Prognostic Impact of Hypoxia-Inducible Factors 1α and 2α in Colorectal Cancer Patients: Correlation with Tumor Angiogenesis and Cyclooxygenase-2 Expression

Hiroshi Yoshimura, Dipok Kumar Dhar, Hitoshi Kohno, Hirofumi Kubota, Toshiyuki Fujii, Shuhei Ueda, Shoichi Kinugasa, Mitsuo Tachibana, and Naofumi Nagasue

Department of Digestive and General Surgery, Shimane Medical University, Izumo, Japan

ABSTRACT

Purpose: **Angiogenesis plays an important role in a multitude of biological processes including those of tumorigenesis and cancer progression. Hypoxia is the prime driving factor for tumor angiogenesis and the family of hypoxia-inducible factors (HIFs) plays a pivotal role in this process. The role of HIF in tumor angiogenesis has been underscored in different carcinomas but yet to be reported for colorectal carcinomas.**

Experimental Design: **In this study, we examined HIF [HIF-1α (HIF1) and HIF-2α (HIF2)] expression in 87 curatively resected colorectal carcinoma samples, and the results were correlated with clinicopathological factors, microvessel density, cyclooxygenase 2 expression, and patient prognosis.**

Results: **HIF1 (44.8%) was more frequently expressed than HIF2 (29.9%). Most of the clinicopathological factors representing the tumor aggressiveness were significantly correlated with overexpression of HIF2 but not with HIF1 expression. HIF2 expression had direct correlation with microvessel density and cyclooxygenase 2 expression. and, in contrast, HIF1 expression had a weak but significant inverse correlation in T_1 and T_2 tumors only. HIF2 expression alone and the combined expression of HIF1 and HIF2 had significant impact on patient survival. In the multivariate analysis, however, only the combined expression of HIF1 and HIF2 remained independently significant.**

Conclusions: **Taken together, our results suggest that HIF2 expression may play an important role in angiogenesis and that the combined expression of HIF1 and HIF2 may play an important role in tumor progression and prognosis of colorectal carcinomas. Therefore, HIF expression could be a useful target for therapeutic intervention.**

INTRODUCTION

The colorectal cancers rank third in terms of the incidence and mortality in the year 2000, with almost equal numbers in men and women (ratio, 1.1:1; Ref. 1). An increasing trend in the incidence of this carcinoma has been noticed in the Asian nations including Japan. Despite all-out efforts to prevent the recurrence of the disease, certain percentages of patients succumb to disease recurrence and ultimately death.

Formation of new blood vessels (neovascularization or angiogenesis) is a prerequisite for tumor growth beyond a few millimeters (2). The newly formed blood vessels are the key regulators of tumor growth and metastasis and are reported to be a useful prognostic marker in almost all carcinomas including colorectal cancers (3–5). Among the several angiogenic growth factors, the *hypoxia-inducible factor (HIF)* gene family is underscored as a prime driving factor for the angiogenic switch that transcribes the downstream genes for angiogenesis (6). HIF is a basic helix-loop-helix Per-ARNT-Sim transcription factor and exists as an αβ heterodimer. HIF-1β expresses constitutively and does not vary according to the hypoxic status of the cells, whereas the α subunit accumulates rapidly inside the hypoxic cells primarily because of the prevention of ubiquitation and proteosomal degradation of the protein, which usually take place in normoxic cells. There are three homologues of the α subunit (HIF-1α, HIF-2α, and HIF-3α). Among them, HIF-1α and -2α are thought to play a significant role in tumor neovascularization. Besides the role in tumor angiogenesis, high expression of HIF has been correlated with frequent mutations of tumor suppressor genes (*von Hippel-Lindau, p53,* and *PTEN*), enhanced cellular proliferation, decreased apoptosis, and development of drug resistance to chemotherapeutic agents.

Both HIF-1α (HIF1) and HIF-2α(HIF2) are widely expressed by various cancers and have been proved to be significant prognostic markers in selected cancers (7–10). To date, only two studies have evaluated the expression of HIF1 in a limited numbers of colorectal carcinomas. However, the precise correlation of HIF (1α and 2α) expression with specific clinicopathological features and the overall impact on patient prognosis have not been evaluated in colorectal cancer patients (11, 12). Recently, we found that cyclooxygenase-2 (COX-2) was overexpressed in tumors with high microvessel density (MVD); however, the exact mechanism of this association is not known (5). To that end, we set out to address these issues in 87 curatively resected colorectal carcinoma patients.

PATIENTS AND METHODS

Eighty-seven curatively resected colorectal carcinoma patients of different Dukes stages were selected randomly from

Received 6/18/03; revised 3/31/04; accepted 4/6/04.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Hiroshi Yoshimura, Department of Digestive and General Surgery, Shimane Medical University, Izumo 693-8501, Japan. Phone: 81-853-202235; Fax: 81-853-202229; E-mail: hyoshi@shimane-med.ac.jp.

patients operated on between 1990 and 1999. None of them had any preoperative radiochemotherapy. Of these patients, 36 were female and 51 were male; their ages ranged from 29 to 91 years. Immediately after resection, all of the specimens were opened in the operating room and photographed for documentation. According to a prospective protocol, tumor and all of the lymph nodes were cut at several levels and embedded in paraffin, and sections were taken for routine H&E staining. Experienced pathologists checked all of these slides and documented the pathological characteristics of the tumor and lymph nodes. Tumor stage was defined according to the Union International Contre Cancer (UICC) Tumor-Node-Metastasis (TNM; 1997) staging system. The follow-up was complete in all of the patients. The data obtained at regular follow-up visits at the outpatient department were stored in a database specially designed for colorectal carcinoma patients. An update inquiry about the present status of all surviving patients was made by telephone call in December 2001.

Immunohistochemistry. Representative paraffin sections containing both the normal mucosa and the invasive front of the tumor tissue were selected for the immunohistochemical staining. Also of the metastatic liver tissue specimens from selected patients were used for the immunohistochemistry. Serial sections were stained for HIF1 and HIF2 with the Catalyzed Signal Amplification system (Dako Corporation, Carpinteria, CA). Briefly, slides were deparaffinized, rehydrated in graded alcohols, and placed in Tris-buffered saline solution. Antigen retrieval was done by autoclaving the slides for 10 min in citrate buffer (pH 6.0; Dako, Denmark). Endogenous peroxidase was blocked by incubating the slides in 0.3% H_2O_2 for 15 min. Sections were incubated for 15 min with normal serum from donor species of secondary antibody followed by an overnight incubation with the primary antibodies [anti-HIF1 (clone ESEE122) and anti-HIF2 (clone ep190b); Novus Biologicals, Inc., Littleton, CO; 1:1000 dilution]). Link antibody was applied for 15 min. Sections were incubated for 15 min with streptavidin biotin complex and then another 15 min with an amplification reagent. Streptavidin-peroxidase solution was applied, and the sections were incubated for 15 min. Finally, peroxidase activity was developed with AEC (3-amino-9-ethylcarbazole) solution. In between the steps, slides were washed in Tris-buffered saline. Counterstaining was done with Meyer's hematoxylin. A positive and a negative control slide were always included in each immunostaining.

Tumor blood vessels and COX-2 were stained by a universal immunoenzyme polymer method as described previously (5). Mouse monoclonal antibodies against CD34 (CD34 Class II; DAKO, Glostrup, Denmark; dilution 1:50 for 1.5 h at room temperature) and COX-2 (Cayman Chemical Co., Ann Arbor, MI; dilution 1:300, for 14 h at 4°C) were used.

Evaluation of Staining. Two individual authors (H. Y. and D. K. D.) separately investigated all of the slides. The accordance of the results in HIF1 and HIF2 staining between the two authors was 78.2 and 85.1%, respectively. Using a conference microscope, we evaluated discordant cases and reached a final decision without difficulty. Intra-observer variability was not determined. The extent and intensity of the cytoplasmic staining were measured separately, and a final staining score was calculated by multiplying the score of extent and intensity of staining as reported previously (5). When nuclear staining was present in ≥5% of the tumor cells, the sample was considered nuclear positive. Because of variable nuclear and cytoplasmic staining, the cases with nuclear positivity and/or with the highest staining score for cytoplasmic staining were considered to be HIF positive.

For MVD, hypervascular areas were selected under low magnification, and the three most hypervascular areas were counted at ×200 magnification. Any single endothelial cell or cluster of endothelial cells that were positive for CD34 and that were with or without any lumen were counted as a single microvessel. The mean value for three fields was regarded as the MVD for each tumor. COX-2 positivity was determined as described previously (5).

Statistical Analysis. The correlation between HIF expression and various clinicopathological parameters was determined by the Student *t* test, Fisher's exact test, or the Mann-Whitney *U* test as appropriate. Correlation between two continuous variables was determined by the Spearman's rank test. The survival curves were plotted using the Kaplan-Meier method, and the statistical significance between groups was determined by the log- rank test. The end points for analysis were the disease-free survival and the overall survival starting from the day of operation. Independent variables predicting survival were evaluated by the multiple stepwise regression analysis using the Cox model. The Statview 4.5J (Abacus Concepts, Berkeley, CA) software was used for data analysis.

RESULTS

HIF Expression in Normal Mucosa and Cancers. Both HIF1 and HIF2 were expressed occasionally in the luminal border of the normal mucosa. The staining was weak and cytoplasmic, and there was no nuclear staining in any instance in the normal mucosal cells. In contrast, the staining pattern was variable in tumors. HIF expression was noticed in the cytoplasm and/or nucleus of the tumor cells (Fig. 1, *A* and *B*). The staining was prominent in the advancing border of the tumor (Fig. 1C). This was true for both HIF1 and HIF2. Interstitial cells, most likely macrophages, were frequently positive for both HIF1 and HIF2 (Fig. 1*E*). Similarly, although it was not as frequent, distinct staining for HIF was noticed in the endothelial cells lining the blood vessels in the vicinity of tumors (Fig. 1*F*). Occasionally, the cytoplasmic staining for HIF2 was noticed as aggregates at the luminal border of the tumor cells (Fig. 1*D*).

Relationship between HIF and Clinicopathological Findings. Thirty-nine (44.8%) and 26 (29.9%) tumors were positive for HIF1 and HIF2, respectively. Both HIF1 and HIF2 were positive in 12 cases, and both were negative in 34 cases. There was no direct correlation between HIF1 and HIF2 expression by Spearman's rank test. The associations between HIF expression and clinicopathological variables are shown in Table 1. HIF1 had no correlation with any of the clinicopathological parameters as evaluated in this study. There were tendencies of higher expressions in females and in early stages of cancers, but neither reached a statistical significance. In contrast, HIF-2 expression increased significantly with the advancement of the T stage ($P < 0.01$) and the Dukes stage ($P < 0.05$) of the tumor and lymphatic/vascular invasion ($P < 0.05$). There was a

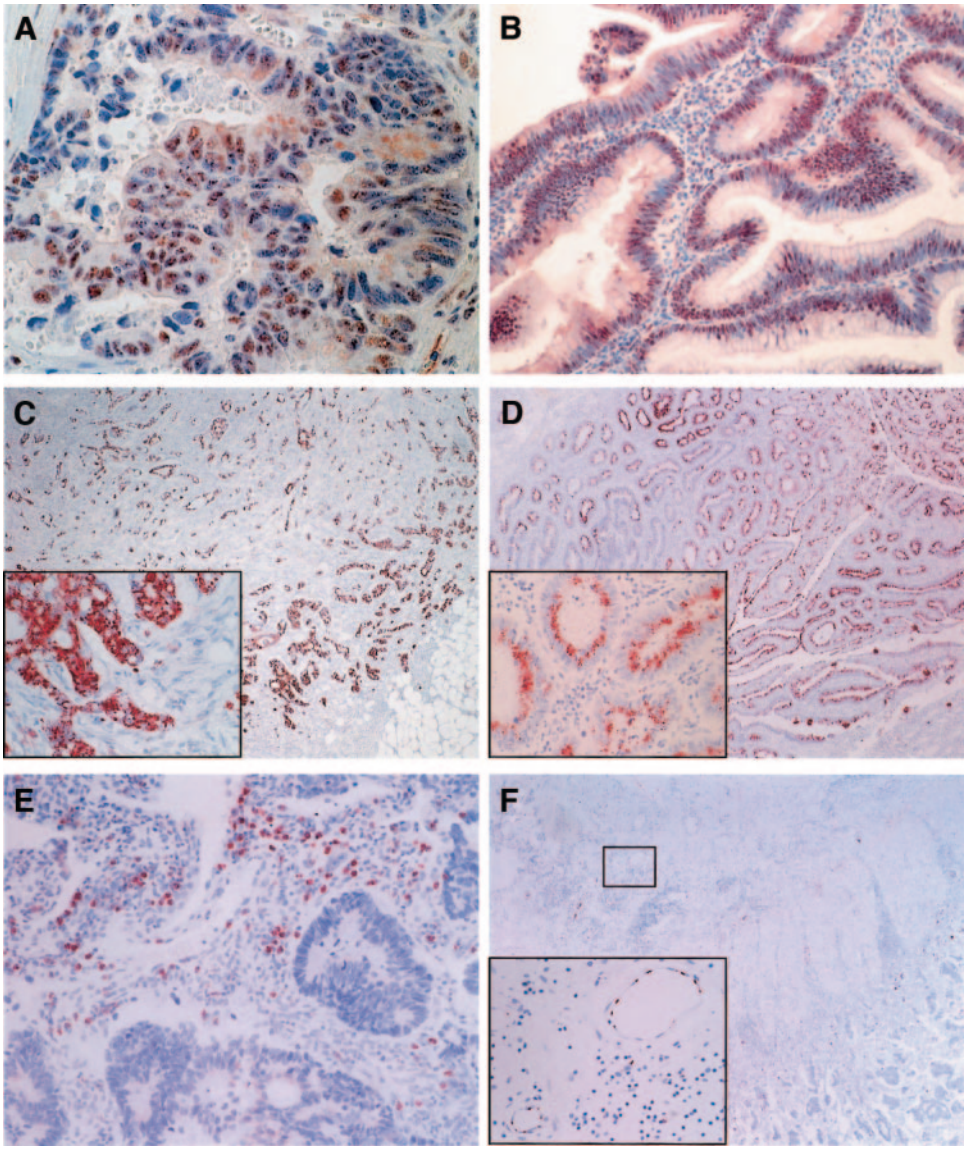

Fig. 1 The expression patterns of HIF-1α (HIF1) and HIF-2α (HIF2) are shown. Prominent nuclear staining was noticed for both HIF1 (×100; *A*) and HIF2 (×40; *B*) in colorectal carcinomas. *C,* a tumor sample with strong cytoplasmic staining for HIF2 in the advancing border of tumor is shown (×20; *inset,* ×200). *D,* cytoplasmic staining for HIF2 accumulating near the luminal border is shown (×40; *inset,* ×200). *E,* tumor infiltrating cells strongly stained for HIF2 are shown (×40). *F,* endothelial cells of the tumor-associated blood vessels stained for HIF2 are shown (×20; *inset,* ×200).

significant ($P < 0.01$) correlation with the differentiation status of the tumor, with an increased prevalence of expression in the moderately and poorly differentiated types of tumors. The prevalence of HIF2 positive tumors was significantly ($P < 0.05$) higher in the left sided tumors than those of the right sided tumors (Table 1).

Correlation with MVD and COX-2 Expression. The MVD was significantly higher in HIF2-positive tumors than those in HIF2-negative ones ($P < 0.01$, Student's t test). A significant ($P < 0.01$, Fisher's exact test) correlation was also noticed between HIF2 and COX-2 expressions (Table 1). If HIF2 and COX-2 expressions are considered as continuous variables, there were significant direct correlation between the MVD and both HIF2 ($P < 0.01$, $r = 51.0$, Spearman's rank test) and COX-2 ($P < 0.01$, $r = 42.2$, Spearman's rank test) expressions (data not shown). In contrast to HIF2, tumors with HIF1 overexpression had slightly low MVD (78.9 ± 41.5 *versus* 84.5 ± 45.7). When this analysis was restricted among the T_1 and T_2 tumors, there was a weak but significant ($P < 0.05$, F test; not significant, Student t test) inverse correlation between the MVD and HIF1 expression.

Relationship between HIF Status and Patient Survival. HIF2 expression had a significant ($P < 0.05$, log-rank test) impact on patient prognosis in terms of the overall survival but had a trend of poor disease-free survival in the HIF2-positive cases, although this did not reach a statistically significant level. The 5-year overall survival rates for HIF2-negative and -positive patients were 80.7% and 61.4%, respectively, whereas the 5-year disease-free survival rates for HIF2-negative and -positive patients were 64.8% and 54.6%, respectively. A tendency for poor overall and disease-free survival was noticed in patients with HIF1-positive tumors but there were no statistical significance between the HIF1- positive and HIF1-negative groups. The combined expression of HIF1 and HIF2 had a significant

Table 1 Correlation of HIF[a] with the clinicopathological factors

	HIF1			HIF2		
	-positive ($n = 39$)	-negative ($n = 48$)	*P*	-positive ($n = 26$)	-negative ($n = 61$)	*P*
Age (yr)	65.1 ± 12.4	66.9 ± 10.9	NS	68.0 ± 8.3	65.3 ± 12.6	NS
Sex						
Male	19	32	NS	15	36	NS
Female	20	16		11	25	
Tumor size, cm, mean ± SD	4.6 ± 1.7	4.6 ± 2.3	NS	5.1 ± 2.0	4.3 ± 2.0	NS
Location						
Right	12	13	NS	12	13	<0.05
Left	27	35		14	48	
Differentiation						
Well	20	21	NS	6	35	<0.01
Others	19	27		20	26	
N stage						
0	25	28	NS	12	41	NS
1	9	14		11	12	
2	5	6		3	8	
T stage						
1	8	10	NS	1	17	<0.01
2	4	6		1	9	
3	27	32		24	35	
Lymphatic/venous invasion						
Negative	7	8	NS	1	14	<0.05
Positive	32	40		25	47	
Dukes stage						
A	12	13	NS	2	23	<0.05
B	12	13		9	16	
C	10	15		10	15	
D	5	7		5	7	
Microvessel density						
All cases	78.9 ± 41.5	84.5 ± 45.7	NS	101.9 ± 47.3	73.5 ± 39.5	<0.01
T_1 and/or T_2 tumors	58.6 ± 26.6	76.4 ± 52.7	NS[b]	103.3 ± 55.6	66.1 ± 42.9	NS[c]
COX-2 expression						
Negative	10	13	NS	1	23	<0.01
Positive	29	35		25	38	

[a] HIF, hypoxia-inducible factor; NS, not significant; N, node; T, tumor; Cox-2, cyclooxygenase 2.
[b] *F* test, $P < 0.05$.
[c] *F* test, NS.

(*$P < 0.01$*) impact on patient survival (Fig. 2). In the multivariate analysis, only the Dukes stage had a significant independent impact on patient prognosis and both HIF1 and HIF2 were eliminated from the stepwise multivariate analysis. In a different set of analyses, HIF remained as an independent prognostic marker along with the Dukes stage only when patients positive for both HIF1 and HIF2 were considered as the HIF-positive cases (Table 2).

DISCUSSION

Colorectal cancer remains a formidable disease worldwide. Although the overall outcome of the disease has become better because of the early diagnosis and emergence of new treatment options, nevertheless 52% (492,000 of 945,000) of the patients with colorectal cancer died in the year 2000 (1). This indicates that alternative treatment options that will restrict the tumor growth should be introduced in the treatment protocols. Such a concept stemmed originally from Folkman's innovative proposition that tumor growth beyond a few millimeters is dependent on tumor angiogenesis, and, therefore, blocking angiogenesis would be an attractive option for treating cancer patients. The advantage of this therapy is that it is neither toxic nor affected by drug resistance, because of the nearly normal genetic composition of the endothelial cells, in contrast to the frequent genetic mutations observed in tumor cells. The knowledge of the expression profile of angiogenic growth factors in a specific tumor is valuable for designing an effective antiangiogenic therapy.

Results of this study indicate that a subgroup of patients with aggressive colorectal carcinomas had frequent positive expression of HIF2, and this was an independent prognostic marker in colorectal carcinoma patients. As in other studies (7, 10), the prevalence of HIF1 expression was higher than HIF2 expression in this study. In contrast to frequent expression of HIF2 in more advanced tumor stages, HIF1 expression was noticed equally in the early and advanced tumor stages. This stage-specific expression pattern of HIF2 may have some effect on the expression pattern of the downstream angiogenic factors in colorectal carcinomas. Recently, we have shown that vascular endothelial growth factor (VEGF) expression was uniformly distributed in adenomas and in noninvasive and invasive colorectal carcinomas, whereas basic fibroblast growth factor

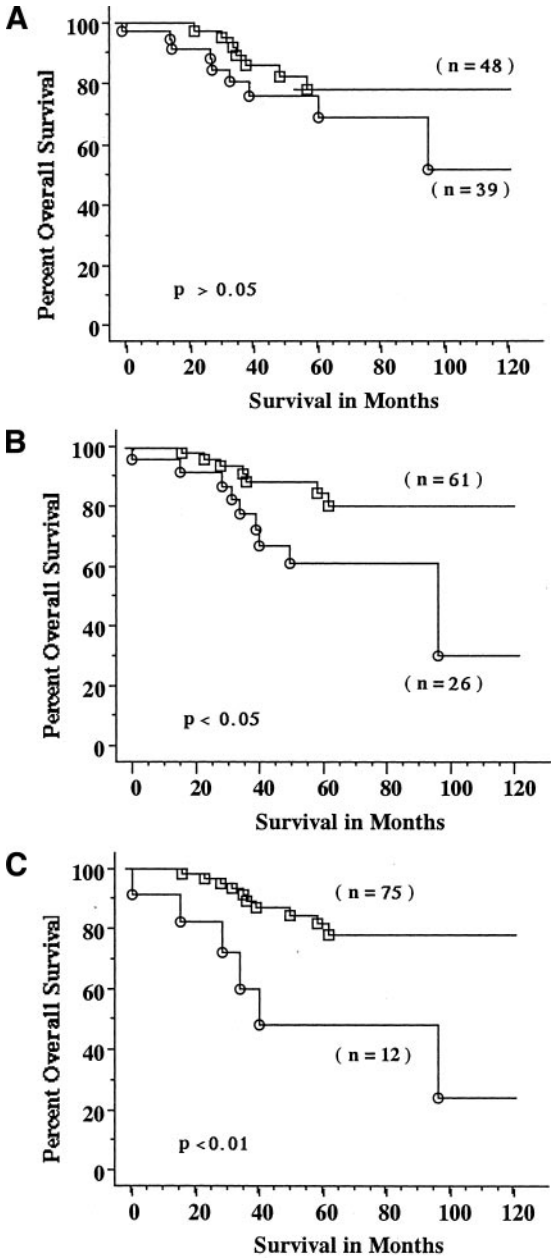

Fig. 2 Cumulative survival curves stratified by HIF-1α (HIF1) expression (*A*), HIF-2α (HIF2) expression (*B*), and combined expressions of HIF1 and HIF2 (c). Significantly worse survival rates were noticed in patients with HIF2 overexpression alone ($P < 0.05$, log-rank test) and in those with combined overexpression of HIF1 and HIF2 ($P < 0.01$, log rank test).

(bFGF) and thymidine phosphorylase (TP) were expressed preferentially in the invasive carcinomas (13). From such an expression pattern, it might be assumed that in colorectal cancers, VEGF expression is preferentially regulated by the HIF1, whereas bFGF, TP, and COX-2 expressions are regulated by HIF2. In fact, this type of association was reported by Fang *et al.* (14) in a recent study in which HIF1 mediated up-regulation of VEGF but not of bFGF in the switch to the angiogenic phenotype during early tumorigenesis. Similarly, in another study, Sivridis *et al.* (7) showed that VEGF and TP were significantly correlated with HIF1 and HIF2 in endometrial carcinomas, respectively. It was reported that tumor-infiltrating macrophages induced angiogenesis in colorectal cancers by releasing TP (15). It is worth mentioning that, in the present study, macrophages were strongly stained for HIF. Very recently, Leek *et al.* (16) found that HIF2 expression by macrophages was a strong denominator of tumor angiogenesis in breast carcinomas. Therefore, it seems that HIFs transcribe the major angiogenic factors differentially during the early and late phases of tumor angiogenesis (Fig. 3). Additional studies will be necessary to address this issue.

Although there some controversies exist, in general, tumor angiogenesis plays a significant role in tumor growth and metastasis of the colorectal cancers, and several angiogenic factors, including VEGF, bFGF, TP, and COX-2, are implicated in this process (3–5, 15). The relative importance of HIF1 and HIF2 in tumor angiogenesis is perhaps different in different types of cancers. In endometrial carcinoma, HIF1 had a significant correlation with tumor MVD, whereas in lung carcinoma only HIF2 expression was significantly correlated with tumor MVD (7, 10). The results of the present study indicate that HIF2 rather than HIF1 might be a surrogate marker for hypervascular tumors in colorectal carcinomas. The relationship between the microvessel formation and the angiogenic factor expression is much more complicated than was thought previously. Recently, Giatromanolaki *et al.* (10) reported a significantly higher expression of HIF2 in tumors with low MVD than in those with moderate MVD. Interestingly, such a relationship between MVD and HIF1 was noticed in the present study. In a subset of patients with T_1 or T_2 tumors ($n = 28$), the MVD was weak but significantly lower in HIF1-positive tumors than in those with negative ones ($P < 0.05$, F test; Table 1). This may indicate that HIF1 expression was induced by tumor hypoxia and worked as an initiator of angiogenesis during the early stages of exponential tumor growth.

A growing body of evidence suggests an important role for COX enzyme in the tumor progression and microvessel formation (17). Recently, we reported that COX-2 plays a pivotal role in tumor angiogenesis and prognosis of colorectal carcinoma

Table 2 Multivariate analysis of prognostic factors

	Model 1 (HIF2)[a]		Model 2 (HIF1–HIF2)	
Prognostic factors	χ	*P*	χ	*P*
Dukes stage	8.37	<0.01	6.80	<0.01
HIF2 expression	2.44	NS		
HIF1–HIF2 expression			5.33	<0.05
Age	0.25	NS	0.364	NS
Sex	0.40	NS	0.08	NS
Tumor differentiation	0.48	NS	0.90	NS
Tumor size	1.67	NS	0.63	NS
Lymphatic invasion	0.02	NS	0.02	NS
Tumor location	0.10	NS	0.10	NS

[a] HIF2, hypoxia-inducible factor 2α; HIF1, hypoxia-inducible factor 1α; HIF1–HIF2, combination of HIF-1α and HIF-2α; NS, not significant.

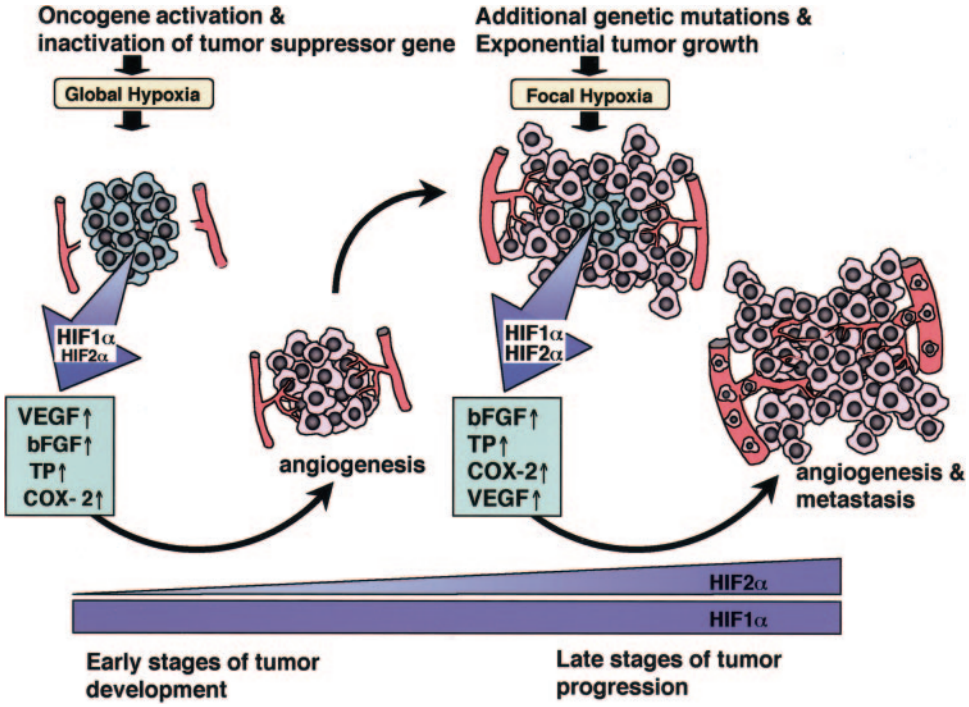

Fig. 3 Hypothetical schematic diagram of the angiogenic growth factors expression profile during colorectal carcinogenesis. *Blue colored cells,* hypoxic cells; *pink colored cells,* well oxygenated cells. *HIF,* hypoxia-inducible factor; *VEGF,* vascular endothelial growth factor; *bFGF,* basic fibroblast growth factor; *TP,* thymidine phosphorylase; *COX-2,* cyclooxygenase 2.

patients (5). Results of the present study indicate that HIF2 might be one of the several factors responsible for modulation of COX-2 expression. To the best of our knowledge, this is the first report showing a direct correlation between HIF2 and COX-2 expression in clinical materials. Very recently, Jones *et al.* (18) reported that COX-2 inhibitors suppressed HIF1 expression through increasing the expression of *von Hippel-Lindau* tumor suppressor gene in endothelial cell lines. Whether such a relation also exists for HIF2 remains to be determined. This indicates that the COX inhibitors might inhibit tumor angiogenesis through HIF repression and will become a choice of treatment in colorectal cancer patients.

Several studies showed that HIF1 either had a significant impact on patient survival or became a predictor of responsiveness of the adjuvant therapy (8, 9, 19, 20). To the best of our knowledge, only two studies addressed the prognostic significance of both HIF1 and HIF2 in the clinical materials (7, 10). In endometrial carcinoma, HIF1 positivity had a prognostic impact, whereas in the lung carcinoma, HIF2 became the prognostic indicator. As it was in lung carcinoma, HIF2 became an independent predictor for prognosis of colorectal carcinoma patients, whereas HIF1 had no impact on patient survival. The lack of prognostic impact of HIF1 could be explained by an intricate relationship between HIF1 and the mutation of tumor suppressor genes. In ovarian carcinoma, HIF1 overexpression alone was of no prognostic value and became a strong prognostic marker only in combination with nonfunctional p53 protein (19). Such a correlation may also exist in colorectal carcinomas and needs additional studies to address this issue.

In conclusion, results of this study indicate that HIF2 was overexpressed in aggressive colorectal carcinomas and had a significant direct correlation with tumor microvessel count and COX-2 expression. Conversely, HIF1 overexpression was noticed in hypoxic early colorectal carcinomas. Effective treatment strategies could be developed by targeting specific angiogenic factors at different stages of colorectal tumor development. Development of small molecular inhibitors or oligonucleotides targeting the HIF dimerization with or without COX-2 inhibitors may be attractive approaches for treating patients with colorectal carcinomas.

REFERENCES

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–43.

2. Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992;267:10931–4.

3. Tanigawa Y, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K. Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res 1997;57:1043–6.

4. Chio HJ, Hyun MS, Jung GJ, Kim SS, Hong SH. Tumor angiogenesis as a predictor in colorectal carcinoma with special reference to mode of metastasis and recurrence. Oncology 1998;55:575–81.

5. Masunaga R, Kohno H, Dhar DK, et al. Cyclooxygenase-2 expression correlates with tumor neovascularization and prognosis in human colorectal carcinoma patients. Clin Cancer Res 2000;6:4064–8.

6. Semenza GL. Expression of hypoxia-inducible factor 1: mechanism and consequences. Biochem Pharmacol 2000;59:47–53.

7. Sivridis E, Giatromanolaki A, Gatter KC, Harris AL, Koukourakis MI. Association of hypoxia-inducible factors 1α and 2α with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. Cancer (Phila) 2002;95:1055–63.

8. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000;60:4693–6.

9. Birner P, Gatterbauer B, Oberhuber G, et al. Expression of hypoxia-inducible factor-1α in oligodendrogliomas. Cancer (Phila) 2001;92:165–71.

10. Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Relation of hypoxia inducible factor 1α and 2α in operable non-small cell lung cancer to angiogenic/molecular profile of tumors and survival. Br J Cancer 2001;85:881–90.

11. Zhong H, Marzo AMD, Laughner E, et al. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. Cancer Res 1999;59:5830–5.

12. Talks KL, Turley H, Gatter KC, et al. The expression and distribution of the hypoxia-inducible factors HIF-1α and HIF-2α in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000;157:411–21.

13. Tabara H, Kohno H, Dhar DK, et al. Concurrent expression of angiogenic growth factors and neovascularization during tumourigenesis in colorectal carcinoma patients. Acta Oncol 2001;40:622–8.

14. Fang J, Yan L, Shing Y, Moses A. HIF-1α mediated up-regulation of vascular endothelial growth factor, independent of basic fibroblast growth factor, is important in the switch to the angiogenic phenotype during early tumorigenesis. Cancer Res 2001;61:5731–5.

15. Matsumura M, Chiba Y, Lu C, et al. Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression correlated with tumor angiogenesis and macrophage infiltration in colorectal cancer. Cancer Lett 1998;128:55–63.

16. Leek RD, Talks KL, Pezzella F, et al. Relation of hypoxia-inducible factor-2α (HIF2α) expression in tumor-infiltrative macrophages to tumor angiogenesis and oxidative thymidine phosphorylase pathway in human breast cancer. Cancer Res 2002;62:1326–9.

17. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 1997;94:3336–40.

18. Jones MK, Szabo IL, Kawanaka H, Husain SS, Tarnawski AS. von Hippel Lindau tumor suppressor and HIF-1α: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. FASEB J 2002;16:264–6.

19. Birner P, Schindl M, Obermair A, Breitenecker G, Oberhuber G. Expression of hypoxia-inducible factor 1α in epithelial ovarian tumors: its impact on prognosis and response to chemotherapy. Clin Cancer Res 2001;7:1661–8.

20. Koukourakis MI, Giatromanolaki A, Skarlatos J, et al. Hypoxia inducible factor (HIF-1α and HIF-2α) expression in early esophageal cancer and response to photodynamic therapy and radiotherapy. Cancer Res 2001;61:1830–2.

Clinical Cancer Research

Prognostic Impact of Hypoxia-Inducible Factors 1α and 2α in Colorectal Cancer Patients: Correlation with Tumor Angiogenesis and Cyclooxygenase-2 Expression

Hiroshi Yoshimura, Dipok Kumar Dhar, Hitoshi Kohno, et al.

Clin Cancer Res 2004;10:8554-8560.