Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;48:516-527
DOI: 10.1159/000491806
Published online: July 17, 2018
Accepted: May 04, 2018

Original Paper

Association Between Hypoxia-Inducible Factor-2α (HIF-2α) Expression and Colorectal Cancer and Its Prognostic Role: a Systematic Analysis

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Key Words
Hif-2α • Expression • CRC • Prognosis • Tumor

Abstract
Background/Aims: Although some studies showed that HIF-2α expression was correlated with an unfavorable prognosis in colorectal cancer (CRC), the prognostic results remain conflicting in CRC. The present study was performed to evaluate the association between HIF-2α expression and the clinicopathological features of this disease and to examine the potential prognostic role of HIF-2α expression in CRC. ***Methods:*** Pooled odds ratios (ORs) or hazard ratios (HRs) were calculated from available publications, The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) datasets. Trial sequential analysis (TSA) was used to estimate the required sample information. ***Results:*** HIF-2α protein expression was more frequent in CRC than in normal colonic tissues (OR = 150.49, $P < 0.001$), higher in male than female CRC patients (OR = 1.47, $P = 0.008$), and lower in high-grade than low-grade CRC (OR = 0.49, $P = 0.029$). TSA verified the reliability of the above results. HIF-2α expression was not linked to the prognosis of CRC in overall survival (OS), disease-specific survival (DSS), metastasis-free survival, and relapse-free survival, and no significant correlation was found between HIF-2α alteration and OS or disease-free survival (DFS) of CRC. Expression of both HIF-2α and vascular endothelial growth factor (VEGFA, VEGFB, or VEGFC) was associated with a poor metastasis-free survival of CRC (HR = 6.95, HR = 113.51, and HR = 8.11, respectively). No association was observed between HIF-2α expression and DFS in other cancers, but HIF-2α expression was correlated with a worse DFS of CRC (HR = 1.23, $P = 0.037$). Moreover, HIF-2α expression was linked to a good survival benefit in some cancers (B-cell lymphoma and lung adenocarcinoma: OS, multiple myeloma: DSS, breast cancer: distant metastasis-free survival, liposarcoma: distant recurrence-free survival) (all HRs < 1, $Ps < 0.05$). ***Conclusions:*** HIF-2α expression may be associated with the carcinogenesis of CRC, which is higher in males

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than in females, negatively linked to tumor differentiation, and correlated with a worse DFS of CRC. Additional prospective studies are needed.

Introduction

Colorectal cancer (CRC) remains a major public health problem and a common cause of morbidity and mortality [1]. According to GLOBOCAN estimates, approximately 1.4 million new cases of CRC were clinically diagnosed in 2012 among all human cancers, leading to approximately 693,900 deaths worldwide [2]. Although the recent diagnostic and therapeutic strategies have some significant improvements, approximately 50% of cases with CRC have overt metastases [3, 4]. Hence, the patients with advanced stage still have a poor 5-year survival rate [5].

Numerous studies have shown the molecular mechanisms linked to CRC [6-9]. Tumor hypoxia is a pathological hallmark that may be correlated with metabolism, the activation of cell signaling, angiogenesis, differentiation, necrosis or cell apoptosis, tumor development and aggressiveness, etc [10-12].. Additionally, tumor hypoxia can have an adverse impact on the prognosis of some cancers (i.e., invasive breast cancer or cervical cancer) and the efficacy of chemo- and radiotherapy [13, 14]. Hypoxia-inducible factor-2α (HIF-2α), also named the endothelial PAS domain protein 1 (EPAS1), a member of the hypoxia-inducible factors (HIFs), is an essential marker, which mediates the transcriptional response to hypoxia stress [15-17]. HIF-2α was not observed under normoxic conditions among multiple organs, while HIF-2α was markedly induced under hypoxia in various organs, including lung, kidney, liver, and intestine [18, 19]. HIF-2α expression was detected in a variety of human tumors, and its expression may be correlated with the poor outcome of some tumors, such as gastric cancer, breast cancer, glioblastoma, neuroblastoma, head and neck squamous carcinoma, and non-small cell lung cancer [10, 20]. Some studies have reported that HIF-2α expression can also be detected in CRC [21-24]. However, there has been no systematic analysis regarding the role of HIF-2α expression in CRC. Thus, the present study analyzed the association of HIF-2α expression and the clinicopathological features of CRC, and its prognostic effect, which provide potentially useful information for the prognosis and treatment of CRC.

The existing studies could not provide sufficient evidence on the significance of HIF-2α expression in CRC. For example, Jubb 2009 *et al*. reported that HIF-2α expression was correlated with an unfavorable OS in CRC [24]. HIF-2α expression was not associated with the prognosis of CRC in OS by Baba 2010 *et al* [23].. Therefore, on the basis of the currently available evidence, we performed a systematic analysis from numerous databases to better understand the prognostic role of HIF-2α expression in CRC. We also evaluated the association between HIF-2α expression and CRC.

Materials and Methods

Literature search

This meta-analysis was performed based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement criteria [25] (for all online suppl. material, see www.karger.com/doi/10.1159/000491806, Table S1). The PubMed, Embase, EBSCO, Web of Science, and Scopus databases were systematically searched to obtain eligible publications assessing the expression of HIF-2α protein in CRC patients. All previously published papers were identified by using the following combination of key words and search terms prior to June 18th, 2017: 'colorectal cancer OR colorectal tumor OR colorectal carcinoma OR colorectal neoplasm OR CRC', 'endothelial PAS domain-containing protein 1 OR EPAS1 OR hypoxia-inducible factor 2α OR hypoxia-inducible factor-2α OR HIF-2 alpha OR HIF-2α OR HIF2A OR HIF 2 alpha OR HIF 2A OR BHLHE73 OR PASD2 OR HLF OR MOP2 OR ECYT4', 'expression OR overexpression OR hyperexpression OR expressed'. The references of the included articles were also carefully screened to identify additional studies.

Selection criteria

The eligible studies fulfilled the following inclusion criteria: 1) cohort or case-control studies in human with CRC reported the information of HIF-2α expression; 2) HIF-2α protein expression was detected by using immunohistochemistry (IHC); 3) full-text studies were published in English; 4) studies had sufficient data to evaluate the association of HIF-2α protein expression between CRC and nonmalignant controls (benign lesions or normal tissue samples); 5) studies provided sufficient information to assess the correlation of HIF-2α expression with the clinical features of patients with CRC; and 6) studies reported the survival data (OS and /or DFS, etc.). Only paper with the most complete information was used when authors published more than one article using overlapping study population data.

The main exclusion criteria were as follows: 1) reviews, letters, case studies, or conference papers; 2) studies on cell lines, animals, or other solid tumors; 3) the detection method of HIF-2α expression was not IHC; 4) patients preoperatively received chemotherapy, radiotherapy, chemoradiotherapy, or targeted therapy; and 5) studies lacking substantiated data of HIF-2α expression and CRC.

Quality assessment

The Newcastle-Ottawa-Scale (NOS) was developed by the University of Newcastle and the University of Ottawa to evaluate the quality of nonrandomized studies to be included in meta-analyses [26, 27]. The quality of each eligible study was estimated by using the NOS for case-control or cohort studies, with a range from 0 to 9 [28]. NOS scores consisted of three parameters of quality: selection (4), comparability (2), and outcome or exposure assessment (3). Studies with six or more scores were classified as high quality [29, 30].

Data extraction

We abstracted the following data from available publications: surname of first author, year of publication, country, ethnicity, mean or median age, tumor stage, staining patterns, cut-off values of IHC method, the frequency of HIF-2α protein expression, number of the study population, survival data of multivariate analysis, and clinical features. The data of clinical characteristics consisted of age (≥ 60 years vs. < 60 years), gender (male vs. female), tumor grade (high-grade of 3-4 vs. low-grade of 1-2), clinical stage (stage 3–4 vs. stage 1–2), vascular invasion (yes vs. no), depth of tumor invasion (pT3–4 vs. pT1–2), lymph node status (positive vs. negative), distant metastasis (yes vs. no), tumor location (colon vs. rectum), vascular endothelial growth factor (VEGF) expression, and microvessel density. Any inconsistent information was resolved by a discussion between all authors.

Survival analysis of HIF-2α alteration

The data from the Cancer Genome Atlas Research Network (http://cbioportal.org) were analyzed to evaluate the potential correlation between HIF-2α alteration and the prognosis of CRC patients in OS and DFS [31, 32].

Statistical analysis

The strength of the association between HIF-2α protein expression and CRC was estimated by the overall odds ratios (ORs) with 95% confidence intervals (95% CIs). The relationship between HIF-2α protein expression and the clinical characteristics of CRC was also analyzed by the pooled ORs and 95% CIs. The overall hazard ratios (HRs) and 95% CIs were calculated to assess the impact of HIF-2α expression on the prognosis of CRC, when possible. The heterogeneity among the eligible studies was measured by using Cochran's Q test [33]. The random-effects model (the most common method: DerSimonian-Laird) was applied in the present meta-analysis [34, 35]. For the positive results with more than two studies (substantial heterogeneity: $P < 0.1$), sensitivity analyses were performed to determine whether these removing studies changed the pooled OR and heterogeneity [36]. Potential publication bias was measured by using Egger's linear regression test for the results with greater than nine studies [37]. Trial sequential analysis (TSA) was performed to reduce the risk of type I error, which could estimate the sample size needed with an adjusted threshold when the statistical evidence is conclusive and reliable [38, 39]. Monitoring boundary was constructed to decide whether sufficient evidence in a trial had been achieved. A cumulative z-value greater than the boundary suggested that a trial may be terminated early [40, 41]. The pooled data of HIF-2α expression were analyzed by using Stata software, version 12.0 (Stata Corp., College Station, TX, USA) and R software, version 3.4.0 (The R Foundation for Statistical Computing; Vienna, Austria). For analyses

with fewer than four studies, the combined sensitivity, specificity, and the summary receiver operator characteristic (SROC) curve (AUC) values were calculated to estimate the potential diagnostic role of HIF-2α protein expression in CRC vs. control group (Meta-Disc software, version 1.4; Unit of Clinical Biostatics, the Ramón and Cajal Hospital, Madrid, Spain) [42].

Results

Characteristics of the eligible studies

Fig. 1 lists a detailed selection procedure for the eligible studies by searching online electronic databases. After careful screening based on the above inclusion criteria, finally, ten papers were examined for HIF-2α expression by using the IHC method in patients with CRC [21-24, 43-48], including 1854 participators for the present meta-analysis. Among the eligible publications, three articles assess the correlation of HIF-2α protein expression between CRC and normal tissue samples [22, 43, 46]. Six papers involving 1294 patients evaluated the relationship of HIF-2α protein expression with the clinical features of CRC [21, 23, 43, 44, 47, 48]. Four papers reported the prognostic information of HIF-2α protein expression by using multivariate analysis [23, 24, 45, 48], including 1074 patients with CRC. Ten publications were high quality by using NOS. The general characteristics of the included studies are listed in Table 1.

Association of HIF-2α protein expression between CRC and normal controls

In the comparison of 290 CRCs and 68 normal tissue samples, the data showed that HIF-2α protein expression in CRC was significantly higher than that in normal tissues (OR = 150.49, 95% CI = 16.45-1376.80, $P < 0.001$) (Table 2).

Association of HIF-2α protein expression with some clinical features of CRC

As shown (see online suppl. material) in Table S2, no correlation was found between HIF-2α protein expression and age factor, tumor location (colon vs. rectum), or microvessel density.

The data from three studies of 872 CRC patients showed that HIF-2α protein expression was significantly correlated with gender (male vs. female: OR = 1.47, 95% CI = 1.11, 1.94, $P = 0.008$) (Table 2).

The data from two studies involving 138 CRCs demonstrated that vascular endothelial growth factor (VEGF) expression was correlated with HIF-2α status (OR= 2.56, 95% CI = 1.22-5.38, $P = 0.013$) (Table 2).

Association of HIF-2α protein expression with other clinicopathological features of CRC

HIF-2α protein expression was not associated with clinical stage, lymph node status, depth of tumor invasion,

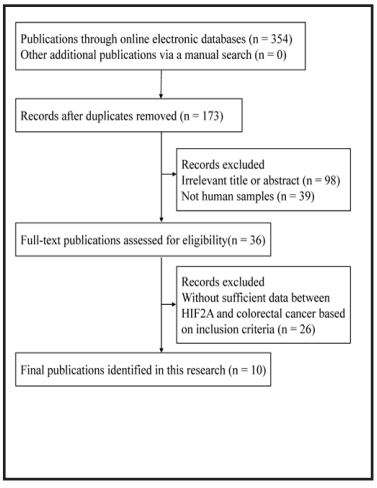

Fig. 1. PRISMA flow diagram of the potential publications.

Table 1. General characteristics of the eligible publications. NA: not applicable; Ab: antibody; IHC: immunohistochemistry; N: nucleus; C: cytoplasm; E: expression; MA: multivariate analysis; OS: overall survival; DSS: disease-specific survival; NOS: Newcastle–Ottawa Scale

First author	Country	Age	M/F	Primary Ab	Time	Design	Stage	IHC-Cut off	CRC Total (E %)	Control Total (E %)	Clinical features	MA-survival	NOS
Yoshimura 2004 [48]	Japan	29-91	51/36	Dilution: 1:1000, clone ep190b; Novus Biologicals, Inc., Littleton, CO	Overnight	Prospective	A-D	N 5%; C highest	87 (29.9)		Yes	NS	9
Koukourakis 2005 [47]	Greece	NA	NA	Dilution 1:2, the EP190b (IgG1 Mo Ab)	Overnight	Retrospective	B-C	N 10%; C 50%	75 (42.7)		Yes	NA	6
Koukourakis 2006 [46]	Greece	NA	NA	EP 190b (Neat), Oxford University	Overnight	Retrospective	NA	N+C: 50%	70 (42.9)	20 (0.0)	No	NA	7
Cleven 2007 [45]	The Netherlands	NA	55/78	Dilution: 1: 500, ab8365 Abcam, UK	100 min	Prospective	1-4	N: 5%	133 (83.5)		No	OS	8
Imamura 2009 [44]	USA	NA	26/37	Dilution: 1:200, ep190b, Novus Biologicals, Littleton, CO	Overnight	Retrospective	1-4	N: Strong	63 (44.4)		Yes	NA	8
Rasheed 2009 [43]	UK	59	56/34	Dilution 1: 100, NB100-132D3, Novus Biologicals	NA	Retrospective	A-C	N+C: NA	90 (64.4)	25 (0.0)	Yes	NA	9
Jubb 2009 [24]	UK	NA	97/62	EP190/E10, UK	NA	Retrospective	A-D	N: Any positivity	159 (53.5)		No	OS	9
Baba 2010 [23]	USA	NA	261/470	Dilution: 1:250, anti-EPAS-1, Santa Cruz Biotechnology	Overnight	Prospective	1-4	C: Weak to strong	695 (46.3)		Yes	OS, DSS	9
Li 2011 [22]	China	NA	NA	Sigma	NA	Retrospective	NA	N: 10%	130 (96.9)	23 (0.0)	No	NA	6
Wu 2015 [21]	China	NA	NA	Dilution: 1:100, ab199, Abcam	Overnight	Retrospective	1-4	NA: 10%	284 (64.4)		Yes	NA	7

vascular invasion, or metastasis (see online suppl. material, Table S2).

Data from two studies comprising 782 patients with CRC indicated that HIF-2α protein expression was negatively associated with tumor differentiation (OR = 0.49, 95% CI = 0.25-0.93, *P* = 0.029) (Table 2).

No obvious evidence of heterogeneity was observed for positive results in CRC vs. normal controls, male vs. female, high grade vs. low grade, and in relation to VEGF expression (Table 2).

Prognostic role of HIF-2α protein expression using multivariate analysis

One study involving 87 patients with CRC reported that HIF-2α protein expression was not associated with the prognosis in OS [48]. The data from three studies involving 987 CRC patients showed no relationship between HIF-2α protein expression and 5-year OS (HR = 1.54, 95% CI = 0.81-2.92, *P* = 0.186) (see online suppl. material, Fig. S1). One study reported that HIF-2α protein expression was not correlated with a 5-year disease-specific survival (DSS) among 695 CRCs (HR = 0.88, 95% CI = 0.66-1.17, *P* = 0.381) [23] (see online suppl. material, Fig. S1).

TSA

TSA was applied for quantification of the required information size in cancer vs. normal controls, and in relation to clinical features with more than one study. The type I error rate of 5% and type II error rate of 20% were set in this analysis.

HIF-2α protein was not expressed in normal tissue samples in this meta-analysis, based on the accrued information size (AIS) method, a TSA was performed by using the assumed intervention effect of relative risk reduction (RRR) of -50%. The results demonstrated that the cumulative Z-curve crossed trial sequential monitoring boundary (see online suppl. material, Fig. S2), which was a true positive result. Thus, there may be no essential evidence for conducting further studies.

When male CRC patients were compared to female CRC patients, a TSA by using the optimal a priori anticipated information size (APIS) method (the assumed intervention effect of RRR of 20%) showed that the cumulative Z-curve crossed trial sequential monitoring boundary. The estimated required information size was 1014 participants (Fig. 2).

In relation to VEGF expression, according to APIS method (RRR = 20%), a TSA showed that the

Table 2. The summary of the significant association between HIF-2α protein expression and colorectal cancer (publications). HIF-2α: hypoxia-inducible factor-2 α; VEGF: vascular endothelial growth factor; OR: odds ratio; 95% CI: 95% confidence interval

Comparison and studies	Case groups	Frequency	Control groups	Frequency	OR with 95% CI	*P* value	Heterogeneity (I^2)
Cancer vs. Normal							
Koukourakis 2006 [46]	30/70	42.9	0/20	0.0	30.88 (1.80, 530.85)		
Rasheed 2009 [43]	58/90	64.4	0/25	0.0	91.80 (5.41, 1557.94)		
Li 2011 [22]	126/130	96.9	0/23	0.0	1321.22 (68.83, 25362.80)		
Total	214/290	73.8	0/68	0.0	150.49 (16.45, 1376.80)	< 0.001	0.169
Cancer (Male vs. Female)							
Yoshimura 2004 [48]	15/51	29.4	11/36	30.6	0.95 (0.37, 2.40)		
Rasheed 2009 [43]	37/56	66.1	21/34	61.8	1.21 (0.50, 2.92)		
Baba 2010 [23]	130/242	53.7	192/453	42.4	1.58 (1.15, 2.16)		
Total	182/349	52.2	224/523	42.8	1.47 (1.11, 1.94)	0.008	0.536
High-grade vs. low-grade							
Rasheed 2009 [43]	8/13	61.5	50/77	64.9	0.86 (0.26, 2.90)		
Baba 2010 [23]	17/62	27.42	305/630	48.4	0.40 (0.23, 0.72)		
Total	25/75	33.3	355/707	50.2	0.49 (0.25, 0.93)	0.029	0.265
VEGF expression (high HIF-2α vs. low HIF-2α)							
Koukourakis 2005 [47]	13/32	40.6	9/43	20.9	2.58 (0.93, 7.16)		
Imamura 2009 [44]	12/28	42.9	8/35	22.9	2.53 (0.85, 7.51)		
Total	25/60	41.7	17/78	21.8	2.56 (1.22, 5.38)	0.013	0.978

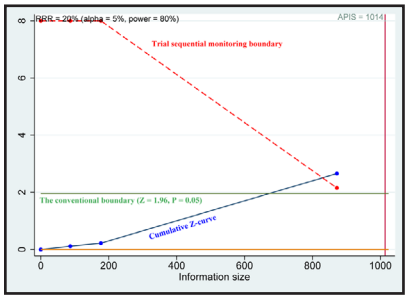

Fig. 2. Trial sequential analysis assessing the association between HIF-2α expression and gender, male vs. female CRC patients, the optimal a priori anticipated information size (APIS) method with 80% power, RRR of 20%, the cumulative Z-curve crossed trial sequential monitoring boundary, suggesting that the cumulative evidence is reliable.

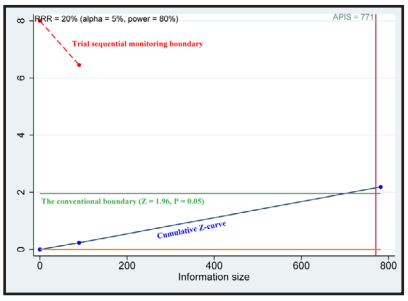

Fig. 3. Trial sequential analysis assessing the association between HIF-2α expression and tumor differentiation, the optimal a priori anticipated information size (APIS) method, RRR = 20%, power = 80%, the cumulative Z-curve crossed the conventional boundary, and the cumulative information size was more than the required information size, indicating that the cumulative evidence is conclusive.

Table 3. The summary of the significant association between HIF-2α expression and the prognosis in cancers (PrognoScan database). HIF-2α: hypoxia-inducible factor-2 α; HR: hazard ratio; 95% CI: 95% confidence interval

N	Dataset	Probe ID	HR (95% CI)	Endpoint	Disease
51	GSE12945	200878_at, 200879_s_at	0.62 (0.13 - 3.11), 0.24 (0.00 - 20.61)	Disease-free survival	Colorectal cancer
145	GSE17536	200878_at, 241055_at, 200879_s_at	1.49 (0.67 - 3.31), 1.86 (0.13 - 25.97), 1.10 (0.46 - 2.64)	Disease-free survival	Colorectal cancer
226	GSE14333	200878_at, 241055_at, 200879_s_at	1.27 (0.75 - 2.15), 1.39 (1.05 - 1.84), 1.26 (0.76 - 2.08)	Disease-free survival	Colorectal cancer
55	GSE17537	200878_at, 241055_at, 200879_s_at	1.14 (0.54 - 2.42), 0.15 (0.00 - 5.34), 0.56 (0.27 - 1.15)	Disease-free survival	Colorectal cancer
Total: 477			Pooled HR with 95%: 1.23 (1.01-1.48), P = 0.037		
115	GSE19615	200878_at, 241055_at, 200879_s_at	1.08 (0.39 - 3.02), 1.33 (0.48 - 3.70), 2.14 (0.87 - 5.27)	Distant Metastasis Free Survival	Breast cancer
87	GSE6532-GPL570	200878_at, 241055_at, 200879_s_at	0.81 (0.51 - 1.26), 3.55 (0.43 - 29.46), 0.91 (0.18 - 4.51)	Distant Metastasis Free Survival	Breast cancer
77	GSE9195	200878_at, 241055_at, 200879_s_at	0.58 (0.21 - 1.54), 0.06 (0.00 - 5.20), 0.13 (0.01 - 1.34)	Distant Metastasis Free Survival	Breast cancer
136	GSE12093	200878_at, 200879_s_at	0.51 (0.26 - 1.00), 0.71 (0.40 - 1.25)	Distant Metastasis Free Survival	Breast cancer
200	GSE11121	200878_at, 200879_s_at	0.48 (0.27 - 0.86), 0.52 (0.36 - 0.74)	Distant Metastasis Free Survival	Breast cancer
286	GSE2034	200878_at, 200879_s_at	0.80 (0.54 - 1.20), 0.79 (0.59 - 1.08)	Distant Metastasis Free Survival	Breast cancer
117	E-TABM-158	200878_at, 200879_s_at	0.91 (0.47 - 1.76), 0.60 (0.11 - 3.27)	Distant Metastasis Free Survival	Breast cancer
125	GSE2990	200878_at, 200879_s_at	0.87 (0.46 - 1.63), 0.42 (0.14 - 1.22)	Distant Metastasis Free Survival	Breast cancer
54	GSE2990	200878_at, 200879_s_at	0.67 (0.38 - 1.17), 0.82 (0.45 - 1.46)	Distant Metastasis Free Survival	Breast cancer
198	GSE7390	200878_at, 200879_s_at	1.18 (0.86 - 1.60), 1.13 (0.85 - 1.51)	Distant Metastasis Free Survival	Breast cancer
Total: 1395			Pooled HR with 95%: 0.80 (0.68-0.95), P = 0.009		
82	jacob-00182-CANDF	200878_at, 200879_s_at	0.32 (0.12 - 0.83), 0.71 (0.31 - 1.63)	Overall Survival	Lung adenocarcinoma
84	HARVARD-LC	38230_at	0.61 (0.38 - 0.98)	Overall Survival	Lung adenocarcinoma
79	jacob-00182-HLM	200878_at, 200879_s_at	0.81 (0.43 - 1.52), 0.92 (0.55 - 1.54)	Overall Survival	Lung adenocarcinoma
86	MICHIGAN-LC	U81984_at	0.47 (0.22 - 1.04)	Overall Survival	Lung adenocarcinoma
104	jacob-00182-MSK	200878_at, 200879_s_at	0.67 (0.30 - 1.49), 0.75 (0.34 - 1.68)	Overall Survival	Lung adenocarcinoma
117	GSE13213	A_23_P210210, A_23_P430120	0.70 (0.46 - 1.09), 0.58 (0.36 - 0.93)	Overall Survival	Lung adenocarcinoma
204	GSE31210	200878_at, 241055_at, 200879_s_at	0.24 (0.08 - 0.73), 0.99 (0.68 - 1.43), 0.59 (0.29 - 1.18)	Overall Survival	Lung adenocarcinoma
178	jacob-00182-UM	200878_at, 200879_s_at	0.91 (0.48 - 1.70), 0.79 (0.49 - 1.27)	Overall Survival	Lung adenocarcinoma
Total: 934			Pooled HR with 95%: 0.72 (0.62-0.83), P < 0.001		
140	GSE30929	200878_at, 200879_s_at	0.65 (0.47 - 0.89), 0.32 (0.16 - 0.64)	Distant Recurrence Free Survival	Liposarcoma
Total: 140			Pooled HR with 95%: 0.49 (0.25-0.97), P = 0.039		
158	GSE4475	200878_at, 200879_s_at	0.56 (0.32 - 1.00), 0.77 (0.27 - 2.22)	Overall Survival	B-cell lymphoma
Total: 158			Pooled HR with 95%: 0.60 (0.36-0.99), P = 0.047		
559	GSE2658	200878_at, 241055_at, 200879_s_at	0.72 (0.59 - 0.87), 1.00 (0.79 - 1.28), 0.70 (0.52 - 0.94)	Disease-specific survival	Multiple myeloma
Total: 559			Pooled HR with 95%: 0.80 (0.64-1.00), P = 0.047		

cumulative Z-curve crossed the conventional boundary (Z = 1.96, *P* = 0.05), but it did not cross trial sequential monitoring boundary (see online suppl. material, Fig. S3), suggesting that this analysis on VEGF expression is a false positive result. More studies with large populations are necessary to further confirm this finding (the estimated required sample size of 2605 patients).

In relation to tumor differentiation, TSA (APIS method: RRR = 20%) revealed that the cumulative Z-curve crossed the conventional boundary, and the number of the cumulative study population was more than the required information size (Fig. 3). Therefore, further relevant studies were unnecessary.

Prognostic role of HIF-2α expression from PrognoScan database

Data on the prognostic significance of HIF-2α expression were also used by PrognoScan database [49]. The pooled data showed that HIF-2α expression was not significantly associated with OS and DSS among 294 and 226 CRCs, respectively (*P* > 0.1) (see online suppl. material, Table S3). HIF-2α expression was correlated with a poor DFS among 477 patients with CRC (HR = 1.23, 95% CI = 1.01-1.48, *P* = 0.037) (Table 3). Additional study populations further suggested that HIF-2α expression was not associated with the OS and DSS of CRC.

Prognostic role of HIF-2α expression from PROGGeneV2 database

To evaluate the prognostic role of HIF-2α expression for CRC in metastasis-free survival and relapse-free survival, PROGGeneV2 database was used [50]. No association was found between HIF-2α expression and the metastasis-free survival and relapse-free survival of CRC ($P > 0.1$) (see online suppl. material, Table S4).

The association between both HIF-2α and VEGFA, VEGFB, or VEGFC expression and the prognosis of CRC was also analyzed in OS, metastasis-free survival and relapse-free survival (see online suppl. material, Table S4 and Fig. 4). Expression of both HIF-2α and VEGFA was linked to a poor relapse-free survival of CRC (751 cases) (HR = 1.93, $P = 0.038$) (Fig. 4), and the expression of HIF-2α and different VEGF subtypes was associated with an unfavorable metastasis-free survival of CRC (247 cases) (HIF-2α and VEGFA: HR = 6.95, $P = 0.009$, HIF-2α and VEGFB: HR = 113.51, $P < 0.001$, HIF-2α and VEGFC: HR = 8.11, $P = 0.009$) (Fig. 4).

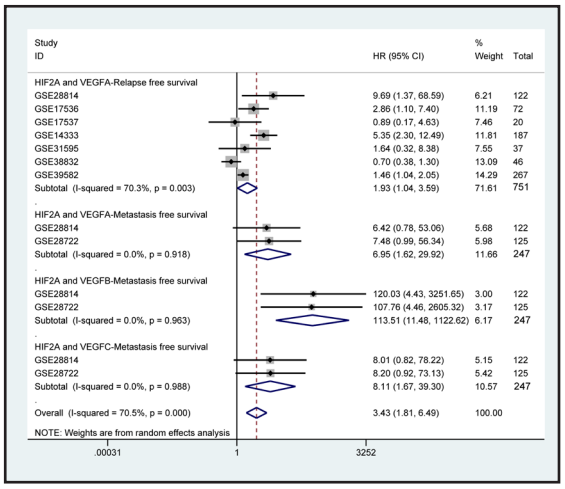

Fig. 4. Forest plot of the significant association of expression of both HIF-2α and different vascular endothelial growth factor (VEGF) subtypes for the prognosis of CRC.

Prognostic role of HIF-2α alteration from the Cancer Genome Atlas Research Network

HIF-2α is altered in 19 samples (3.0%), including 633 CRC patients from the Cancer Genome Atlas Research Network. No significantly statistical significance was observed between HIF-2α alteration and OS ($P = 0.086$) (see online suppl. material, Fig. S4). HIF-2α alteration was not significantly linked to DFS ($P = 0.110$) (see online suppl. material, Fig. S5).

Diagnostic role of HIF-2α protein expression in CRC vs. normal tissue samples

When CRC was compared to normal tissue samples, the pooled sensitivity, specificity, and AUC of HIF-2α protein expression were 0.74 (95% CI = 0.68-0.79), 1.00 (95% CI = 0.95-1.00) and 0.973, respectively (see online suppl. material, Fig. S6).

Prognostic role of HIF-2α expression in other cancers

We finally analyzed the association between HIF-2α expression and the prognosis in other human cancers from PrognoScan database, including bladder cancer, blood cancer (acute myeloid leukemia, B-cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, multiple myeloma), brain cancer (astrocytoma, glioblastoma, glioma, meningioma), breast cancer, uveal melanoma, head and neck squamous cell carcinoma, lung adenocarcinoma, non-small-cell lung carcinoma, lung squamous cell carcinoma, ovarian cancer, melanoma, liposarcoma, prostate cancer, and renal cell carcinoma (see online suppl. material, Table S3). HIF-2α expression may be associated with a favorable OS in 158 patients with B-cell lymphoma (HR = 0.60, $P = 0.047$) and 934 lung adenocarcinoma patients (HR = 0.72, $P < 0.001$), a favorable prognosis of 559 cases with multiple myeloma in DSS (HR = 0.80, $P = 0.047$), a survival benefit of breast cancer (1395 cases) in distant metastasis-free survival (HR = 0.80, $P = 0.009$), and a good prognosis of liposarcoma (140 patients) in distant recurrence-free survival (HR = 0.49, $P = 0.039$) (Table 3). No association was observed between HIF-2α expression and DFS in other cancers.

Discussion

Rapid cell proliferation and the formation of abnormal blood vessels result in hypoxia, and hypoxia has been confirmed in solid tumors. HIF-2α is a type of HIFs involved in body response to oxygen level [51]. The expression of HIF-2α in various cancers has been recorded and detected by many studies [10, 52]. HIF-2α expression may have a longer overall survival in hepatocellular carcinoma by Yang *et al* [53].. Bangoura *et al*. reported that HIF-2α expression was correlated with a shortened overall survival of hepatocellular carcinoma [54]. The present study found that HIF-2α was frequently expressed in patients with CRC [22, 43, 46], and HIF-2α expression was more common in CRC than in normal tissue samples, with undetectable expression of HIF-2α in normal colonic tissues. Additionally, TSA indicated that the evidence of the result of CRC vs. normal controls was reliable. The present finding suggested that HIF-2α expression may be linked to the carcinogenesis of CRC. HIF-2α stimulates the proto-oncogene c-Myc activity and induces progression via the cell cycle [55], which may lead to carcinogenic effects.

Next, the correlation of HIF-2α protein expression with the clinical characteristics of CRC was investigated. No relationship between HIF-2α expression and these clinical features of patients with CRC was observed, including age factor, tumor location (colon vs. rectum), microvessel density, clinical stage, lymph node status, depth of tumor invasion, vascular invasion, and metastasis. Two studies with small populations (fewer than 100 cases per study) showed no correlation between HIF-2α expression and gender (male vs. female) [43, 48], but in a large population (695 cases), Baba 2010 *et al*. reported that HIF-2α expression was associated with gender in CRC [23]. HIF-2α expression was negatively linked to tumor differentiation (high-grade vs. low-grade) by Baba 2010 *et al*. (692 CRC patients) [23], but there was no significant association between HIF-2α expression and tumor differentiation in 90 patients with CRC [43]. These findings, based on more studies, suggested that HIF-2α expression was notably higher in male CRC patients compared with female CRC patients but was lower in patients with high-grade compared with low-grade CRC patients. Further TSA showed that the results on gender and tumor differentiation were not necessary for conducting additional studies in the future.

VEGF expression was not significantly related to HIF-2α expression status in two studies (less than 80 cases per study) [44, 47]. In the present analysis (138 CRCs), VEGF expression was notably higher in high HIF-2α-reactive patients than in low HIF-2α expression patients, suggesting that HIF-2α could increase the expression of VEGF, therefore promoting angiogenesis. While TSA revealed that additional studies with large CRC patients are needed to further validate this false positive result (the estimated required sample information: 2605 patients). Moreover, the expression of both HIF-2α and different VEGF subtypes was linked to an unfavorable metastasis-free survival of CRC (247 cases) (HIF-2α and VEGFA: HR = 6.95, $P = 0.009$, HIF-2α and VEGFB: HR = 113.51, $P < 0.001$, HIF-2α and VEGFC: HR = 8.11, $P = 0.009$), and the HR value of both HIF-2α and VEGFB expression was higher than both HIF-2α and VEGFA, and both HIF-2α and VEGFC, indicating that the expression of both HIF-2α and VEGFB may be more strongly associated with a decreased metastasis-free survival for CRC. More prospective studies on the prognostic association between HIF-2α and different VEGF subtypes in CRC are needed.

Finally, we analyzed the prognostic role of HIF-2α expression or alteration in CRC. Two studies recorded that HIF-2α expression was associated with a decreased 5-year OS using multivariate analysis (fewer than 160 CRC patients per study) [24, 45]. Baba 2010 *et al*. reported no association between HIF-2α expression and the 5-year OS or DSS of CRC in multivariate analysis among a larger population of 695 cases [23]. In addition, HIF-2α gene alteration from the cBioPortal database was not significantly correlated with the prognosis of CRC in OS and DFS. These findings suggested that HIF-2α expression or alteration was not notably associated with OS of patients with CRC. Further analyses from PrognoScan database conformed no relationship between HIF-2α expression and the prognosis of CRC in OS and DSS. PROGGeneV2 database showed no correlation between HIF-2α expression and

metastasis-free survival and relapse-free survival of CRC. However, HIF-2α expression was correlated with a worse DFS for CRC, and a significant relationship was not found between HIF-2α expression and DFS in other cancers, which suggested that HIF-2α expression may become a potential specific marker for the prognosis of CRC in DFS. Future prospective studies are essential to confirm the prognostic effect of HIF-2α expression in DFS.

HIF-2α expression was associated with a different survival benefit among some cancers (B-cell lymphoma and lung adenocarcinoma: OS, multiple myeloma: DSS, breast cancer: distant metastasis-free survival, liposarcoma: distant recurrence-free survival) (all HRs < 1, *Ps* < 0.05), indicating that HIF-2α expression may be a novel prognostic marker and potential therapeutic target for different cancer patient stratification. Moreover, we did not find the relevant drug information for the HIF-2α gene from the Drug-Gene Interaction Database (DGIdb) [56, 57]. Further prospective and well-designed (multicenter, randomized controlled) studies are essential to translate the use of these findings into the clinical applications.

Limitations

Several limitations should be acknowledged. First, although the present study found that the combined sensitivity, specificity, and AUC of HIF-2α protein expression were 0.74, 1.00, and 0.97, respectively, in 290 CRCs vs. 68 normal tissue samples, a frequency of approximately 56% was shown by using IHC in 1786 CRC samples, which further suggested that HIF-2α expression could not be a potential diagnostic marker for CRC. Second, we did not have sufficient studies to analyze the difference of HIF-2α protein expression between CRC and benign lesions (such as adenoma). Third, VEGF positivity was higher in positive HIF-2α-reactive CRCs than that in negative CRCs (OR = 2.56, *P* = 0.013), but too unreliable to obtain this definitive result based on TSA. Additional studies are needed to further confirm the association between VEGF expression and HIF-2α expression status in the future.

Conclusion

The present comprehensive evaluation of available data showed that HIF-2α IHC is notably higher in CRC than in normal colonic tissue samples, and higher in male compared with female patients with CRC. However, HIF-2α IHC is lower in high-grade compared with low-grade CRC. No relationship was found between HIF-2α expression or alteration and OS of CRC. There is no association between HIF-2α expression and DSS, metastasis-free survival, or relapse-free survival of CRC and between HIF-2α alteration and DFS of CRC. HIF-2α expression is correlated with an unfavorable DFS of CRC but is not associated with a DFS in other cancers. HIF-2α expression is linked to a favorable survival in B-cell lymphoma and lung adenocarcinoma (OS), multiple myeloma (DSS), breast cancer (distant metastasis-free survival), and liposarcoma (distant recurrence-free survival). Further prospective clinical studies are necessary to validate these findings based on multicenter design.

Abbreviations

HIF-2α (hypoxia-inducible factor-2α); CRC (colorectal cancer); OR (odds ratio); HR (hazard ratio); TCGA (The Cancer Genome Atlas); GEO (the Gene Expression Omnibus datasets); TSA (trial sequential analysis); OS (overall survival); DSS (disease-specific survival); DFS (disease-free survival); VEGF (vascular endothelial growth factor); EPAS1 (endothelial PAS domain protein 1); PRISMA (the preferred reporting items for systematic reviews and meta-analyses); IHC (immunohistochemistry); NOS (Newcastle-Ottawa Scale); 95% CI (95% confidence interval); SROC (the summary receiver operator characteristic curve); AUC (the summary receiver operator characteristic curve); AIS (the accrued information size); APIS (the optimal a priori anticipated information size); RRR (relative risk reduction); DGIdb (the Drug-Gene Interaction Database).

Acknowledgements

Although this study was not primary research involving human samples, it was a secondary analysis of human subject data in the public domain.

Fenggang Hou, Jianlin Ren, Susu Han, and Tao Huang contributed to the conception and design of this research. Susu Han, Shanshan Liu, Wen Li, Wei Yang, and Qi Shi contributed to writing the article and the final approval of the submitted version. Susu Han, Tao Huang, Shanshan Liu, Wen Li, Wei Yang, Qi Shi, Hongjia Li, Shaoqi Zong, Jianlin Ren, and Fenggang Hou contributed to the analyses and interpretation of the data and completion of the figures and tables. All authors read and approved the final manuscript.

The present study was financially supported by a grant from the Natural Science Foundation of China (81473624).

Disclosure Statement

The authors declare that they have no competing interests.

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