Hypoxia-inducible factor-1α expression predicts the response to 5-fluorouracil-based adjuvant chemotherapy in advanced gastric cancer

JUN NAKAMURA[1], YOSHIHIKO KITAJIMA[1], KEITA KAI[2], MAYUMI MITSUNO[1], TAKAO IDE[1], KAZUYOSHI HASHIGUCHI[1], MASATSUGU HIRAKI[1] and KOHJI MIYAZAKI[1]

Departments of [1]Surgery and [2]Pathology and Biodefence, Saga University Faculty of Medicine, Nabeshima, Saga 849-8501, Japan

Received May 18, 2009; Accepted June 23, 2009

DOI: 10.3892/or_00000489

Abstract. Hypoxia frequently occurs in various solid tumors, thereby accelerating cancer progression and treatment resistance. Hypoxia-inducible factor-1α (HIF-1α) plays a central role in tumor hypoxia by up-regulating the gene expression related to angiogenesis, cancer invasion and anti-apoptosis. The present study immunohistochemically investigated HIF-1α expression in 63 gastric cancer specimens. Those specimens were obtained from 44 patients that received 5-FU chemotherapy post-operatively whereas the remaining 19 patients did not. The immunostaining pattern of HIF-1α was classified into 3 patterns: diffuse-positive within the tumor (DP), positive at the invasive front of the tumor (FP) and negative (N). Thirty-six of 63 (57.1%) patients exhibited DP, 24 (38.1%) revealed FP and the remaining 3 (4.8%) patients were judged as N. The HIF-1α expression pattern grouped into DP and FP/N correlated with the clinicopathological factors and survival. As a result, the HIF-1α expression did not show a significant correlation with the clinicopathological factors, such as the depth of invasion, lymph node metastasis and tumor stage, nor patient survival in the 63 patients. However, in the 44 patients that underwent chemotherapy, patients with the FP/N pattern showed longer survival than those with the DP pattern. On the other hand, no significant difference in survival was found between the 2 patterns among 19 patients without the chemotherapy. These results indicated that the diffuse expression of HIF-1α in gastric tumors might lead to drug resistance against adjuvant chemotherapy using 5-FU. In conclusion, the assessment of the HIF-1α expression in the resected tissues might predict the drug response to adjuvant 5-FU chemotherapy in advanced gastric cancer patients.

Correspondence to: Dr Kohji Miyazaki, Department of Surgery, Saga University Faculty of Medicine, 5-1-1 Nabeshima, Saga 849-8501, Japan
E-mail: miyazak2@cc.saga-u.ac.jp

Key words: hypoxia-inducible factor-1α, gastric cancer, adjuvant chemotherapy, 5-fluorouracil

Introduction

Gastric cancer is one of the most common malignancies in the world (1) and it represents the second highest cause of cancer-related deaths (1 million deaths per year). Although a surgical resection is essential to cure this malignancy, adjuvant (post-operative) chemotherapy is also important for reducing the rate of recurrence and improving patient survival (2,3). Among several chemotherapeutic agents, 5-fluorouracil (5-FU) has been widely used for adjuvant chemotherapy for gastric cancer. Recently, S-1, a modified oral fluorinated pyrimidine prodrug was developed and to date it has been a first line drug for the treatment of advanced gastric cancer in Japan. S-1 is an oral anti-cancer agent combined with tegafur (FT), 5-chloro-2,4-dihydroxipyridine (CDHP) and potassium oxonate (Oxo) in a molar ratio of 1:0.4:1 (4). According to a randomized control study, a Japanese group has reported that adjuvant chemotherapy with S-1 decreased the proportion of cancer recurrence in patients who have undergone a curative resection for locally advanced gastric cancer (2).

Hypoxia is frequently present in various solid tumors and it is recognized to be a microenvironment resulting in treatment resistance to anti-cancer drugs and a poor prognosis (5,6). Hypoxia-inducible factor-1 (HIF-1) is a transcription factor which was originally reported to up-regulate the oxygen response activator of erythropoietin under hypoxia (7). The HIF-1 expression is regulated by oxygen tension and it plays an essential role in oxygen homeostasis (8,9). HIF-1 is a heterodimer composed of HIF-1α and HIF-1ß subunits. Under normoxic conditions, the expression of HIF-1α is maintained at low levels due to oxygen-dependent polyubiquitination by the von Hippel-Lindau (VHL) tumor suppressor protein, which targets the HIF-1α for proteasomal degradation, whereas HIF-1ß is constitutively expressed. However, the HIF-1α degradation pathway is inhibited in hypoxic conditions, thus leading to the stabilization of the HIF-1α protein (10-14). Stabilized HIF-1α is dimerized with HIF-1ß, then translocates to the nucleus and transactivates the expression of a number of

genes by binding to the hypoxia-responsive element (HRE) on the target genes. More than 60 genes which are involved in glucose transport, angiogenesis, erythropoiesis, vasomotor regulation and survival of cancer cells harbor HREs on the regulatory regions and are activated by HIF-1α (15,16). Numerous studies have demonstrated a significant association between the overexpression of HIF-1α protein and tumor aggressiveness or a poor prognosis in several tumors, including gastric cancer (17-24). Furthermore, growing evidence suggests that hypoxia in tumors selects for cells with decreased potential for apoptosis through the overexpression of anti-apoptotic proteins and decreased killing effects through the up-regulation of drug transporter proteins, thus indicating the involvement of HIF-1α in resistance to standard radiation therapy and chemotherapy under hypoxia (25-29). However, few clinical studies have so far assessed whether the HIF-1α expression in tumors affects drug sensitivity to adjuvant chemotherapy.

The present study investigated the HIF-1α expression in resected cancer tissue specimens from 63 gastric cancer patients, 44 of whom received adjuvant chemotherapy with 5-FU following a gastrectomy. The HIF-1α expression was compared with cancer recurrence and patient survival in order to clarify whether the HIF-1α expression can predict the effects of 5-FU-based adjuvant chemotherapy.

Materials and methods

Patients. Sixty-three patients with advanced gastric cancer who underwent a curative resection at the Department of Surgery, Saga University Hospital (Saga, Japan) from June 2000 to July 2007 were enrolled. Patients who received neoadjuvant (pre-operative) chemotherapy were excluded. None of the patients had hepatic, peritoneal, or distant metastasis or tumor cells in the peritoneal fluid on cytology analysis. The stage classification and the evaluation of resected specimens were performed according to the guidelines of the Japanese Gastric Cancer Association (30). The curative potential of a resection was classified based on surgical and histological observations as follows: Cur A (no residual disease with a high probability of cure), Cur B (no residual disease but not fulfilling the criteria for Cur A) and Cur C (definite residual disease). All of the 63 patients were histologically diagnosed to be Cur B. The 63 patients included 43 (68.3%) men and 20 (31.7%) women, ranging from 26 to 91 years old (mean, 66.9±12.9 years). The prognosis and cancer recurrence in the patients were followed for >6 months (median, 30.1 months; range, 7.9–97.8 months). Among the 63 patients who underwent a surgical resection, 44 (69.8%) received 5-FU-based adjuvant chemotherapy (adjuvant group) and the remaining 19 (30.2%) did not receive the treatment (surgery group) because of advanced age or several complications. The 5-FU-based drugs were 5-FU, FT, S-1 and doxifluridine (5'-DFUR). S-1 alone was orally administered to 34 of 44 (77.3%) patients in the adjuvant group. Oral FT was administered to 2 patients (4.5%) and oral 5'-DFUR to 2 patients (4.5%). Oral FT with intravenous paclitaxel was administered to 1 patient (2.3%). 5-FU with cisplatin (CDDP) was intravenously administered to 1 patient (2.3%). Oral S-1 along with intravenous CDDP was given to 2 patients (4.5%), and oral S-1 with intravenous paclitaxel was administered to 2 patients (4.5%). The median duration of drug administration was 4.63 months (range, 1-47 months). Informed consent for the use of the specimens, which was written on a form approved by the Ethics Committee, was obtained from all patients.

Immunohistochemistry. Immunohistochemical staining was performed according to the procedures described in a previous study with slight modifications (31,32). Briefly, the paraffin-embedded samples were cut into 4-μm thick sections and then were deparaffinized in xylene and rehydrated in a graded series of ethanol. For antigen retrieval, the tissue sections were treated by microwave boiling in 1 mM EDTA (pH 8.0) for 5 min. After quenching the endogenous peroxidase activity in methanol containing 3% hydrogen peroxide for 10 min, the slides were incubated with 10% normal goat serum to block any non-specific binding of the immunoreagents. Next, the primary anti-HIF-1α antibody (clone HI-67, NB100-105, 1:200 dilution; Novus Biologicals, Littleton, CO) was placed onto the slides and the slides were then incubated at room temperature for 2 h. After washing in phosphate-buffered saline (PBS), the slides were incubated with biotinylated anti-mouse antibody conjugated to a peroxidase-labeled dextran polymer (Dako EnVision+, Carpinteria, CA) for 30 min at room temperature. The slides were then washed in PBS, followed by incubation for 3 min at room temperature with chromogen solution from a liquid DAB (3,3-diaminobenzidine) substrate kit (Nichirei Co., Tokyo, Japan). Finally, nuclear counterstaining was done using Mayer's hematoxylin solution. A positive HIF-1α expression was determined if nuclear staining was observed in >10% of the tumor cells. Concomitant cytoplasmic staining was not counted because HIF-1 is a transcription factor functioning in the nucleus. The HIF-1α expression was assessed at the center as well as the invasive front of the tumor in each section.

The staining pattern of HIF-1α was classified into 3 patterns. When the HIF-1α expression was positive in the nucleus at both the cancer central and invasive front, then the staining pattern was designated to be diffuse-positive (DP). When nuclear staining of HIF-1α was found only at the invasive front, then the staining was judged to be front-positive (FP). Finally, a section without any nuclear HIF-1α staining in the cancer cells was assessed as being negative (N).

Statistical analysis. Differences in the mean values were evaluated by Student's t-test and differences in frequencies were analyzed by either Fisher's exact test or the Chi-squared test. Disease-specific survival (DSS) and disease-free survival (DFS) were calculated by the Kaplan-Meier method and then were compared using the log-rank test. In addition, p-values of <0.05 were considered to be statistically significant.

Results

Immunohistochemical staining of HIF-1α. HIF-1α was positive at the center of the tumor in 36 of 63 (57.1%) patients, whereas at the invasive front of the tumor, HIF-1α was judged to be positive in 60 (95.2%) patients. The staining pattern in 36 of 63 (57.1%) patients was diffuse-positive (DP), 24 (38.1%) patients were front-positive (FP), while the remaining 3 (4.8%)

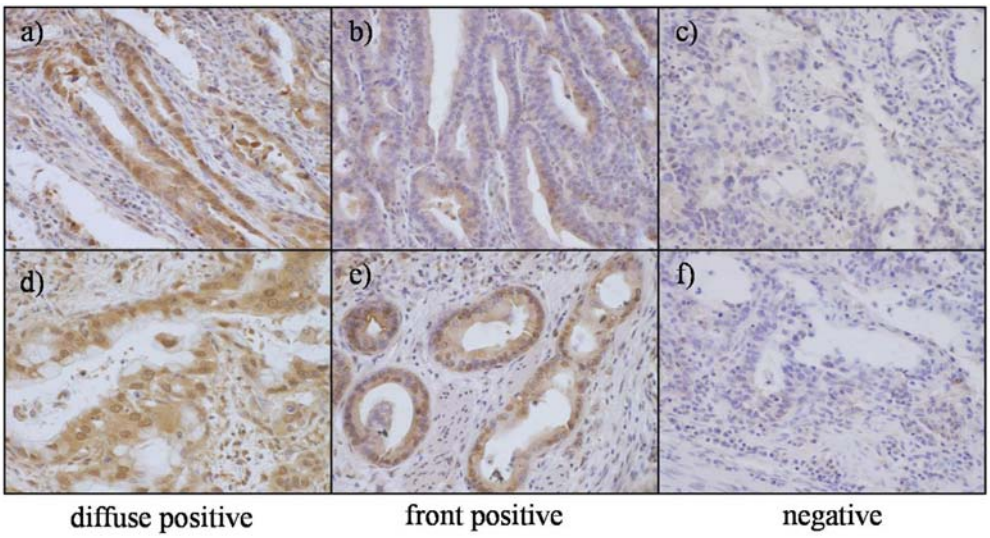

Figure 1. Immunohistochemical analysis of HIF-1α expression in advanced gastric cancer (magnification, x200). Upper panels (a, b and c) show the tumor center and lower (d, e and f) show the invasive front. Each of (a and d), (b and e) and (c and f) are from same case (a and d). The diffuse positive pattern: nuclear staining of HIF-1α is observed in cancer cells at both the tumor center and invasive front (b and e). The front positive pattern: nuclear staining of HIF-1α is found only at the invasive front (c and f). The negative pattern: nuclear staining of HIF-1α is not observed in the tumor center or the invasive front.

Table I. Clinicopathological characteristics of 63 patients.

HIF-1α staining	DP (n=36)	FP/N (n=27)	P-value
Age (average ± SD)	68.28±12.97	65.07±12.90	0.3337
Gender			
M	24	19	
F	12	8	0.7546
Histology			
Differentiated	13	8	
Undifferentiated	23	19	0.5892
T (depth of invasion)			
1/2	11	14	
3/4	25	13	0.0873
N (lymph node metastasis)			
-	3	4	
+	33	23	0.6854
ly (lymphatic invasion)			
-	3	3	
+	33	24	0.9506
v (vascular invasion)			
-	12	13	
+	24	14	0.2343
Stage			
I/II	5	5	
III/IV	31	22	0.8813

patients were negative (N) (Fig. 1). No HIF-1α expression was observed in the normal epithelium of the 63 specimens.

HIF-1α expression pattern and clinicopathological features. Table I shows the relationship between the clinicopathological characteristics and the HIF-1α expression pattern in 63 advanced gastric cancer patients. There were no statistically significant differences between the DP group and FP/N group regarding various factors including gender, histology, depth of cancer invasion (T), lymph node metastasis (N), lymphatic invasion (ly), vascular invasion (v) and tumor stage. Furthermore, the HIF-1α expression pattern did not contribute to the prognosis (data not shown).

Comparison between the surgery group and adjuvant group. The clinicopathological factors and HIF-1α expression were compared between the surgery and adjuvant group (Table II). The patients who received adjuvant chemotherapy were significantly younger than the surgery group (p=0.0037). However, there was no significant difference between the 2 groups regarding the other factors (Table II). In the DSS and DFS curves, the adjuvant group showed better prognosis than the surgery group, however the differences were not statistically significant (p=0.1851, 0.0724, respectively; Fig. 2).

Kaplan-Meier survival analysis of the adjuvant and surgery groups. The relationship between patient survival and the HIF-1α expression pattern was statistically analyzed in the adjuvant and surgery groups (Fig. 3). In the adjuvant group, the DSS as well as the DFS of patients with the DP pattern were significantly worse than patients with FP/N pattern (p=0.0289, 0.0482, respectively). On the other hand, the HIF-1α expression pattern in the surgery group did not correlate with either the DSS or DFS. Finally, the patient survival was compared between the surgery and adjuvant groups in each of the HIF-1α expression patterns (Fig. 4). No significant difference in the DSS and DFS was found between the surgery and adjuvant group in the 36 patients with the DP pattern (Fig. 4). In the 27 patients with FP/N pattern, patients with adjuvant chemotherapy showed significantly longer DSS and DFS (p=0.0039, 0.0036, respectively), than those without chemotherapy.

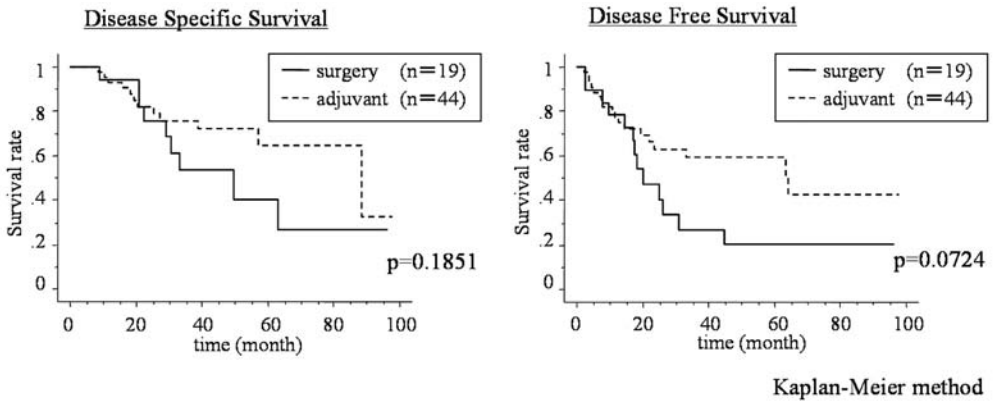

Figure 2. Disease-specific survival (DSS) and disease-free survival (DFS) curves were estimated by the Kaplan-Meier method in all 63 patients. Patients who received adjuvant chemotherapy showed a better prognosis than those who did not, however, the differences were not statistically significant.

Table II. Comparison between surgery group and adjuvant group.

	Surgery group (n=19)	Adjuvant group (n=44)	P-value
Age (average ± SD)	73.95±9.06	63.86±13.24	0.0037
Gender			
M	12	31	
F	7	13	0.5680
Histology			
Differentiated	9	12	
Undifferentiated	10	32	0.2070
T (depth of invasion)			
1/2	7	18	
3/4	12	26	0.7620
N (lymph node metastasis)			
-	2	5	
+	17	39	0.7341
ly (lymphatic invasion)			
-	1	5	
+	18	39	0.7722
v (vascular invasion)			
-	9	16	
+	10	28	0.5900
Stage			
I/II	4	6	
III/IV	15	38	0.7161
HIF-1 expression			
DP	12	24	
FP/N	7	20	0.5261

Discussion

Solid tumors contain hypoxic regions (5), in which HIF-1 protein is stabilized and activated. HIF-1α up-regulates a series of genes involved in angiogenesis, cancer invasion and metastasis, leading to acceleration of cancer malignancy (15,16). We previously demonstrated that tumor-stromal cell interactions under hypoxia increase the invasiveness of pancreatic cancer cells through up-regulated HGF/c-Met signaling via HIF-1α (31). We further reported a significant correlation between the HIF-1α expression and poor prognosis in patients with pancreatic cancer (32). Other studies have also reported a significant association between HIF-1α expression and prognosis in a variety of human cancers including stomach (17), esophagus (18), pancreas (19), lung (20), breast (21), upper urinary tract (22), uterine cervix (23) and ovarian cancer (24). However, few studies have so far addressed the clinical implications of the HIF-1α expression in regard to either chemosensitivity or chemoresistance in cancer patients. A correlation between HIF-1α expression and the effect of adjuvant chemotherapy has been reported in esophageal and breast cancer (33,34). Kurokawa *et al* reported that in 52 patients with esophageal squamous cell carcinoma treated with adjuvant chemotherapy or radiotherapy, an overexpression of HIF-1α was found to be significantly correlated with an unfavorable prognosis (33). Furthermore, Generali *et al* reported that in 187 breast cancer patients who post-operatively received four cycles of the four weekly i.v. CMF regimen (cyclophosphamide, methotrexate and 5-FU), HIF-1α expression was associated with a statistically significant shorter DFS, whereas overall survival was not affected (34). To date, there have been no studies assessing whether or not the HIF-1α expression correlates with the response to post-operative chemotherapy in gastric cancer. The present study investigated HIF-1α expression by assessing the nuclear staining in the central region as well as invasive front of 63 gastric cancer tissues. HIF-1α expression was observed in the central region in 36 of 63 (57.1%) tumors, whereas positive HIF-1α expression at invasive front was observed in 60 of 63 (95.2%) tumors. The staining pattern of HIF-1α was classified into 3 patterns; DP, FP and N (Fig. 1). The patterns were further grouped into DP and FP/N and subjected to comparative analysis with the clinicopathological factors and patient survival. No significant correlation was observed between the HIF-1α expression and the depth of tumor invasion, lymph node metastasis or tumor stage. These results suggested that the patients analyzed in this study were

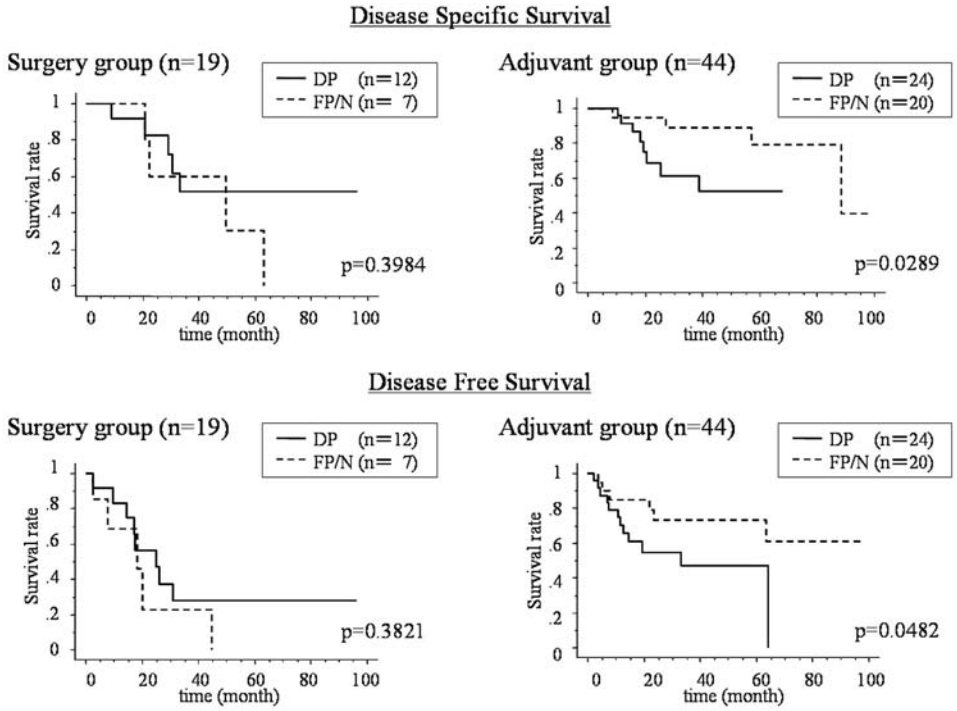

Figure 3. Disease-specific survival (DSS) and disease-free survival (DFS) curves estimated by the Kaplan-Meier method in the surgery group (n=19) and adjuvant group (n=44). In the adjuvant group, the DSS as well as DFS in patients with the DP pattern were significantly worse than those in the FP/N group.

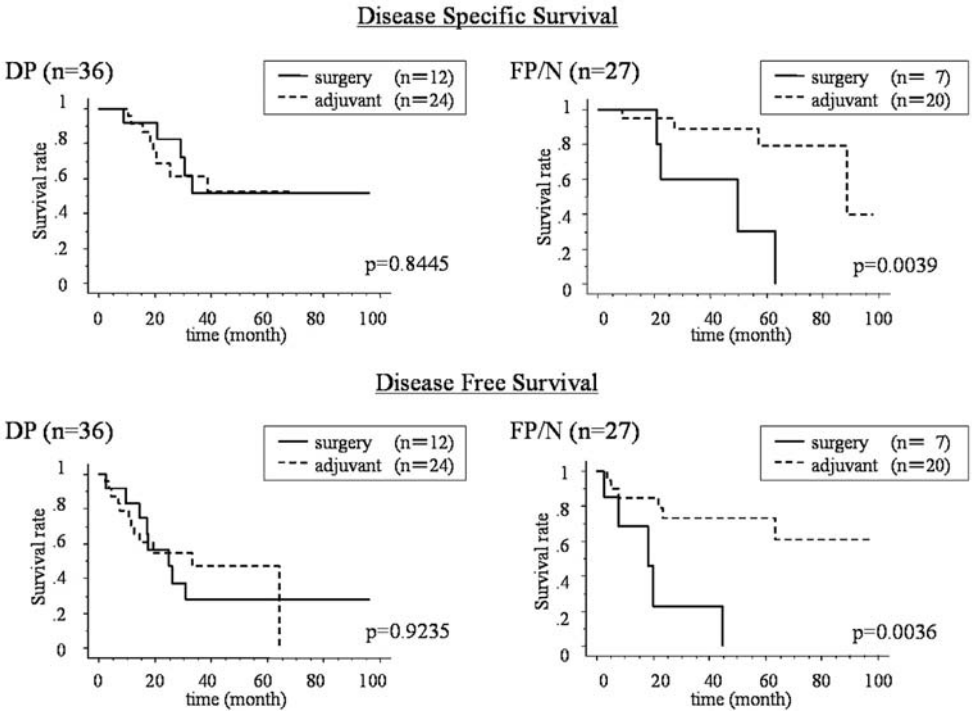

Figure 4. Disease-specific survival (DSS) and disease-free survival (DFS) curves estimated by the Kaplan-Meier method in DP cases (n=36) and FP/N cases (n=27). In the FP/N pattern, the DSS as well as DFS in the surgery group were significantly worse than those in the adjuvant group.

restricted to Cur B (>T3 or >N2) and the similar pathological background resulted in no significant correlation with such factors. In the 44 patients who underwent adjuvant chemotherapy after the operation, the DSS and DFS of patients with the DP pattern were significantly worse than those with the FP/N pattern (p=0.0289, 0.0482, respectively; Fig. 3). This result indicates that the patients with the DP pattern might be more resistant to adjuvant 5-FU treatment and thus experienced earlier cancer recurrence, in comparison to those with the FP/N pattern. Furthermore, in the 27 patients with the FP/N pattern, the DSS and DFS of patients in the adjuvant group were significantly longer than those in the surgery group

(p=0.0039, 0.0036, respectively; Fig. 4). In contrast, no significant difference was found between the adjuvant and surgery group among the 36 patients expressing DP pattern (Fig. 4). Taken together, the results directly indicated that adjuvant chemotherapy using 5-FU is effective in patients with the FP/N, but not those with DP pattern of HIF-1α. HIF-1α is involved in hypoxia induced drug resistance by suppressing drug-induced apoptosis by enhancing the Bcl-2/Bax ratio (25). In addition, HIF-1α expression reduces vincristine-induced apoptosis in gastric cancer, through modulation of the expression of apoptotic proteins such as Bcl-2, Bid, leading to resistance to chemotherapeutic agents (35). Furthermore, the expression of the anti-apoptotic protein IAP-2 (the inhibitor of apoptosis protein 2), which inhibits the translocation of the proapoptotic protein Bax to the mitochondria, is also induced by hypoxia (36). These *in vitro* studies suggest the possibility that gastric cancer cells with the DP HIF-1α pattern express anti-apoptotic factors and reveal resistance against adjuvant chemotherapy more than those with the FP/N. The current study analyzed 63 patients who were post-operatively diagnosed to be CurB, suggesting that several cancer cells were possibly viable even after the curative operation. The residual cells with the DP pattern might exhibit more resistance against 5-FU treatment and cause earlier recurrence, in comparison to those with the FP/N pattern. On the other hand, the question of whether hypoxia exists in all of the cancer cells expressing the DP pattern must be considered. Currently, other studies have demonstrated hypoxia independent induction of HIF-1α (37-41). Transforming growth factor-ß1 (TGF-ß1) induces HIF-1 stabilization through the selective inhibition of HIF-1α associated prolyl hydroxylase 2 (PHD2) expression under normoxic conditions (38). Therefore, some factor, other than hypoxia may contribute to either the HIF-1α overexpression or stabilization in gastric cancer cells expressing the DP pattern.

In conclusion, we demonstrated for the first time that HIF-1α expression is a predictive marker of the response to adjuvant chemotherapy for advanced gastric cancer.

A favorable effect of 5-FU adjuvant treatment might therefore be expected in the patients with the FP/N pattern of HIF-1α expression, however, additional treatment using other drugs such as HIF-1α inhibitor should be considered in patients with the DP pattern.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics, 2008. CA Cancer J Clin 58: 71-96, 2008.
2. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, Furukawa H, Nakajima T, Ohashi Y, Imamura H, Higashino M, Yamamura Y, Kurita A and Arai K: ACTS-GC Group: Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 357: 810-820, 2007.
3. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, Haller DG, Ajani JA, Gunderson LL, Jessup JM and Martenson JA: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 345: 725-730, 2001.
4. Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K and Fukushima M: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7: 548-557, 1996.
5. Hockel M and Vaupel P: Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93: 266-276, 2001.
6. Griffiths EA, Pritchard SA, Welch IM, Price PM and West CM: Is the hypoxia-inducible factor pathway important in gastric cancer? Eur J Cancer 41: 2792-2805, 2005.
7. Semenza GL and Wang GL: A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12: 5447-5454, 1992.
8. Wang GL and Semenza GL: General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci USA 90: 4304-4308, 1993.
9. Wang GL and Semenza GL: Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 270: 1230-1237, 1995.
10. Huang LE, Arany Z, Livingston DM and Bunn HF: Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 271: 32253-32259, 1996.
11. Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Smenza GL and Bedi A: Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 14: 34-44, 2000.
12. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER and Ratcliffe PJ: The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399: 271-275, 1999.
13. An WG, Kanekal M, Simon MC, Maltepe E, Blagosklonny MV and Neckers LM: Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. Nature 392: 405-408, 1998.
14. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim Av, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW and Ratcliffe PJ: Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292: 468-472, 2001.
15. Semenza GL: HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 8: s62-s67, 2002.
16. Semenza GL: Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3: 721-732, 2003.
17. Sumiyoshi Y, Kakeji Y, Egashira A, Mizokami K, Orita H and Maehara Y: Overexpression of hypoxia-inducible factor 1alpha and p53 is a marker for an unfavorable prognosis in gastric cancer. Clin Cancer Res 12: 5112-5117, 2006.
18. Koukourakis MI, Giatromanolaki A, Skarlatos J, Corti L, Blandamura S, Piazza M, Gatter KC and Harris AL: Hypoxia inducible factor (HIF-1α and HIF-2α) expression in early esophageal cancer and response to photodynamic therapy and radiotherapy. Cancer Res 61: 1830-1832, 2001.
19. Sun HC, Qiu ZJ, Liu J, Sun J, Jiang T, Huang KJ, Yao M and Huang C: Expression of hypoxia-inducible factor-1 alpha and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. Int J Oncol 30: 1359-1367, 2007.
20. Giatromanolaki A, Koukourakis MI, Sivridis E, Pastorek J, Wykoff CC, Gatter KC and Harris AL: Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. Cancer Res 61: 7992-7998, 2001.
21. Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM, Semenza GL, van Diest PJ and van der Wall E: Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 97: 1573-1581, 2003.
22. Nakanishi K, Hiroi S, Tominaga S, Aida S, Kasamatsu H, Matsuyama S, Matsuyama T and Kawai T: Expression of hypoxia-inducible factor-1alpha protein predicts survival in patients with transitional cell carcinoma of the upper urinary tract. Clin Cancer Res 11: 2583-2590, 2005.
23. Burri P, Djonov V, Aebersold DM, Lindel K, Studer U, Altermatt HJ, Mazzucchelli L, Greiner RH and Gruber G: Significant correlation of hypoxia-inducible factor-1alpha with treatment outcome in cervical cancer treated with radical radiotherapy. Int J Radiat Oncol Biol Phys 56: 494-501, 2003.
24. Birner P, Schindl M, Obermair A, Breitenecker G and Oberhuber G: Expression of hypoxia-inducible factor 1alpha in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. Clin Cancer Res 7: 1661-1668, 2001.

25. Liu L, Ning X, Sun L, Zhang H, Shi Y, Guo C, Han S, Liu J, Sun S, Han Z, Wu K and Fan D: Hypoxia-inducible factor-1 alpha contributes to hypoxia-induced chemoresistance in gastric cancer. Cancer Sci 99: 121-128, 2008.
26. Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP and Schuetz JD: The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with Heme. J Biol Chem 279: 24218-24225, 2004.
27. Papandreou I, Krishna C, Kaper F, Cai D, Giaccia AJ and Denko NC: Anoxia is necessary for tumor cell toxicity caused by a low-oxygen environment. Cancer Res 65: 3171-3178, 2005.
28. Kinoshita M, Johnson DL, Shatney CH, Lee YL and Mochizuki H: Cancer cells surviving hypoxia obtain hypoxia resistance and maintain anti-apoptotic potential under reoxygenation. Int J Cancer 91: 322-326, 2001.
29. Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, Miller C, Demonacos C, Stratford IJ and Dive C: Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Mol Cell Biol 24: 2875-2889, 2004.
30. Japanese Gastric Cancer Association: Japanese classification of gastric carcinoma. 2nd English edition. Gastric Cancer 1: 10-24, 1998.
31. Ide T, Kitajima Y, Miyoshi A, Ohtsuka T, Mitsuno M, Ohtaka K, Koga Y and Miyazaki K: Tumor-stromal cell interaction under hypoxia increases the invasiveness of pancreatic cancer cells through the hepatocyte growth factor/c-Met pathway. Int J Cancer 119: 2750-2759, 2006.
32. Ide T, Kitajima Y, Miyoshi A, Ohtsua T, Mitsuno M, Ohtaka K and Miyazaki K: The hypoxic environment in tumor-stromal cells accelerates pancreatic cancer progression via the activation of paracrine hepatocyte growth factor/c-Met signaling. Ann Surg Oncol 14: 2600-2607, 2007.
33. Kurokawa T, Miyamoto M, Kato K, Cho Y, Kawarada Y, Hida Y, Shinohara T, Itoh T, Okushiba S, Kondo S and Katoh H: Over-expression of hypoxia-inducible-factor 1alpha(HIF-1alpha) in oesophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. Br J Cancer 89: 1042-1047, 2003.
34. Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, Gandolfi V, Dogliotti L, Bottini A, Harris AL and Fox SB: Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res 12: 4562-4568, 2006.
35. Zhou J, Schmid T, Schnitzer S and Brune B: Tumor hypoxia and cancer progression. Cancer Lett 237: 10-21, 2006.
36. Dong Z, Nishiyama J, Yi X, Venkatachalam MA, Denton M, Gu S, Li S and Qiang M: Gene promoter of apoptosis inhibitory protein IAP2: identification of enhancer elements and activation by severe hypoxia. Biochem J 364: 413-421, 2002.
37. Frede S, Berchner-Pfannschmidt U and Fandrey J: Regulation of hypoxia-inducible factors during inflammation. Methods Enzymol 435: 405-419, 2007.
38. McMahon S, Charbonneau M, Grandmont S, Richard DE and Dubois CM: Transforming growth factor beta1 induces hypoxia-inducible factor-1 stabilization through selective inhibition of PHD2 expression. J Biol Chem 281: 24171-24181, 2006.
39. Hellwig-Bürgel T, Rutkowski K, Metzen E, Fandrey J and Jelkmann W: Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 94: 1561-1567, 1999.
40. Blouin CC, Pagé EL, Soucy GM and Richard DE: Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha. Blood 103: 1124-1130, 2004.
41. Metzen E, Zhou J, Jelkmann W, Fandrey J and Brüne B: Nitric oxide impairs normoxic degradation of HIF-1alpha by inhibition of prolyl hydroxylases. Mol Biol Cell 14: 3470-3481, 2003.