumor suppressor genes such as *HER2*, *p53*, *VHL*, and *PTEN*, overexpression of HIF-1α is induced by hypoxia. Increased HIF-1α expression is associated with malignant potential, and with patient prognosis and response to chemoradiotherapy in some cancer types.**

Methods. **We investigated the association between HIF-1α expression and clinicopathological characteristics, including the expression of VEGF and p53 proteins, in gastric cancer. Furthermore, we analyzed the impact of HIF-1α, VEGF, and p53 protein expression on resistance to chemotherapy in advanced gastric cancer.**

Results. **Among 146 specimens from patients with gastric adenocarcinoma, 89 (61.0%), 52 (35.6%), and 102 (69.9%) were positive for HIF-1α, p53, and VEGF expression, respectively. The increased expression of HIF-1α protein correlated significantly with the increased expression of p53 ($P < 0.0001$) and VEGF ($P = 0.0007$). However, overexpression of these proteins was not associated with prognosis or clinicopathological status, with the exception of infrequent distant metastases. Furthermore, overexpression of these proteins was not associated with chemosensitivity in these patients with gastric cancer.**

Conclusion. **Our results indicate that overexpression of HIF-1α correlates significantly with p53 and VEGF protein expression in patients with gastric cancer; however, this overexpression shows no association with clinicopathological status, patient prognosis, or chemosensitivity.**

Key words Hypoxia-inducible factor 1α · p53 · VEGF · Gastric cancer · Immunohistochemistry

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Introduction

Hypoxia is a common feature of rapidly growing solid tumors, because oxygen is only able to diffuse 100–180 mm from the blood capillaries to cells [1]. Therefore, cellular adaptation to hypoxia and altered glucose metabolism are fundamental to the biology of cancer cells. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor for many genes known to control the delivery of oxygen and nutrients through the induction of angiogenesis and glycolysis [2–5]. HIF-1α activates the transcription of vascular endothelial growth factor (VEGF), a key factor in tumor angiogenesis, and the expression of glucose transporters, glycolytic enzymes, and growth factors, which may promote tumor cell survival under hypoxic conditions [5–7].

The expression of HIF-1α is regulated by two different mechanisms. First, the expression of HIF-1α mRNA is upregulated by a signal transduction pathway from a receptor tyrosine kinase such as human epidermal growth factor type 2 (HER2) to downstream molecules such as phosphatidylinositol-3-kinase (PI3K)-protein kinase B (AKT)-FK-506-binding protein-rapamycin associated protein (FRAP) [9–11]. Second, the expression of HIF-1α protein is controlled by the activation and inactivation of oxygen-dependent degradation. The binding of the von Hippel-Lindau tumor suppressor (VHL) protein under normoxic conditions [12–14] and p53 protein expression activate the ubiquitin-dependent degradation of HIF1-α [4].

HIF-1α overexpression has been detected by immunohistochemistry in several human cancers, including those of the brain, bladder, breast, colon, ovary, pancreas, kidney, and prostate [15–23]. Furthermore, HIF-1α overexpression correlates significantly with highly aggressive disease and poor prognosis in some cancer types, such as breast, ovarian, oligodendroglioma, esophageal, and oropharyngeal cancer [16,18,19,24–26]. In addition, the increased expression of HIF-1α

correlates significantly with a poorer response to chemotherapy and/or radiotherapy in esophageal [27], oropharyngeal [26], neck and head cancer [28], and ovarian cancer [24].

However, the clinical significance of HIF-1 expression has not been well studied in gastric adenocarcinoma. In this retrospective study, we examined, by immunohistochemistry the expression of HIF-1α and its downstream molecule; namely, VEGF, and also p53 protein, which associates with HIF-1α protein degradation, in surgical specimens of gastric cancer. We also assessed the relationship between HIF-1α, VEGF, and p53 protein expression and clinicopathological findings of tumors and patient prognosis. Furthermore, we examined the association between the protein expression of HIF-1, VEGF, and p53 and the response to preoperative chemotherapy for advanced gastric cancer.

Patients, materials, and methods

Patients and materials

Paraffin-embedded tumor specimens from 146 patients, representing all patients who had undergone gastrectomy at Osaka University Hospital and affiliated hospitals between 1995 and 2003, were used to assess the clinical significance of protein expression. None of the patients had received preoperative treatment. For the assessment of chemosensitivity, biopsied specimens from 31 patients who had had neoadjuvant chemotherapy were obtained through pretreatment endoscopy. All 31 patients had been treated with combination chemotherapy that included 5-fluorouracil (5-FU) and cisplatin (CDDP; Table 1). Written informed consent was obtained from all patients before collection of specimens.

Evaluation of clinical responses

Clinical responses to chemotherapy were assessed in accordance with WHO criteria as follows: (1) complete response (CR), disappearance of all known disease; (2) partial response (PR), 50% or less decrease in entire tumor burden; (3) no change (NC), less than 50% decrease or less than 25% increase in entire tumor burden; (4) progressive disease (PD), 25% or more increase in the entire tumor burden or the appearance of new lesions. The entire tumor burden was measured by computed tomography scan and endoscopy before and after chemotherapy.

Immunohistochemistry

Paraffin-embedded tissues were cut into 4-μm-thick sections and subjected to immunohistochemical analysis performed by the avidin-biotin-peroxidase complex (ABC) method, using a Histofine SAB-PO Kit (Nichirei, Tokyo, Japan). The primary antibody for HIF-1α protein used in this study was monoclonal IgG 2b (clone H1α 67) (1:50; Novus Biologicals, Littleton, CO, USA). Primary antibodies used for p53 and VEGF proteins were rabbit polyclonal Ab A-20 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and p53 protein (DO-7) mouse monoclonal antibody (1:200; Novocastra Laboratories, Newcastle, UK). After deparaffinization and rehydration, sections were immersed in antigen retrieval buffer at 95 °C for 45 min. Subsequent steps were performed as indicated by the manufacturer. The expression of HIF-1α and p53 was defined as positive if nuclear staining was observed in more than 10% of cancer cells. Cytoplasmic staining for VEGF expression was scored as positive for VEGF overexpression if observed in more than 10% of cancer cells.

Statistical analysis

The correlation between HIF-1α expression and p53 or VEGF was examined using Spearman's correlation. Associations of clinicopathological variables were compared for positive and negative categories of HIF-1α, p53, and VEGF expression, using the χ^2 test or Spearman's test. Overall survival curves were plotted according to the Kaplan-Meier method and were evaluated using the Log-rank test. The significance level was set at 5% for each analysis.

Results

HIF-1α, p53, and VEGF protein expression in gastric cancer

Eighty-nine (61.0%) of 146 gastric cancer specimens were positive for HIF-1α expression, as determined

Table 1. Combination chemotherapeutic regimens used for 31 patients

Combination chemotherapy	Patients
Epirubicin + methotrexate + 5-FU + cisplatin	11
Pirarubicin + leucovorin + mitomycin C + 5-FU + cisplatin	3
Docetaxel + 5-FU + cisplatin	17

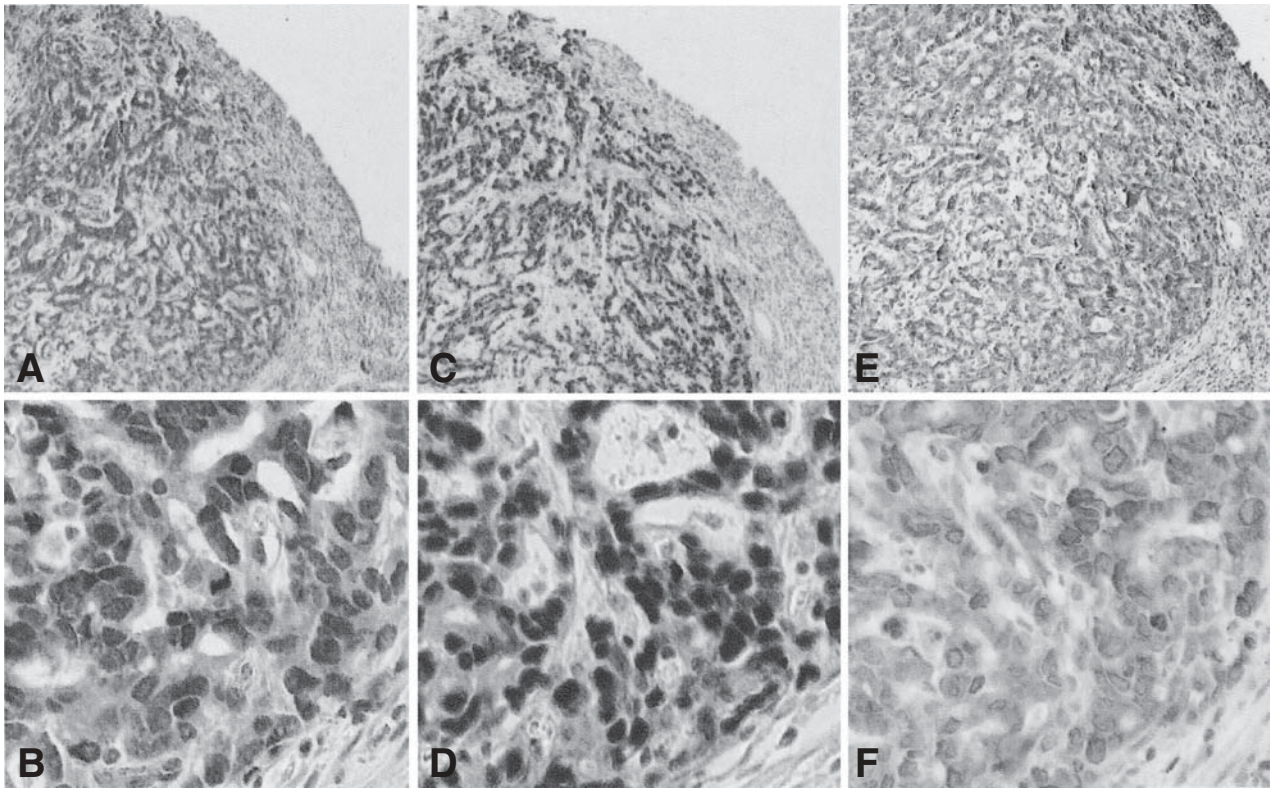

Fig. 1A–F. Immunohistochemical staining for **A** and **B** hypoxia-inducible factor (HIF), **C** and **D** p53, and **E** and **F** vascular endothelial growth factor (VEGF) in representative gastric cancer tissues. **A**, **C**, and **D** ×100; **B**, **D**, and **F** ×400

by immunohistochemistry (Fig. 1A,B). Fifty-two specimens were positive for p53 (Fig. 1C,D), and 102 were positive for VEGF (Fig. 1E,F) expression. The increased expression of HIF-1α protein correlated significantly with the increased expression of p53 ($P < 0.0001$) and VEGF ($P = 0.0007$; Table 2).

Clinicopathological significance of protein expression

HIF-1α protein expression was not associated with histological type, depth of invasion into the gastric wall, lymph node metastasis, blood vessel invasion, lymphatic vessel invasion, or clinical stage (Table 2). Negativity for HIF-1α expression was significantly associated with the development of distant metastasis ($P = 0.0143$). Similarly, negativity for p53 expression was significantly related to distant metastasis ($P < 0.0001$).

Prognostic significance of HIF-1α, p53, and VEGF expression

There was no significant association between the expression of HIF-1α, p53, and VEGF proteins and overall patient survival (Fig. 2).

Association between HIF-1α, p53, and VEGF expression and chemosensitivity

Of the 31 patients assessed for chemosensitivity, 15, 18, and 25 biopsied specimens were positive for HIF-1α, p53, and VEGF expression, respectively. However, there was no association between the expression of these proteins and the clinical response to chemotherapy (Table 3).

Discussion

HIF-α overexpression has been detected in the majority of solid tumors, such as brain, bladder, breast, colon, ovarian, pancreatic, and esophageal cancers [15–23]. Furthermore, in several cancers, HIF-α overexpression has been reported to be associated with poor prognosis and resistance to chemotherapy or radiation therapy [16,18,19,24–28]. However, there have been no reports on HIF-1α status and its clinical significance in gastric adenocarcinoma.

In this study, we examined, by immunohistochemistry, the expression of HIF-1α, VEGF, and p53 proteins in surgical specimens of gastric cancer, and we assessed the clinical significance of the protein expression. Our

Table 2. Correlation between the expression of hypoxia-induced factor (HIF), p53, and vascular endothelial growth factor (VEGF) proteins and various clinicopathological parameters

	HIF protein expression			p53 protein expression			VEGF protein expression		
	(+)	(–)	*P* value	(+)	(–)	*P* value	(+)	(–)	*P* value
Histology									
Differentiated	54	28	0.1714	33	49	0.1288	61	21	0.2253
Undifferentiated	35	29		18	46		41	22	
T									
1	39	18		18	39		32	24	
2	26	18	0.3420	17	27	0.7070	36	8	0.0549
3	18	13		12	19		25	6	
4	6	8		4	10		9	5	
N									
+	36	30	0.1685	25	41	0.4351	49	17	0.2237
–	52	27		25	54		52	26	
M									
0	87	50	0.0143	51	86	<0.0001	97	39	0.3175
1	2	7		0	9		5	4	
Stage									
≦II	46	25	0.3577	22	49	0.3322	46	24	0.2399
IIIa≦	43	32		29	46		56	19	
Vessel invasion									
+	13	11	0.1817	8	16	0.4616	20	4	0.4277
–	72	44		42	74		79	36	
Lymphatic invasion									
+	36	28	0.3885	21	43	0.6542	50	14	0.0545
–	47	27		27	47		46	27	
p53 protein expression									
+	37	14	<0.0001						
–	52	43							
VEGF protein expression									
+	71	31	0.0007						
–	17	26							

Table 3. Association between response to chemotherapy and expression of hypoxia-induced factor (HIF), p53, and vascular endothelial growth factor (VEGF) proteins

	Response to chemotherapy			
Protein expression	PR	NC	PD	*P* value
HIF-1				
(+)	5	8	2	0.8269
(–)	6	8	2	
p53				
(+)	6	10	2	0.9294
(–)	5	6	2	
VEGF				
(+)	9	13	3	0.8248
(–)	2	3	1	

PR, partial response; NC, no change; PD, progressive disease

results showed that increased expression of HIF-1α correlated significantly with VEGF expression in gastric cancer tissues, indicating that HIF-1α upregulated VEGF expression [29]. The *VEGF* gene contains a number of HIF-1 binding sites in its regulatory region, and HIF-1 has been shown to activate the VEGF promoter in vitro [30]. The inhibition of HIF-1α function results in the reduction of VEGF secretion in vitro, and in the inhibition of tumor growth and angiogenesis in vivo [31]. VEGF and HIF-1α are upregulated by cyclooxygenase-2 (COX-2) overexpression through a prostaglandin E2 (PGE2)-dependent pathway [32]. We found here that the increased expression of HIF-1α and VEGF had no association with the clinicopathological findings on prognosis, except for the rare occurrence of distant metastasis. Interestingly, negativity for HIF-1α expression was associated with distant metastasis, including liver and lung metastases. Kuwa et al. [19] reported that high HIF-1α expression had a correlation

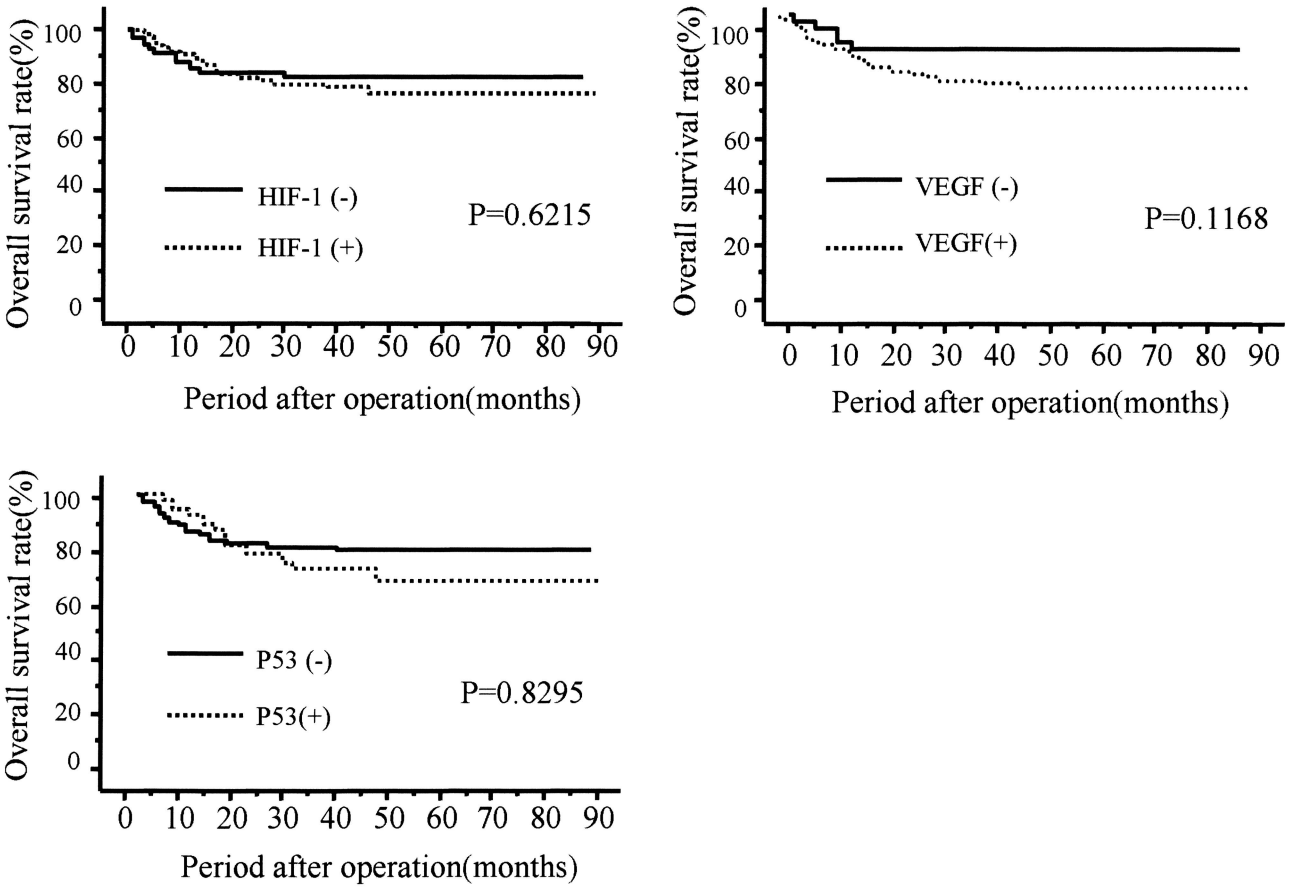

Fig. 2. Association between HIF, p53, and VEGF expression and overall survival rates in 146 patients

with liver metastasis in colorectal cancer and in another study, by Matuyama et al. [25], such a correlation was shown for esophageal carcinoma, but there was no association between HIF-1α expression and distant metastasis [25]. The reason for the less frequent distant metastasis in gastric cancer with HIF-1α overexpression remains unknown. However, highly aggressive gastric tumors with simultaneous distant metastasis may not require the signal transduction pathway of HIF-1α.

Here, we also showed that the overexpression of p53 protein, as determined by immunohistochemistry, correlated significantly with high expression of HIF-1α. HIF-1α is downregulated by the activation of degradation through p53 protein [4]. The accumulation of p53 protein represents a mutation of the *p53* gene, which may cause loss of function of p53 protein. Loss of p53 function may lead to the accumulation of HIF-1α protein because of the decrease in degradation.

We also analyzed the association between HIF-1α expression and chemosensitivity, using biopsied specimens obtained through endoscopy. Overexpression of HIF-1α and VEGF, shown by immunohistochemistry, correlates with therapeutic failure in several cancers [24,26–28]. However, in our study, high expression of HIF-1α, VEGF, and p53 showed no association with resistance to a variety of chemotherapy regimens. Although the number of patient specimens for which chemosensitivity analysis was done was limited ($n = 31$).

In conclusion, we have demonstrated in the present study that HIF-1α protein expression is associated with p53 and VEGF protein expression in gastric carcinoma. Furthermore, lack of HIF-1α and p53 overexpression was significantly associated with the development of simultaneous distant metastases, but not with the development of lymph node and peritoneal metastases. High expression of these proteins in gastric cancer patients did not correlate with patient prognosis or response to chemotherapy. These results suggest that the clinical significance of HIF-1α in gastric cancer may be limited.

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