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Original Article

The Correlation of Expression Levels of HIF-1α and HIF-2α in Hepatocellular Carcinoma with Capsular Invasion, Portal Vein Tumor Thrombi and Patients' Clinical Outcome

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Objectives: The roles of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α in the development of hepatocellular carcinoma have not been fully elucidated. Here, we aim to uncover the relationship between the prognosis of hepatocellular carcinoma patients and the expression of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α in tumor tissues.
Methods: The protein levels of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α were detected by immunohistochemistry on paraffin sections of 126 paired hepatocellular carcinoma tissue and peritumoral tissue samples. The mRNA levels of them were detected by quantitative real-time polymerase chain reaction.
Results: High expression of hypoxia-inducible factor-1α was found in 57.1% (72/126) of tumor specimens, compared with 5.6% (7/126) in peritumoral tissues, while high expression of hypoxia-inducible factor-2α was found in only 13.5% (17/126) of tumors, compared with 47.6% (60/126) of peritumoral tissues. There was high expression of hypoxia-inducible factor-1α protein in hepatocellular carcinoma tissues closely associated with capsular infiltration and portal vein invasion, and thus lower overall survival and disease-free survival of hepatocellular carcinoma patients ($P < 0.05$). No significant association has been found between the expression of hypoxia-inducible factor-2α protein and capsular infiltration, portal vein invasion, overall survival and disease-free survival ($P > 0.05$). However, patients with high expression of both hypoxia-inducible factor-1α and hypoxia-inducible factor-2α have a significantly worse outcome than patients with low expression of both hypoxia-inducible factor-1α and hypoxia-inducible factor-2α ($P < 0.05$).
Conclusions: The discordant results on expression of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α suggest that these two proteins are differentially regulated *in vivo*, thus reflecting distinctive protein expression and stabilization mechanisms. The association between hypoxia-inducible factor-1α expression and unfavorable outcome indicates the importance of using hypoxia-inducible factor-1α as a treatment target in hepatocellular carcinoma.

Key words: hepatocellular carcinoma – HIF-1α – HIF-2α – overall survival – disease-free survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide (1). Although HCC is one of the most hypervascularized tumor types with rich blood perfusion, it still contains hypoxic regions due to rapid cell proliferation and aberrant blood vessel formation, especially in patients with liver cirrhosis (2). Intrahepatic hypoxia results in aggressive tumor progression, resistance to chemotherapy and radiation, as well as poor prognosis (2,3). Tumor cells and tissues adapt to a hypoxic microenvironment via the activation of numerous hypoxia-related molecules, among which hypoxia-inducible factors (HIFs) are the predominant ones (2). HIFs regulate hundreds of genes as they encode proteins that play vital roles in many aspects of cancer biology, including cell survival, tumor angiogenesis, metastasis and resistance to radiation and chemotherapy (4,5).

HIFs are heterodimers that consist of a constitutively expressed HIF-1β subunit and an O2-regulated subunit, mainly including HIF-1α and HIF-2α. In well-oxygenated cells, the hydroxylation of proline residues by prolyl hydroxylase domain protein 2 promotes the interaction of HIF-1α and HIF-2α with the von-Hippel–Lindau tumor suppressor protein, which recruits an E3 ubiquitin-protein ligase and thus targets HIF-1α and HIF-2α for degradation by the ubiquitin–proteasome system (6). Under hypoxic conditions, however, the low availability of oxygen inhibits the activity of hydroxylase, leading to the stability of HIF-1α and HIF-2α. Accumulated HIF-1α and HIF-2α translocate to nuclei and dimerize with HIF-1β to form a functional transcription factor capable of DNA binding at hypoxia response elements (HREs) and the transcriptional activation of target genes (5,7). Although these two subunits have similar construction and common HREs, their target genes are not fully the same (8). Moreover, the transcriptional activity of HIF-1α and HIF-2α is different, even though they target the same genes (8,9). Therefore, HIF-1α and HIF-2α may play different roles in the microenvironment of hypoxia for the development of HCC.

Indeed, the expression of HIF-1α and HIF-2α protein was also different, and their roles in the development of HCC have not been fully elucidated. Many studies focused on the expression of either HIF-1α or HIF-2α in HCC; however, comparison of the expression of both of them in HCC with that in paired peritumoral tissues and the correlation of their expression with the patient's prognosis have not been done (10–14), although the importance of this is obvious. In this study, therefore, we compared the expression of HIF-1α and HIF-2α in HCC and paired peritumoral tissues from 126 clinical samples, and thus aim to reveal the roles of HIF-1α and HIF-2α in hepatocarcinogenesis.

PATIENTS AND METHODS

Patients and Specimens

Tissues were obtained from 76 HCC patients who undergo surgical resection at Department of Hepatobiliary Surgery, Affiliated Hospital of Guiyang Medical College (Guiyang, China) and 50 HCC patients who undergo surgical resection at Department of General Surgery, the Center Hospital of Huanggang (Huanggang, China) between October 2005 and June 2009. Informed consents were obtained from the patients, and the experiment protocol was approved by Human Ethics Committee of the Guiyang Medical College and the Center Hospital of Huanggang. All tissue samples were obtained from the patients before any medical treatment. Peritumoral tissues were obtained at least 2 cm away from the primary site of the tumor. All patients were tested positive for HBsAg and negative for antibodies to the hepatitis C virus (anti-HCV) and human immunodeficiency virus (anti-HIV). Of the total number of patients, 110 were male and 16 were female. The mean age was 48.8 years (range 19–66 years). Sixty patients had HCC <5.0 cm in maximum diameter and 66 patients had HCC ≥ 5.0 cm. Twenty-five patients were found to have portal vein tumor thrombus, which extended to the second or first portal vein branch, but not to the main portal or opposite-side portal branch by preoperative imaging studies and postoperative pathological examination. Follow-up data after liver resection were available for all of the patients. The clinicopathological features of 126 patients with HCC are listed in Table 1.

RNA Extraction and First-strand cDNA Synthesis

A sample of 150–200 mg of 60 fresh frozen tumors and adjacent liver tissues was used to isolate total RNA with TRIzol reagents (Invitrogen, Carlsbad, CA, USA). The amount and quality of the extracted RNA were determined by the Nano Drop Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The cDNA synthesis was performed under the condition of 42°C for 60 min in a volume of 25 μl containing 2 μg of RNA, 1.6 μM Oligo (dT)18, 0.6 μM dNTP and 200 U M-MLV reverse transcriptase (Promega, Madison, WI, USA) in reaction buffer. We performed all potential risk experiments in biological safety cabinets and strictly complied with the laboratory biosafety manual. All researchers received biosafety training, were vaccinated the Hepatitis B vaccine series and tested for antibodies to the hepatitis B antigen (HBsAg).

Real-time Polymerase Chain Reaction

Following reverse transcription, the cDNA samples were diluted 1 : 5 in RNase-free water. The primer sequences used herein were HIF-1α forward: 5′-ACTTCTGGATGCTGGTG ATTTG-3′, reverse: 5′-GCTTCGCTGTGTGTTTTGTTCT-3′; HIF-2α forward: 5′-TCATGCGACTGGCAATCAGC-3′, reverse: 5′-GTCACCACGGCAATGAAACC-3′; GAPDH forward: 5′-CAAAGCTGGTGTGGGAGG-3′, reverse: 5′-CT CCTGGAAGATGGTGATGG -3′. Each reaction used SYBR Green mix, cDNA template, and 10 μM forward primer, 10 μM reverse primer and double distilled H_2O in a total volume of 20 μl, and performed on real-time polymerase chain reaction (PCR) System7500 (Applied Biosystems,

Table 1. Correlations of HIF-1α and HIF-2α protein expression in surgical specimens of HCC with clinicopathological characteristics

Parameters	HIF-1α		*P* value	HIF-2α		*P* value
	Low ($n = 54$)	High ($n = 72$)		Low ($n = 109$)	High ($n = 17$)	
Age (years)						
<50	14	20	0.817	28	6	0.394
≥50	40	52		81	11	
Gender						
Male	46	64	0.537	96	14	0.453
Female	8	8		13	3	
Cirrhosis						
Absence	34	47	0.788	73	8	0.111
Presence	20	25		36	9	
Tumor size (cm)						
<5	28	32	0.410	51	9	0.637
≥5	26	40		58	8	
AFP (μg/l)						
<400	24	38	0.354	50	12	0.058
≥400	30	34		59	5	
Histological grade						
Well	7	10	0.942	14	3	0.421
Moderate	39	50		79	10	
Poor	8	12		16	4	
Capsular infiltration						
Absence	45	44	0.007*	75	14	0.391
Presence	9	28		34	3	
Portal vein invasion						
Absence	48	53	0.033*	86	15	0.521
Presence	6	19		23	2	

HIF, hypoxia-inducible factor; HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein.
*$P < 0.05$.

Foster City, CA, USA). Reaction conditions were 94°C denaturation for 1 min, 94°C for 60 s within 40 circulations, annealing at 55°C for 60 s and extension at 72°C for 60 s. The program was set to automatically record the average fluorescence value of the last 10% in the last cycle, which is equal to the amount of amplification at the end of each cycle. After reactions were completed, baseline and threshold were adjusted in the ABI 7500 software system where the cycle threshold (CT) value of each reaction hole was read. Data were analyzed according to the comparative CT value method and were normalized according to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in each sample.

Immunohistochemistry Analysis

The immunohistochemistry (IHC) analysis of the expression of HIF-1α and HIF-2α was performed on serial sections of each sample of tumor and adjacent liver tissues. After deparaffinization and rehydration, endogenous peroxidase activity was blocked by 3% H_2O_2 in methanol solution. The sections were then processed to unmask antigens by heating in a microwave oven in citrate buffer for 15 min (pH 6.0). Afterwards, the sections were then incubated with primary antibodies, including monoclonal anti-HIF-1α antibody (clone H1alpha67, Santa Cruz Biotechnology, CA, USA) and polyclonal anti-HIF-2α antibody (Boster Biological Technology, Wuhan, China), at 37°C for 1 h and then overnight at 4°C. After washing for three times with phosphate buffered saline, the sections were incubated with corresponding biotinylated secondary antibodies (Santa Cruz Biotechnology), and then followed with the incubation of horseradish peroxidase-labeled streptavidin (Boster Biological Technology, Wuhan, China) for 20 min. Detection of immunoreactivity was carried out by the 3,3′-diaminobenzidine assay. All specimens were evaluated by two

investigators who were blinded to the patients' clinical information. Specimens scored differently by the two investigators were reinvestigated together using a multiheaded microscope.

IHC analysis of the expression of HIF-1α and HIF-2α was also performed on frozen sections of each sample of tumor and adjacent liver tissues to confirm the integrity of HIF-1α and HIF-2α antigenicity with formalin fixation. We performed all potentially risky experiments in the Biosafety Level 2 laboratory, while strictly following the laboratory biosafety manual during the frozen section cutting. When the experiments were finished, the freezing microtome and the room was thoroughly cleaned and disinfected without delay.

The protein levels of HIF-1α and HIF-2α were scored according to the number of cells exhibiting the cytoplasmic and nuclear staining using the following classification system: I, no staining; II, nuclear staining in $<10\%$ of cells and/or with weak cytoplasmic staining; III, nuclear staining in 10–50% of cells and/or with distinct cytoplasmic staining; IV, nuclear staining in $>50\%$ of cells and/or with strong cytoplasmic staining (13,15–17). In the following analysis, for HIF-1α and HIF-2α, cases of Scores I and II were considered low expression patterns while the remaining cases were considered as high expression patterns (13,15–17).

Protein Preparation and Western Blotting Analyses

Approximately 50 mg of HCC and peritumoral tissue specimens were minced to small pieces using surgical scissors and sonicated in protein lysis buffer, followed by centrifugation at 13 000 rpm for 15 min at 4°C. Protein concentrations were determined by the Bradford method. A total of 30–50 μg of protein was separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using 10% SDS polyacrylamide gels, transferred to polyvinylidene fluoride membranes, blocked in 5% non-fat milk in TBS-T for 2 h, and incubated with primary antibodies to HIF-1α (1 : 1000, Santa Cruz Biotechnology), HIF-2α (1 : 250, Boster Biological Technology) and β-actin (1 : 3000, Santa Cruz Biotechnology) for 1 h at 37°C, and then overnight at 4°C, followed by 1 h incubation with the appropriate secondary antibody. The bands were visualized using an enhanced chemiluminescence detection system (Therm, Rockford, IL, USA).

Statistical Analysis

Statistical analysis was performed using SPSS version 13.0. Continuous variables were expressed as a mean with standard deviation and analyzed using Student's *t*-test (two-tailed). Comparison of the clinicopathological parameters with HIF-1α and HIF-2α expression was conducted by the two-tailed Mann–Whitney *U*-test. The survival curves were computed by the Kaplan–Meier analysis and the prognostic significance was analyzed by the log-rank test. A P value <0.05 was considered to be statistically significant.

RESULTS

Expression Level of HIF-1α and HIF-2α Showed Opposite Patterns in HCC and Paired Peritumorial Tissues

The protein levels of HIF-1α and HIF-2α were first measured by IHC on paraffin slides of 126 samples of HCC and paired peritumorial tissues. The positive staining of HIF-1α and HIF-2α is located in the cytoplasm and/or the nuclei, with representative staining shown in Fig. 1A. High expression of HIF-1α was found in 57.1% (72/126) of tumor tissues and in 5.6% (7/126) of peritumoral tissues (Table 2). In contrast, high expression of HIF-2α was only detected in 13.5% (17/126) of tumor tissue, compared with 47.6% (60/126) of peritumoral tissues. To confirm the integrity of HIF-1α and HIF-2α antigenicity with formalin fixation, the comparison between the staining of frozen and paraffin sections has also been done, and no significant differences of HIF-1α and HIF-2α expression were found between these two types of sections (data not shown). Therefore, the expression of HIF-1α and HIF-2α showed opposite patterns in HCC and paired peritumoral tissues. To confirm this finding, western blotting was also applied to detect the protein levels of them in four paired HCC specimens. As shown in Fig. 1B, the protein level of HIF-1α in HCC tissues was significantly higher than that in peritumoral tissues, whereas HIF-2α was much higher in peritumoral tissues than that in tumor tissues. Meanwhile, similar tendency on the change of the mRNA levels of HIF-1α and HIF-2α were also found (Fig. 1C). As a result, these results clearly showed that both mRNA and protein expression levels of HIF-1α and HIF-2α were opposite in HCC tissues and the paired peritumoral tissues. However, comparing immunostaining results and mRNA expression in a case-dependent fashion and plotting each case on a graph, HIF-1α immunostaining results and its mRNA expression were basically the same, while no significant correlation was found between HIF-2α immunostaining results and its mRNA expression (Fig. 1D). These results suggested that the expression of HIF-1α and HIF-2α may be regulated by different mechanisms in HCC.

HIF-1α, but Not HIF-2α, Associates with Capsular Infiltration and Portal Vein Invasion of HCC

Next, we analyzed the relationship between the expression of HIF-1α and HIF-2α and clinicopathological features. As shown in Table 1, expression of HIF-1α in the HCC tissues with capsular infiltration and portal vein invasion was significantly higher than that without capsular infiltration or portal vein invasion ($P < 0.05$). However, no correlation was found between HIF-1α expression and other clinicopathological features, including patients' age, gender, cirrhosis, tumor size, serum alpha-fetoprotein (AFP) level and tumor differentiation grade. In contrast, no correlation was found between HIF-2α expression and all of these clinicopathological features. These findings suggest that HIF-1α could be a valuable predictor for early invasion and metastasis of HCC.

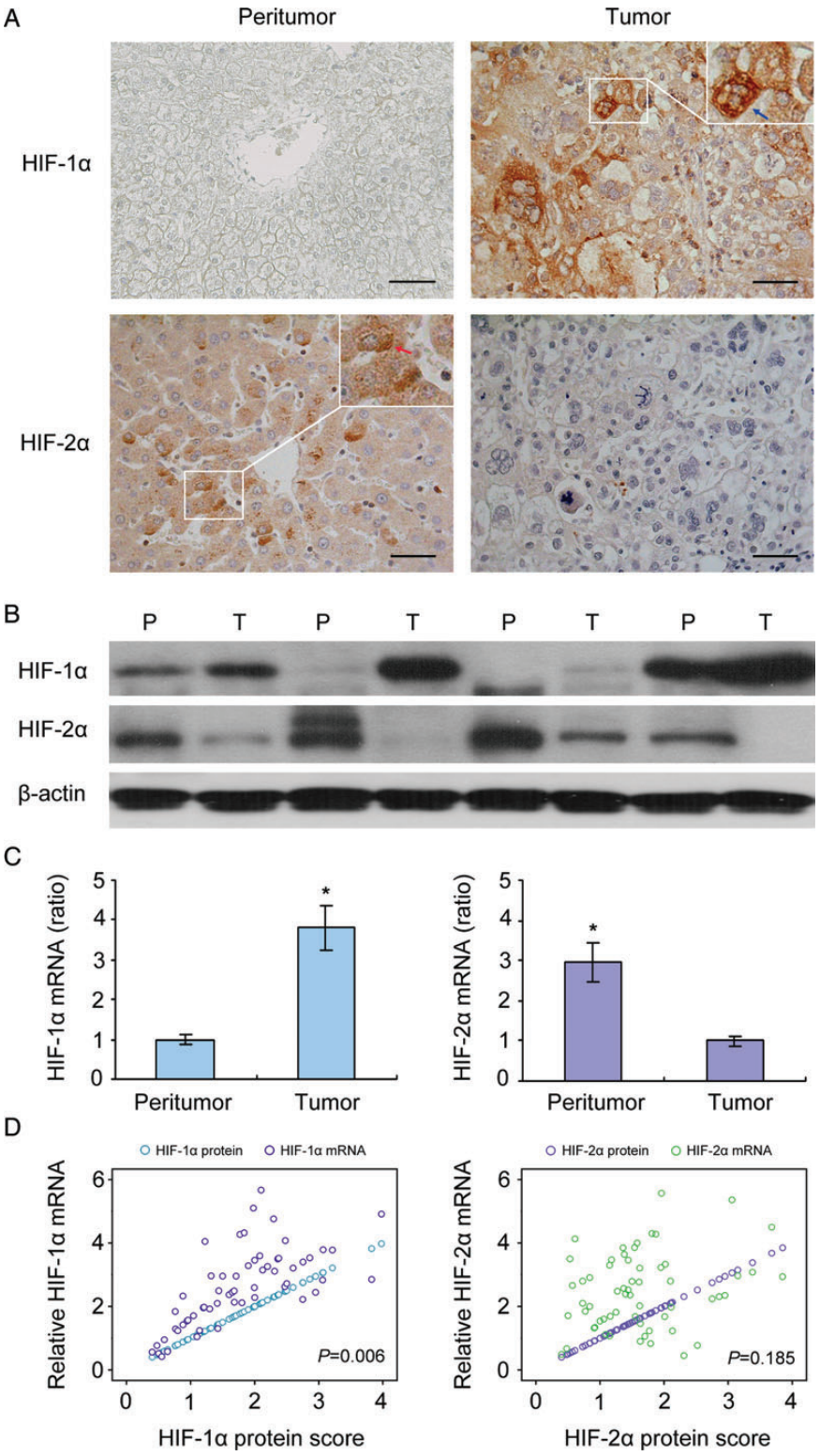

Figure 1. Expression of hypoxia-inducible factor (HIF)-1α and HIF-2α in hepatocellular carcinoma (HCC) patients. (A) Representative images of HIF-1α and HIF-2α staining in HCC tissue and the paired peritumoral tissue. Scale bar, 10 μm. (B) The expression of HIF-1α and HIF-2α protein in HCC tissue and the paired peritumoral tissue was confirmed by western blotting. Beta-actin was applied as a control. (C) Real-time polymerase chain reaction (PCR) analysis of cDNA reverse-transcribed from mRNA of HIF-1α and HIF-2α in HCC tissues and the paired peritumoral tissue. GAPDH was used as a control. Error bars indicate SD ($n = 60$). (D) Overlay scatters indicate the correlation between the protein expression detected by IHC and mRNA levels analyzed by real-time PCR of HIF-1α and HIF-2α. Asterisk indicates $P < 0.05$, two-tailed test. SD, standard deviation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IHC, immunohistochemistry; P, peritumor; T, tumor.

Table 2. Correlations of HIF-1α and HIF-2α protein expression in peritumoral tissues with clinicopathological characteristics

Parameters	HIF-1α		*P* value	HIF-2α		*P* value
	Low ($n = 119$)	High ($n = 7$)		Low ($n = 66$)	High ($n = 60$)	
Age (years)						
<50	33	1	0.673	19	15	0.632
≥50	86	6		47	45	
Gender						
Male	105	5	0.217	56	54	0.368
Female	14	2		10	6	
Cirrhosis						
Absence	80	1	0.008*	43	38	0.832
Presence	39	6		23	22	
Tumor size (cm)						
<5	57	3	1.000	33	27	0.575
≥5	62	4		33	33	
AFP (μg/l)						
<400	59	3	1.000	29	33	0.215
≥400	60	4		37	27	
Histological grade						
Well	16	1		10	7	0.796
Moderate	84	5		45	44	
Poor	19	1		11	9	
Capsular infiltration						
Absence	85	4	0.418	49	40	0.351
Presence	34	3		17	20	
Portal vein invasion						
Absence	95	6	1.000	54	47	0.624
Presence	24	1		12	13	

*$P < 0.05$.

High Expression of HIF-1α in HCC Tissues Indicates Poor Outcome of HCC

The correlation of HIF-1α and HIF-2α expression with long-term patients' survival after hepatectomy was also analyzed. The mean overall survival (OS) time of patients with low HIF-1α expression in tumor tissues was 38.1 ± 11.8 months, while that with high HIF-1α expression was only 30.3 ± 8.4 months ($P = 0.018$). The mean disease-free survival (DFS) time of the patients with low HIF-1α expression in tumor tissues was 23.8 ± 8.1 months, while that with high HIF-1α expression was only 16.2 ± 6.6 months ($P = 0.014$). The Kaplan–Meier analysis showed that patients with high HIF-1α expression had shorter OS and DFS than those with low HIF-1α expression ($P < 0.05$, Fig. 2A). However, no significant correlation has been found between the expression of HIF-2α in HCC tissues and the OS and DFS of HCC patients ($P > 0.05$). The mean OS time of the patients with low HIF-2α expression in tumor tissues was 31.4 ± 18.9 months, while that with high HIF-2α expression was 26.8 ± 21.1 months ($P = 0.441$). The mean DFS time of the patients with low HIF-2α expression in tumor tissues was 25.7 ± 20.6 months, while that with high HIF-2α expression was 22.3 ± 18.0 months ($P = 0.134$, Fig. 2B). For better understanding of the synergetic roles of HIF-1α and HIF-2α in HCC, we categorized the cases into four groups: (A) low levels of both HIF-1α and HIF-2α ($n = 44$); (B) low levels of HIF-1α and high levels of HIF-2α ($n = 10$); (C) high levels of HIF-1α and low levels of HIF-2α ($n = 65$) and (D) high levels of both HIF-1α and HIF-2α ($n = 7$). It has been found that patients with high expression of both HIF-1α and HIF-2α have significantly lower OS and DFS than patients with low expression of both HIF-1α and HIF-2α ($P < 0.05$, Fig. 2C).

DISCUSSION

Although it has been demonstrated that HIF-1α and HIF-2α are overexpressed in numerous tumors, it has not been fully

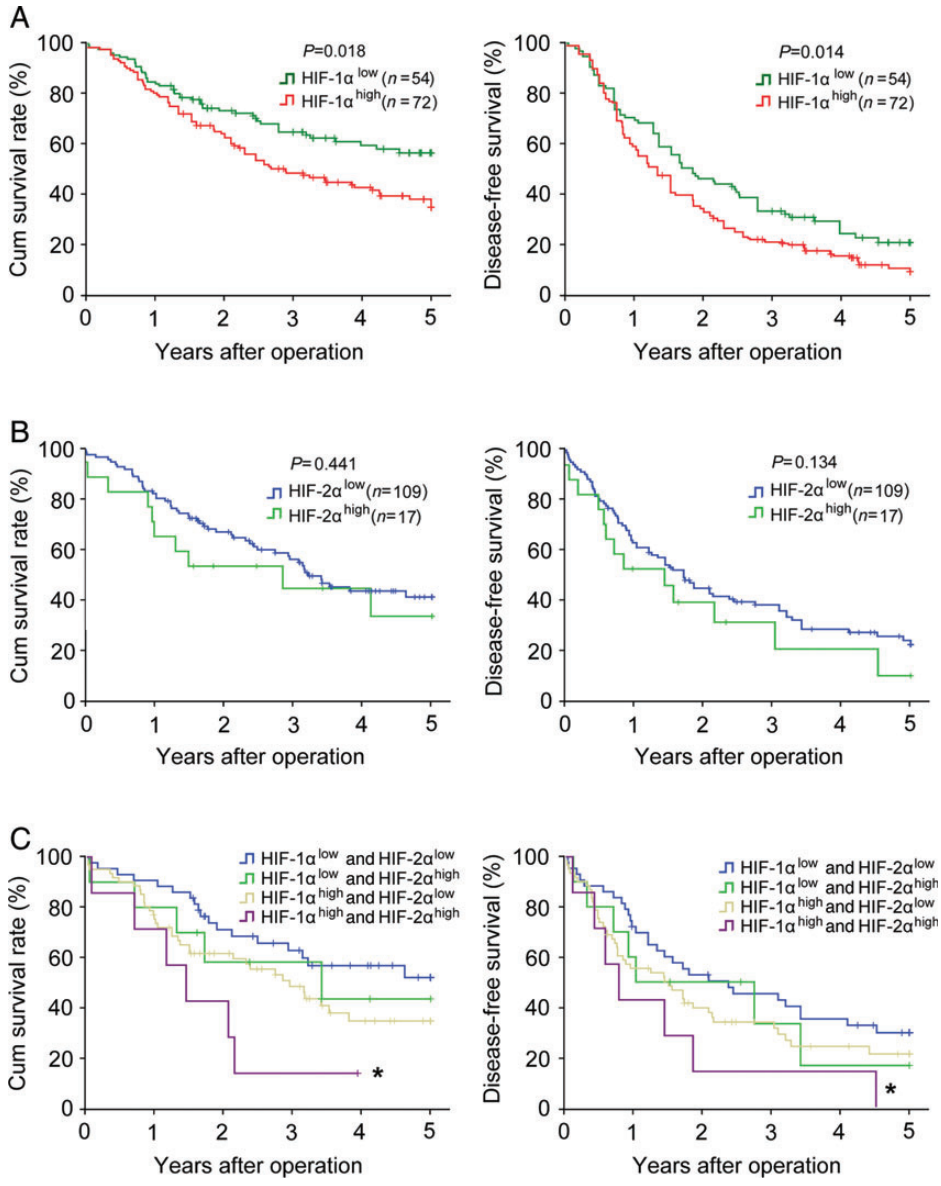

Figure 2. Expression of HIF-1α in HCC tissues associated with the prognosis of HCC patients. The Kaplan–Meier analysis of OS and DFS in 126 HCC patients based on expression of HIF-1α and HIF-2α. (A) High protein levels of HIF-1α protein in HCC tissues indicated both shorter OS and DFS ($P < 0.05$). (B) No difference was found on OS and DFS between high expression of HIF-2α and low expression of HIF-2α ($P > 0.05$). (C) Patients with high expression of both HIF-1α and HIF-2α have significantly lower OS and DFS than the patients with low expression of both HIF-1α and HIF-2α. OS, overall survival; DFS, disease-free survival. Asterisk indicates $P < 0.05$ vs. HIF-1α^{low} and HIF-2α^{low}.

addressed in HCC. Even worse, the existing literature shows conflicting results (10–15,17–20); for examples, Li et al. (12) showed that the expression of HIF-1α was higher in peritumoral liver tissues than in HCC tissues, while others found that HIF-1α was higher in HCC tissues than in peritumoral liver tissues. To address these questions, we analyzed the protein expression level of HIF-1α and HIF-2α in HCC and the paired peritumoral tissues collected from clinical samples. We found that the expression of HIF-1α in HCC was higher than that in paracancerous tissues, while an opposite expression pattern of HIF-2α was found. In addition, mRNA levels of HIF-1α and HIF-2α were also opposite in HCC and paired peritumoral tissues. These findings were supported by Huang et al. (10) and Wada et al. (19) who reported that the expression of HIF-1α was lower in peritumoral liver tissues than that in HCC tissues, and by Sun et al. (14) who showed that the expression of HIF-2α was higher in peritumoral liver tissues than that in HCC tissues. All of these results suggest that although both HIF-1α and HIF-2α are regulated by oxygen tension, they seem to be differently regulated by the tumor microenvironment. This can be due to the different sensitivities of HIF-1α and HIF-2α in response to hypoxia. Meanwhile, HIF-1α and HIF-2α are also affected by many other factors, including glucose metabolism, proto-oncogenes and tumor suppressor gene mutation and the oncogenic signal transduction pathways (21–24). These factors may exert

different effects on the expression of HIF-1α and HIF-2α, and thus lead to upregulation of HIF-1α and downregulation of HIF-2α. Meanwhile, we found that the association of mRNA levels and protein expression between HIF-1α and HIF-2α was also different. There were positive correlations between HIF-1α protein levels detected by immunostaining and its mRNA expression determined by real-time PCR, while no significant correlation between those of HIF-2α. This finding indicated that there may be different mechanisms involved in the regulation of HIF-1α and HIF-2α on mRNA, translation and/or post-translation levels. It is well known that HIFs are easily degraded by 26S proteasome under a non-hypoxic condition (25). This means that the translation from mRNA and the presence of immunoreactivity may not be closely correlated. When the ubiquitin–proteasome system is active, mRNA is expressed but the protein is absent. The persistence of the proteins should be dependent upon the association of the hypoxic state or the activity of von Hippel–Lindau gene products (26). Therefore, the regulation of HIF-1α and HIF-2α could be comprehensively complicated, and further investigations should focus on uncovering the underlying mechanisms.

Through binding to the HRE, HIF-1α has been demonstrated to transactivate a panel of target genes involved in tumor angiogenesis, invasion and metastasis, such as vascular endothelial growth factor (VEGF) (27), MMP9 (28) and E-cadherin (29). Our findings show that high expression of HIF-1α is closely associated with capsular infiltration and portal vein invasion of HCC, but not age, gender, tumor diameter, serum AFP levels or histological grade. High expression of HIF-1α in HCC is also significantly associated with poor DFS and OS rate in HCC patients. Our data suggest that HIF-1α is an unfavorable prognostic factor in HCC patients, which is in agreement with other *in vitro* studies (10,13,15,19). For example, in rat HCC models, tumor progression after hypoxia and chemotherapy was related to upregulation of HIF-1α and subsequent VEGF production, and transcriptional blockade of HIF-1α could enhance their therapeutic efficacy (30). In cell lines, HCC cell proliferation was inhibited by the HIF-1α antisense oligonucleotide (31).

An increasing number of reports are focusing on the role of HIF-2α in carcinogenesis and tumor progress with inconsistent conclusions. Bangoura et al. (20) showed that high expression of HIF-2α was associated with tumor angiogenesis and a poor outcome in HCC, whereas Sun et al. (14) found that a high protein level of HIF-2α in HCC correlated with a good outcome of HCC patients because of higher levels of apoptosis and expression of proapoptotic proteins, which inhibited cell and tumor growth. In the present study, we found that the expression of HIF-2α was higher in the peritumoral tissues than that of HCC primary tumors. We did not show a relationship between HIF-2α and capsular infiltration and portal vein invasion. Meanwhile, no significant correlation has been found between the expression of HIF-2α in HCC tissues and the OS and DFS of HCC patients. But when survival analysis was based on the combinations of HIF-1α and HIF-2α expression, we found that patients with high expression of both HIF-1α and HIF-2α have significantly worse outcome than the patients with low expression of both HIF-1α and HIF-2α. Therefore, application of combinations of HIF-1α and HIF-2α may contribute to a more accurate prediction of prognosis. Owing to the small sample size of our study, further studies based on a large sample size would be required to confirm these results.

In addition to HIF-1α and HIF-2α, two other hypoxia family members HIF-1β and HIF-3α are also involved in cellular responses to hypoxia. HIF-1β is an essential subunit which makes HIF-1 and HIF-2 possess transcription activity (32). HIF-3α can compete with HIF-1α and HIF-2α to bind with HIF-1β subunits, causing the reduction of HIF-1 and HIF-2 levels, thereby inhibing the up-regulation of target gene expressions induced by HIF-1 and HIF-2 (33). So they may also be involved in the development and progression of HCC, but we need to conduct more extensive studies to understand the roles they play in this process.

In conclusion, our findings of the opposite expression patterns of HIF-1α and HIF-2α in HCC tissue suggest that the regulation of HIF-1α and HIF-2α is different in HCC, and HIF-1α can serve as an unfavorable prognostic indicator in HCC patients.

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Conflict of interest statement

None declared.

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