Prognostic Value and Clinicopathological Differences of HIFs in Colorectal Cancer: Evidence from Meta-Analysis

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Abstract

Background: The prognostic value of HIFs in colorectal cancer was evaluated in a large number of studies, but the conclusions were inconclusive. Meanwhile, clinicopathologic differences of HIF-1α and HIF-2α were rarely compared in recent studies.

Methodology: Identical search strategies were used to search relevant literatures in the PubMed and Web of Science databases. The prognostic significances and clinicopathological differences of HIFs in CRC were analyzed.

Principal Findings: A total of 23 studies comprising 2984 CRC patients met the inclusion criteria. The results indicated that overexpressed HIFs were significantly associated with increase of mortality risk, including overall survival (OS) (HR 2.06, 95% CI 1.55–2.74) and disease free survival (HR 2.84, 95% CI 1.87–4.31). Subgroup analysis revealed that both overexpressed HIF-1α and HIF-2α had correlations with worse prognosis. The pooled HRs were 2.01 (95% CI: 1.55–2.6) and 2.07 (95% CI: 1.01–4.26). Further subgroup analysis on HIF-1α was performed by study location, number of patients, quality score and cut-off value. The results showed that HIF-1α overexpression was significantly associated with poor OS, particularly in Asian countries (HR 2.3, 95% CI: 1.74–3.01), while not in European or other countries. In addition, overexpression of HIF-1α was closely related with these clinicopathological features, including Dukes’ stages (OR 0.39, 95% CI: 0.17–0.89), UICC stages (OR 0.42, 95% CI: 0.3–0.59), depth of invasion (OR 0.71, 95% CI: 0.51–0.99), lymphnode status (OR 0.49, 95% CI: 0.32–0.73) and metastasis (OR 0.29, 95% CI: 0.11–0.81). While overexpression of HIF-2α was only associated with grade of differentiation (OR 0.48, 95% CI: 0.29–0.81).

Conclusions: This study showed that both HIF-1α and HIF-2α overexpression were associated with an unfavorable prognosis. HIF-1α overexpression seemed to be associated with worse prognosis in Asian countries. Additionally, HIF-1α and HIF-2α overexpression indicated distinct clinicopathologic features.

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Introduction

Colorectal cancer is the third most common malignancy worldwide, and one of the leading causes of cancer-related deaths [1]. An increasing trend in the incidence of this carcinoma has been noticed in the Asian nations. Despite recent therapeutic advances, its 5-year survival rate is still pessimistic due to its recurrence and drug resistance [2]. Growing evidence suggests that hypoxia plays a pivotal role in disease progression and therapy resistance in most solid tumors, including colorectal cancer [3,4]. Rapid oxygen consumption and aberrant tumor angiogenesis and blood flow result in a hypoxic tumor environment. Owing to the fundamental importance of oxygen for metabolism and survival, cells have evolved intricate response mechanisms to respond to hypoxia. The most important regulators mediating the primary transcriptional responses to hypoxic stress are hypoxia-inducible factors (HIFs). Given that hypoxia promotes tumor progression and therapy resistance, HIFs are expected to be useful biomarkers associated with progress disease and poor prognosis in CRC. Increased expression of HIFs has also been observed in a broad range of human cancer cell types, and has been associated with poor prognosis in many cases [5], but the prognostic value of HIFs for CRC patients is inconclusive.

HIFs are heterodimers composed of an inducible α-subunit (HIF-1α, HIF-2α or HIF-3α), and a constitutive HIF-1β subunit (also known as aryl hydrocarbon receptor nuclear translocator or
ARNT), which together form the HIF-1, HIF-2 and HIF-3 transcriptional complexes, respectively. Of the three HIF family members, HIF-1 and HIF-2 are the most well-characterized. HIF-1α and HIF-2α are usually detected to measure tumor oxygen levels because the HIF-1β subunit is constitutive. HIF-1α and HIF-2α have 48% amino acid sequence identity and similar protein structures, but distinct target genes and mechanisms of regulation. HIF-1α preferentially induces glycolytic pathway, whereas HIF-2α regulates genes involved in tumor growth, cell cycle and maintaining stem cell pluripotency [6]. Thus, HIF1α and HIF2α can promote highly divergent, even opposing, outcomes, which results in distinct clinicopathologic features and prognosis. Multiple xenograft tumor models also support the hypothesis that HIF1α and HIF2α play different roles in tumor progression by regulating both shared and unique target genes [7]. However, clinicopathologic and prognostic differences of HIF1α and HIF2α in CRC were rarely compared in recent studies. Therefore, we made a meta-analysis from eligible studies to investigate the relationship between HIF expression and prognosis of CRC patients. Meanwhile, we performed a subgroup analysis to assess the roles of HIF-1α and HIF-2α in clinicopathologic features and prognosis of CRC.

Materials and Methods

Identification and eligibility of relevant studies

We searched literature from PubMed, WanFang and Web of Science databases using the terms: “HIF”, “colorectal neoplasms”, “colorectal Cancer”, “colon cancer” “rectal cancer”, “prognosis” with all possible combinations. Bibliographies, review articles and other pertinent studies were searched manually for additional eligible studies.

The inclusion criteria for eligibility of a study in the meta-analysis were as follows: (1) evaluating HIF expression in the human CRC tissues; (2) assessing the relationships between HIFs expression with CRC clinicopathologic features or prognosis; (3) articles written in English or Chinese; (4) sufficient information provided to estimate hazard ratio (HR) about overall survival (OS) or disease free survival (DFS), or to estimate odds ratio (OR) about clinicopathologic features. In addition, letters, reviews, conference abstracts, and case reports were not in the scope of our analysis because of the limited data. Overlapping articles were also excluded from this meta-analysis, only the most recent or the most complete study was involved in the analysis.

Data extraction and management

Two investigators (Xin He and Wenjie Xia) reviewed each eligible study independently and extracted data from all the publications meeting the inclusion criteria. Controversial problems were arbitrated by the third investigator (Jinhong Xu). The following information was collected from each study: the first author’s name, year of publication, country of origin, number of patients, gender of patients, HIF isoforms, source and dilution of antibody, cut-off value, tumor characteristics, condition of adjuvant therapy and survival data.

Methodological assessment

Newcastle–Ottawa quality assessment scale (NOS) was used to assess the quality of each study [8]. The score assessed eight items of methodology, categorized into three dimensions including selection, comparability, and outcome. A maximum of 1 score was awarded for each item with the exception of the item related to comparability that allowed the assignment of two scores. A total of 0 and 9 scores were respectively designated as lowest and highest quality, and the studies with 6 scores or more were graded as the high quality ones in the scale. The scores provided by two researchers were compared and a consensus value for each item was achieved.

Statistical methods

For the pooled analysis of the impact of HIF expression on survival outcome, HRs and its 95% CI were used. If these
| First author | Year | Country       | HIF isoforms | Number of total patient (positive) | Expression location | Method | Antibody source     | Antibody dilution | Definition of HIF positive | HR estimation | Quality score |
|--------------|------|---------------|--------------|-----------------------------------|---------------------|--------|---------------------|-------------------|------------------------|---------------|---------------|
| Xie          | 2012 | China         | HIF-1α       | 93 (46)                           | C and N             | IHC    | Santa Cruz          | 1:100             | Multiplying the intensities core by expressions core = median value | NA            | 8             |
| Korkeila     | 2011 | Finland       | HIF-1α       | 168 (68)                          | N                   | IHC    | BD                  | 1:100             | Weak, moderate or strong staining | HR for DFS    | 7             |
| Shioya       | 2011 | Japan         | HIF-1α       | 50 (21)                           | N                   | IHC    | Neomarkers          | 1:20000           | 40%                    | HR for OS and survival curves for DFS | 8             |
| Havelund     | 2011 | Denmark       | HIF-1α       | 86 (39)                           | N                   | IHC    | BD                  | 1:75              | Summing the intensities core and expressions core = 3 | Survival curves for OS | 7             |
| Saigusa      | 2011 | Japan         | HIF-1α       | 52 (NA)                           | NA                  | RT-PCR | NA                  | NA                | 0.0212 for PFS and 0.1274 for OS | HR for OS and DFS | 7             |
| Mohammed     | 2011 | Spain         | HIF-2α       | 154 (NA)                          | NA                  | RT-PCR | NA                  | NA                | NA                     | NA            | 6             |
| Kwon         | 2010 | Korea         | HIF-1α       | 311 (196)                         | N                   | IHC    | Novus              | 1:50              | 10%                    | HR for OS and DFS | 7             |
| Wu           | 2010 | China         | HIF-1α       | 68 (30)                           | C and N             | IHC    | Abcam              | 1:200             | Multiplying the intensities core by expressions core = 3 | NA            | 6             |
| Zheng        | 2010 | China         | HIF-1α       | 62 (39)                           | C and N             | IHC    | Boster             | 1:100             | Summing the intensities core and expressions core = 3 | NA            | 5             |
| Baba         | 2010 | United States | HIF-1α       | 731 (142)                         | C                   | IHC    | Santa Cruz         | 1:500             | Moderate staining ≥ 50% HR for OS or Any strong staining | HR for OS | 7             |
|              |      |               | HIF-2α       | 695 (322)                         | C                   | IHC    | Santa Cruz         | 1:250             | Weak to strong expression, HR for OS | HR for OS | 7             |
| Toiyama      | 2010 | England       | HIF-1α       | 40 (NA)                           | NA                  | RT-PCR | NA                  | NA                | 0.3649                 | HR for DFS    | 5             |
| Gao          | 2009 | China         | HIF-1α       | 71 (39)                           | C and N             | IHC    | Zymed              | 1:50              | Any staining          | HR for OS | 8             |
| Jubb         | 2009 | Austriaia     | HIF-2α       | 155 (60)                          | N                   | IHC    | BD                  | 1:100             | Moderate or strong staining ≥ 10% in nuclear or distinct, Strong staining in cytoplasm | HR for DFS | 4             |
| Rajaganeshan | 2009 | England       | HIF-1α       | 55 (25)                           | C or N              | IHC    | Abcam              | 1:100             | > 10% in nuclear or distinct, Strong staining in cytoplasm | HR for DFS | 4             |
| Schmitz      | 2009 | Germany       | HIF-1α       | 129 (34)                          | N                   | IHC    | Transduction Laboratories | 1:10              | Any staining          | NA            | 8             |
| Rasheed      | 2009 | England       | HIF-1α       | 90 (48)                           | C and N             | IHC    | Novus              | 1:500             | NA                     | HR for DFS    | 7             |
| Cleven       | 2007 | Holland       | HIF-2α       | 133 (70)                          | N                   | IHC    | BD                  | 1:120             | 5%                     | HR for OS | 4             |
| Lu           | 2006 | China         | HIF-1α       | 30 (19)                           | N                   | IHC    | NA                  | 1:200             | 10%                    | Survival curves for OS | 7             |
| Theodoropoulos | 2006 | Greece        | HIF-1α       | 92 (44)                           | N                   | IHC    | Stress Gene        | 1:1200            | Moderate or strong staining | HR for OS and DFS | 7             |
statistical variables were described in a literature, we pooled it directly; otherwise, they were calculated from available numerical data in the articles according to the methods described by Parmar [9]. In brief, if the trials offered the data such as log-rank test p values, number of total events. The number of aberrant HIF expression and number of preserved HIF expression were extracted to allow estimation of the HR and its 95% CI. If only Kaplan Meier graphs were published, Kaplan-Meier curves were read by Engauge Digitizer version4.1 (http://digitizer.sourceforge.net/). Time-to-event data from the Kaplan–Meier curves was extracted and HR and its 95% CI were calculated via SPSS16.0. Odds ratios (ORs) and their 95%CIs were combined to evaluate the association between HIF expression and clinicopathological factors, such as differentiation grade, Dukes’ stages, depth of invasion, lymphnode status and metastasis. An observed HR>1 implies worse survival for the group with overexpressed/negative HIF expression. An observed OR<1 implies unfavorable parameters for the group with overexpressed/negative HIF expression. The impact of overexpressed/negative HIF expression on survival or clinicopathological factors was considered to be statistically significant if the 95%CI did not overlap with 1. Heterogeneity in between-study was assessed by Chi-square based Q statistical test [10]. And the I² statistic to quantify the proportion of the total variation, which is due to inter-study heterogeneity rather than sampling error and is measured from 0% to 100% [11]. Higher values indicate a greater degree of heterogeneity. When the studies were found to be homogeneous(-with P>0.10 for the Q test), the pooled ORs and HRs estimate of each study were calculated by the fixed-effects model (the Mantel-Haenszel method) [12]. Otherwise, we chose the random-effects model (the DerSimonian and Laird method) [13]. We assessed the possibility of publication bias by visually assessing a funnel plot for asymmetry and by quantitatively performing Egger’s test. Publication bias was indicated when p value of Egger’s test <0.05. The meta-analysis was performed using STATA version 12.0 software (Stata Corporation, Collage Station, Texas, USA). All the P values were for a two-side test and considered statistically significant when p<0.05.

Results

Description of studies

As shown in Figure 1, 227 published records were identified from a search of the above databases using the search strategy as described above. After exclusion of the studies that were out of the scope of our systematic review, a total of 23 eligible studies were included in the final meta-analysis [4,5,14–32]. Of these 23 publications, 20 studies assessed the relationships between HIF-1 expression with CRC clinicopathologic features or prognosis, while 6 studies evaluated the association of HIF-2 expression and CRC pathological features or prognosis. The clinical features of these 23 included studies were summarized in Table 1. These studies were published from 2003 to 2013, and total 2984 CRC patients were enrolled. Sample sizes ranged from 30 to 731 patients (mean 130). 14 of these studies enrolled less than 100 patients and 9 studies included more than 100 patients. 6 of these studies evaluated patients from China, 5 from Japan, 3 from England, others from America, Korea, Finland, Germany, Australia, Holand and Greece. 19 of these studies got 6 scores or more in methodological assessment, which meant they had high qualities.
Figure 2. Forrest plot of Hazard ratio (HR) for the association of different HIF isoforms expression with overall survival (OS) and disease free survival (DFS). A. HRs with corresponding 95% CIs of the HIFs expression with OS. B. HRs with corresponding 95% CIs of the HIFs expression with DFS. HR > 1 implied worse survival for the group with increased HIFs/negative expression and overexpressed HIFs was significantly with the worse prognosis of CRC patients.

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Impact of HIFs expression on overall survival and disease free survival of colorectal cancer

The meta-analysis was performed on 15 studies assessing the association of HIFs expression with OS. The pooled HR was 2.06 (95%CI 1.55–2.74; I² 69.1%) (Figure 2A). Nine studies evaluating the correlation of HIFs expression with DFS were all about HIF-1α. The pooled HR was 2.84 (95%CI 1.87–4.31; I² 41%) (Figure 2B). It suggested that overexpressed HIF was significantly associated with increase of mortality risk. In addition, sensitive analysis was performed. We removed one study at a time and evaluated the rest, pooled HR of HIFs overexpression on OS ranged from 1.98(95% CI: 1.5–2.61) to 2.28(95% CI: 1.74–2.98) (Table 2), and combined HR of HIFs overexpression on DFS ranged from 2.34(95% CI: 1.68–3.26) to 3.22(95% CI: 2.08–4.99) (Table 3). We also performed subgroup analysis about association of HIFs expression with OS by HIF isoforms, the results showed that both HIF-1α and HIF-2α were associated with worse prognosis. The pooled HR was 2.01 (95% CI: 1.55–2.6, I² 33.1%) and 2.07(95% CI: 1.01–4.26, I² 86.1%) respectively (Figure 2A). Subgroup analysis about association of different subcellular localization of HIFs expression with OS was performed, and the results showed that the correlation was not changed no matter where HIF located in (nucleus or cytoplasm). The pooled HR was 2.456 (95% CI: 1.694–3.561, I² 49.2%) and 2.049(95% CI: 1.519–2.764, I² 0%), respectively (Figure 2B).

Moreover, further subgroup analysis on HIF-1α was performed by study location, number of patients, antibody dilution, cut-off value. Subgroup analysis indicated a significant relation between HIF-1α overexpression and OS was exhibited in Asian countries (HR 2.3, 95% CI: 1.74–3.01, I² 0%). Other factors comprising number of patients, antibody dilution and cut-off value did not alter the significant OS of overexpressed HIF-1α (Table 4).

Correlation of HIFs expression with clinicopathological parameters

The meta-analysis was also assessed the correlation between HIF-1α expression and clinicopathological characteristics of CRC. As shown in Table 5, overexpression of HIF-1α was significantly associated with Dukes’ stages (OR 0.39, 95% CI: 0.17–0.89), UICC stages (OR 0.42 95% CI: 0.3–0.59), depth of invasion (OR 0.71, 95% CI: 0.51–0.99), lymphnode status (OR 0.49, 95% CI: 0.32–0.73) and metastasis (OR 0.29, 95% CI: 0.11–0.81).

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**Table 2.** HRs (95% CI) of sensitivity analysis for HIFs overexpression on OS.

| Study omitted       | Estimated HR | low value of 95%CI | High value of 95%CI |
|--------------------|--------------|--------------------|--------------------|
| Korkeila (2011)    | 3.08576      | 2.080869           | 4.575933           |
| Shioya (2011)      | 2.716886     | 1.716758           | 4.299657           |
| Kwon (2010)        | 3.219644     | 2.075922           | 4.993494           |
| Saigusa (2011)     | 2.852396     | 1.829702           | 4.446713           |
| Toyama (2010)      | 2.839389     | 1.815004           | 4.441936           |
| Rajaganeshan (2009)| 2.338718     | 1.678648           | 3.258336           |
| Rasheed (2009)     | 2.684669     | 1.706306           | 4.224007           |
| Theodoropoulos (2006)| 2.776092  | 1.734063           | 4.444295           |
| Shimomura (2013)   | 3.028906     | 1.852393           | 4.95266            |
| Combined           | 2.8408349    | 1.8734429          | 4.3077604          |

**Table 3.** HRs (95% CI) of sensitivity analysis for HIFs overexpression on DFS.

| Study omitted       | Estimated HR | low value of 95%CI | High value of 95%CI |
|--------------------|--------------|--------------------|--------------------|
| Shioya (2011)      | 2.0867274    | 1.567879           | 2.777251           |
| Havelund (2011)    | 2.2600963    | 1.7024611          | 3.0003829          |
| Kwon (2010)        | 2.1164002    | 1.5588678          | 2.8733358          |
| Saigusa (2011)     | 2.0795995    | 1.5696353          | 2.7550092          |
| Baba (2010)        | 2.2764547    | 1.7367126          | 2.9839401          |
| Gao (2009)         | 2.1033344    | 1.5671818          | 2.8229115          |
| Lu (2006)          | 2.0664124    | 1.5748442          | 2.7114177          |
| Theodoropoulos (2006)| 2.0455844 | 1.541568           | 2.7143891          |
| Yoshimura (2004)   | 1.9817994    | 1.5027167          | 2.6136189          |
| Shimomura (2013)   | 2.2203045    | 1.6552455          | 2.97826            |
| Yu (2012)          | 2.1814032    | 1.602671           | 2.9691179          |
| Jubb (2009)        | 2.2353566    | 1.6377747          | 3.0509808          |
| Cleven (2007)      | 2.0521758    | 1.554372           | 2.7094064          |
| Combined           | 2.127789     | 1.6131618          | 2.8065913          |
### Stratified analysis of pooled hazard ratios for colorectal cancer patients with overexpressed HIF-1α.

| Stratified analysis | Number of studies | Number of patients | Pooled HR(95% CI) | P value | I²(%) | P value | Model used |
|---------------------|-------------------|--------------------|--------------------|---------|-------|---------|------------|
| Study location      |                   |                    |                    |         |       |         |            |
| Asia                | 8                 | 789                | 3.45 (1.06, 11.19) | 0.000   | 0     | 0.598   | FEM        |
| Europe              | 2                 | 178                | 1.16 (0.64, 2.11)  | 0.239   | 77.7  | 0.034   | REM        |
| Number of patients  |                   |                    |                    |         |       |         |            |
| >100                | 3                 | 1166               | 2.43 (1.40, 4.22)  | 0.000   | 11.69 |         | FEM        |
| ≤100                | 5                 | 532                | 3.65 (1.52, 70.66) | 0.000   | 1.93  |         | FEM        |
| Cut off value       |                   |                    |                    |         |       |         |            |
| Percentage          | 5                 | 602                | 9.15 (1.18, 70.66) | 0.000   | 13.25 | 0.144   | FEM        |
| Staining            | 2                 | 163                | 3.65 (1.52, 70.66) | 0.000   | 13.25 | 0.144   | FEM        |
| Percentage+staining | 3                 | 881                | 4.61 (1.40, 15.50) | 0.000   | 4.70  |         | FEM        |
| Dilution            |                   |                    |                    |         |       |         |            |
| ≤1:500              | 6                 | 686                | 3.34 (1.97, 5.67)  | 0.000   | 12.10 |         | FEM        |
| >1:500              | 4                 | 960                | 2.11 (1.54–2.88)   | 0.000   | 33.35 |         | FEM        |

**NOTE:** Weights are from random effects analysis.

**Figure 3. Forrest plot of Hazard ratio (HR) for the association of HIF in different subcellular localization with overall survival (OS).**
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**Table 4. Stratified analysis of pooled hazard ratios for colorectal cancer patients with overexpressed HIF-1α.**

REM, random-effects model; FEM, fixed-effects model; HR, hazard ratio; CI, confidence interval.
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Furthermore, there was no significant association between HIF-1α expression with grade of differentiation. The pooled OR was 0.97 (95% CI: 0.67–1.39). (Figure 4).

In addition, we evaluated the correlation between HIF-2α overexpression with clinicopathological characteristics of CRC. The result showed that overexpression of HIF-2α was significantly associated with grade of differentiation (OR 0.48, 95% CI: 0.29–0.81). There was no significant association between HIF-2α expression with Dukes’ stages, depth of invasion and lymphnode status. The pooled OR was 0.91 (95% CI: 0.20–4.17), 0.38 (95% CI: 0.04–3.80), and 0.95 (95% CI: 0.42–0.59) respectively (Table 5).

Publication bias

Egger’s test indicated that there was no evidence of significant publication bias after assessing the funnel plot (Figure S1–S3) for the studies included in our meta-analysis.

Discussion

Hypoxia has been recognized as a common feature of solid tumors and a negative prognostic factor for response to treatment and survival of cancer patients. In 1993, Hockel reported that cervix cancer patients with hypoxic tumors (median pO₂<10 mmHg) had a significantly lower overall and recurrence-free survival [39]. Since then, hypoxia has been found to indicate a highly aggressive disease phenotype associated with poor prognosis in many cancers, including brain, breast, prostate, pancreas, cervix, bladder and ovary [34–37]. HIFs are the best characterized markers mediating transcriptional responses to hypoxic stress and expected to be unfavorable prognostic indicators. Hypoxia and consequently HIF activation is regarded as an important stimulus of CRC angiogenesis. HIF binds to the HRE in the VEGF promoter region, leading to up-regulation of VEGF transcription and the formation of new blood vessels [38].

HIFs in Colorectal Cancer

In this meta-analysis, we had dealt with highly significant heterogeneity among the 23 studies. Although we used random effects models to analyze the data, it did not identify the source of heterogeneity. Thus, we performed stratified analysis according to study location, number of patients, antibody dilution, cut-off value. HIF-1α overexpression was significantly associated with poor OS in Asian countries (HR 2.3, 95% CI: 1.74–3.01, Z = 5.76, P = 0.000), while not in European or other countries. It indicated that HIF-1α overexpression seemed to be associated with disease progress and unfavorable prognosis in Asian CRC patients. Other factors did not alter the significant OS of overexpressed HIF-1α. In addition, significant correlations were observed between HIF-1α overexpression with clinicopathological features including Dukes’ stages, UICC stages, depth of invasion, lymphnode status and metastasis. Our results concurred with previous study that HIF-1α expression had a significant inverse correlation in T1 and T2 CRC. On the other hand, overexpression of HIF-2α was significantly associated with grade of differentiation. Thus, HIF-1 and HIF-2 indicate distinct clinicopathologic features.

In this meta-analysis, we had dealt with highly significant heterogeneity among the 23 studies. Although we used random effects models to analyze the data, it did not identify the source of heterogeneity. Thus, we performed stratified analysis according to study location, number of patients, cut-off value. When the analysis on OS was performed without consideration of other factors, heterogeneity was detected (I² = 69.1%, P = 0.000). While when the studies included were classified into three groups, HIF-1 and HIF-2 have distinct target genes, but few studies compared the clinicopathologic and prognostic differences between HIF-1 and HIF-2.

This meta-analysis aimed to examine the association between HIFs expression and the prognosis of CRC patients, and assess the roles of HIF-1α and HIF-2α in clinicopathologic features. Our analysis combined the outcomes of 23 studies comprising 2984 CRC patients, indicating that overexpressed HIF was significantly associated with increase of mortality risk, including OS (2.06 95%CI 1.55–2.74; Z = 4.95; P = 0.000) and DFS (2.84,, 95%CI 1.87–4.31; Z = 4.92; P = 0.000). Additionally, the results of sensitivity analysis showed that the association was not changed after removing any study. Subgroup analysis revealed that both overexpressed HIF-1α and HIF-2α were associated with worse prognosis in CRC. On the basis of different HIF isoforms, further subgroup analysis was performed by study location, number of patients, antibody dilution, cut-off value. HIF-1α overexpression was significantly associated with poor OS in Asian countries (HR 2.3, 95% CI: 1.74–3.01, Z = 5.76, P = 0.000), while not in European or other countries. It indicated that HIF-1α overexpression seemed to be associated with disease progress and unfavorable prognosis in Asian CRC patients. Other factors did not alter the significant OS of overexpressed HIF-1α. In addition, significant correlations were observed between HIF-1α overexpression with clinicopathological features including Dukes’ stages, UICC stages, depth of invasion, lymphnode status and metastasis. Our results concurred with previous study that HIF-1α expression had a significant inverse correlation in T1 and T2 CRC. On the other hand, overexpression of HIF-2α was significantly associated with grade of differentiation. Thus, HIF-1 and HIF-2 indicate distinct clinicopathologic features.
prognostic significance and clinicopathologic differences of prospective studies are required to investigate the precise in Asian CRC patients. Significant correlations were observed that overexpressed HIF-1α was associated with advanced Dukes’ stage of CRC. ORs with corresponding 95% CIs of the HIF-1α overexpression with UICC stage. OR<1 suggested that unfavorable parameters for the group with increased HIF-1α overexpression was associated with advanced stage of CRC. ORs with corresponding 95% CIs of the HIF-1α overexpression with lymph node metastasis. 

Supporting Information

Figure S1 Egger’s publication bias plot showed no publication bias for studies regarding overexpressed HIF-1α and overall survival (OS) in the meta-analysis: the relationship between the effect size of individual studies (HR, vertical axis) and the precision of the study estimate (standard error, horizontal axis). (TIF)

Figure S2 Egger’s publication bias plot showed no publication bias for studies regarding overexpressed HIF-2α and disease free survival (DFS) in the meta-analysis. (TIF)

Figure S3 Egger’s publication bias plot showed no publication bias for studies regarding overexpressed HIF-2α and overall survival (OS) in the meta-analysis. (TIF)

Checklist S1 PRISMA Checklist. (DOC)

Author Contributions

Conceived and designed the experiments: ZC XH. Performed the experiments: JY CN PW. Analyzed the data: DW WX QH. Contributed reagents/materials/analysis tools: ZZ JX FQ. Wrote the paper: ZC JH. Examined and revised the manuscript: FQ.

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