

# Prognostic significance and clinicopathological features of hypoxic inducible factor-2alpha expression in hepatocellular carcinoma

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## ABSTRACT

**الأهداف:** تقييم مدى العلاقة بين معامل ألفا (HIF-2α) المحرض للاعتلال الدماغي بنقص الأوكسجين وسرطان الخلايا الكبدية باستخدام التحليل البعدي.

**الطريقة:** أُجريت هذه الدراسة في مستشفى مقاطعة أنهوي، هيفي، أنهوي، الصين وذلك في فبراير 2014م. لقد قمنا بالبحث في العديد من قاعدات البيانات التي تضمنت الدراسات المنشورة عن الموضوع باللغتين الإنجليزية والصينية حتى فبراير 2014م. وبعدها قمنا بجمع كلاً من نسبة الخطر العامة للنجاة من المرض، بالإضافة إلى النسب الأرجحية وذلك من أجل تقييم السمات السريرية النسيجية لظهور معامل ألفا-2 لدى المرضى المصابين بسرطان الخلايا الكبدية.

**النتائج:** شمل التحليل البعدي ما مجموعه 7 دراسات انطبقت عليها معايير معينة، وقد تضمنت 1066 مريضاً مصاباً بسرطان الخلايا الكبدية. لقد كان جميع المرضى من الصين. وأشارت نتائج الدراسة بأن العلاقة بين ظهور معامل ألفا والقيم التنبؤية بسير تطور المرض قد كانت غير واضحة. في حين ارتبط ظهور هذا المعامل بصورة واضحة من الناحية الإحصائية بارتشاح الكبسولات، وغزو الأوردة، ودرجة النسيج.

**الخلاصة:** أظهرت الدراسة بأنه يمكن أن يكون هنالك علاقة بين ظهور معامل ألفا-2 وغزو الأوردة وانتقال الورم لدى المرضى المصابين بسرطان الخلايا الكبدية وذلك حسب الأدلة القليلة التي شملها التحليل. وبالمقابل لم يكن لظهور هذا المعامل علاقة واضحة من الناحية الإحصائية مع توقعات سير المرض. لذلك فنحن بحاجة إلى المزيد من الدراسات المتحكم بها ذات الجودة العالية ولعينات كبيرة للتأكد من نتائج هذه الدراسة.

**Objectives:** To assess the association between hypoxic inducible factor-2alpha (HIF-2α) and hepatocellular carcinoma (HCC) by meta-analysis.

**Methods:** This study was carried out at Anhui Province Hospital, Hefei, Anhui, China in February 2014. We searched various databases for studies published in English or Chinese up to February 28, 2014. The hazard ratio for overall survival and analyzed odds ratio were combined to evaluate the clinicopathological features of HIF-2α expression in HCC.

**Results:** A total of 7 eligible studies comprising 1066 patients with HCC were identified after our full assessment according to inclusion criteria. All of the patients came from China. The results indicated that the association between HIF-2α expression and prognostic values in HCC was inconspicuous, while the expression of HIF-2α was significantly associated with capsule infiltration, vein invasion, and histological grade.

**Conclusion:** Expression of HIF-2α was associated with invasion and metastasis in HCC, but did not have a distinct significance in prognosis, according to the limited evidence. However, high quality, large sample size, and controlled trials are required.

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Hepatocellular carcinoma (HCC) is the sixth most common cancer of humans, and the third leading cause of cancer-related deaths worldwide.<sup>1</sup> In spite of advances in surgical technique and early diagnosis, the prognosis of HCC is still poor due to high recurrence and metastatic rate. The recurrence rate in the first year after curative surgery reaches up to 40%.<sup>2</sup> However, the mechanism is complex and unclear. Thus, it is essential to identify new molecules targeted in the development of HCC. Hypoxic inducible factors (HIFs) which include HIF-1, HIF-2, and HIF-3, were reported to play a crucial role in HCC development. The latest evidence indicates that HIF-1 $\alpha$  is up-regulated in HCC development, and its expression may be associated with the clinical outcome of HCC.<sup>3</sup> Similarly, HIF-2 $\alpha$  which is encoded by the endothelial PAS domain protein 1 (EPAS1) gene, shares 48% similarity in amino acid sequence and certain overlapping functions with HIF-1 $\alpha$ .<sup>4</sup> Under the condition of hypoxia in vitro, the HIF-2 $\alpha$  protein is gradually accumulated so that it can persistently activate downstream target genes, while HIF-1 $\alpha$  is transiently stabilized and primarily mediates acute responses, which suggests that HIF-2 $\alpha$  plays a more essential role in the hypoxia response in tumors.<sup>5</sup> Increasing evidence indicates that expression of HIF-2 $\alpha$  is deregulated in a variety of solid tumors, and its expression may be associated with clinical progression and outcome of tumors. However, less is known about the expression and roles of HIF-2 $\alpha$  in HCC. The existing studies have not provided conclusive evidence that HIF-2 $\alpha$  overexpression was relevant to the progression of HCC.

In this study, we reviewed the currently available evidence in medical literature on the relationship between HIF-2 $\alpha$  expression and clinical features and prognosis of HCC, and further assessed the strength of association between them to better understand the development and progression of HCC.

**Methods.** Search strategy and literature search. We searched a range of databases including PubMed (1966-2014), Web of Science (1945-2014), ELSEVIER Science Direct (1823-2014), EMBASE (1990-2014), Chinese Biological Medicine (CBM, 1982-2014) and

China National Knowledge Infrastructure (CNKI, 1979-2014) updated to February 28th, 2014 using the terms: “HIF-2 $\alpha$ ” or “hypoxia-inducible factor 2, alpha subunit” or “hypoxia inducible factor 2 $\alpha$ ” or “endothelial PAS domain protein 1” or “EPAS1” with “carcinoma, hepatocellular” or “hepatocellular carcinoma” or “liver cell carcinoma” or “HCC”. All eligible studies were retrieved, while the review of reference lists was also conducted. Some of them were ruled out because of insufficient data. When faced with the same study specimens, we filtered the latest and the most informative study, to avoid duplicate data.

**Criteria for inclusion and exclusion.** 1) studies regarding HCC, 2) studies using immunohistochemical staining (IHC) to detect the expression of HIF-2 $\alpha$ , 3) samples obtained via surgical resection, 4) studies revealing that the expression of HIF-2 $\alpha$  in HCC was relevant to clinical features or the prognostic indexes. We excluded studies complying with the following: 1) studies on cell lines or animals, 2) reviews without any data, case studies, or conference, 3) tumor samples without intratumoral tissues, or just involving the paracarcinoma tissues, 4) the detection method was not IHC.

**Data extraction and synthesis.** The data was independently extracted from all studies by 2 authors, and the following information obtained: authors, publication year, sample size, index of the prognostic and clinical features (Table 1). They also rated the quality of each eligible study using the Newcastle-Ottawa quality assessment scale (NOS).<sup>6</sup> The assessment has 8 items and each of them range from one to 2 points, a total of 10 points. We summed the scores of each study, as shown in Table 1.

**Statistical analysis.** We measured the impact of HIF-2 $\alpha$  expression on HCC in 2 steps. Firstly, we pooled the data of overall survival (OS) by hazard ratios (HRs) and 95% confidence intervals (CIs) to calculate the effective value to assess the correlation between HIF-2 $\alpha$  and prognosis of HCC. If the HR and 95% CI were described in the study, we pooled it directly. When the variables were not given explicitly, they were calculated from available numerical data according to the method described by Parmar.<sup>7</sup> Secondly, to assess the importance of HIF-2 $\alpha$  expression on clinical features in HCC, we compared the rate of HIF-2 $\alpha$  expression and clinicopathological features in HCC by odds ratio (OR) and 95% CI. By convention, an observed HR<1 implies favorable parameters for the test of HIF-2 $\alpha$  overexpression on the variable; otherwise, it implies

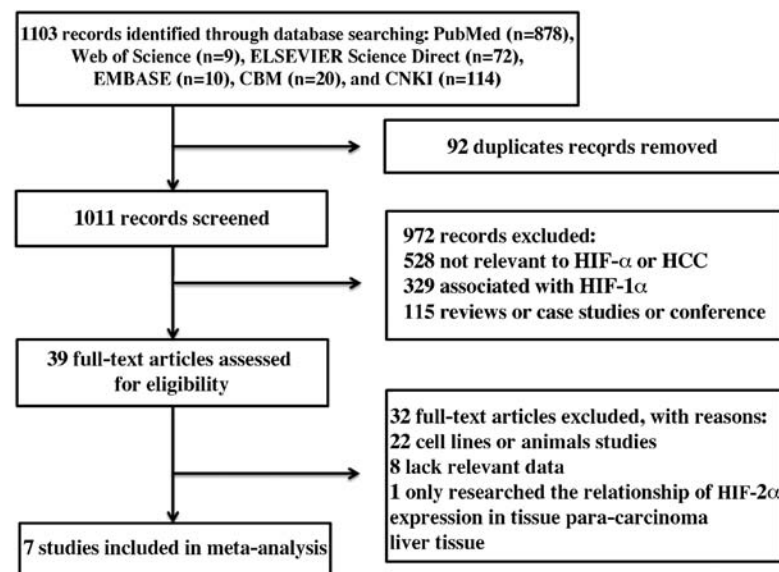
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poorer survival in the group with overexpression of HIF-2 $\alpha$  in HCC. If the 95% CI >1 or <1 completely, it suggests that HIF-2 $\alpha$  expression was statistically significantly different between the negative group on OS or clinicopathological features.

Heterogeneity test was assessed by Chi-square based on Q statistical and I<sup>2</sup> statistical test. If the HR of the studies had fine homogeneity, the fixed effect model (Mantel-Haenszel method) was used to pool the data; otherwise, we used the DerSimonian-Laird method as the random-effect model.<sup>8</sup> We assessed the possibility of publication bias by performing Begg's test. Publication bias was indicated when the *p*-value of Begg's test was <0.05. All of the calculations were performed by using Stata version 11.0 (Stata Corporation, College Station, TX, USA).

**Results.** Study identification and quality. We identified 1103 potentially relevant studies, and 1095 of them were excluded from analysis (Figure 1) after careful extraction. As a result, 7 studies<sup>9-15</sup> were finally included in the meta-analysis, and coincidentally they all derived from China. From these 7 studies, 1066 HCC patients were retrospectively analyzed, and all the tissue samples were examined using IHC.

The study characteristics and the results of quality assessment are shown in Table 1. By assaying the methods and confounders in these studies, we found that the quality scores ranged from 5 to 7 with a median value of 6.1. Five of them described the items prognostic in OS, only 2 studies contained disease free survival rates, we only analyzed the OS by meta-analysis. We then analyzed the correlation of HIF-2 $\alpha$  expression with tumor characteristics, included tumor size,<sup>10-14</sup> liver



**Figure 1** - Study flow chart of data selection of 1103 records identified through database selection. HCC - hepatocellular carcinoma. CBM - Chinese Biological Medicine, CNKI - China National Knowledge Infrastructure

**Table 1** - Characteristics of studies included in the meta-analysis extracted from different studies and quality rating of each study using the Newcastle-Ottawa quality assessment scale.<sup>6</sup>

Study	Year	Population	Sample size	Technique	Survival analysis	HR estimate	HR	95% CIs	Study quality
Bangoura et al <sup>11</sup>	2004	China	97	IHC	No data	No data	No data	No data	5
Bangoura et al <sup>13</sup>	2007	China	315	IHC	OS	Reported	3.699	1.385-10.236	6
Wang et al <sup>10</sup>	2009	China	67	IHC	No data	No data	No data	No data	6
Wang et al <sup>14</sup>	2011	China	104	IHC	OS	Event	7.965	3.839-16.526	7
Zhang et al <sup>15</sup>	2012	China	103	IHC	OS	Event	0.87	0.45-1.69	7
Sun et al <sup>9</sup>	2013	China	254	IHC	OS+DFS	Reported	0.65	0.45-0.943	6
Yang et al <sup>12</sup>	2014	China	126	IHC	OS+DFS	Reported	2.69	1.33-5.44	6

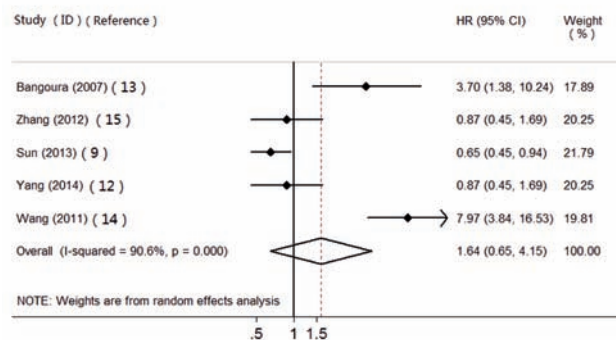
IHC - immunohistochemical staining, OS - overall survival, DFS - disease-free survival, HR - hazard ratio, CI -confidence interval

cirrhosis,<sup>11-14</sup> capsule infiltration,<sup>10-13</sup> vein invasion,<sup>11-13</sup> histological grade,<sup>10-14</sup> and necrosis.<sup>10,11,13</sup>

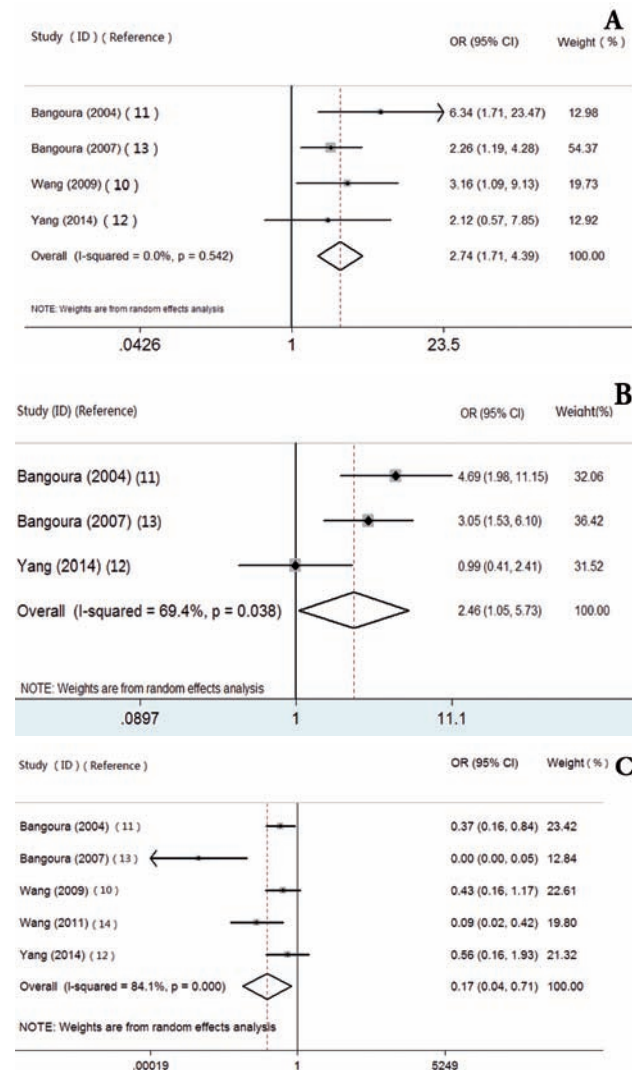
**Meta-analysis of HIF-2 $\alpha$  for HCC. 1. The prognosis value of HIF-2 $\alpha$  expression in HCC.** By assuming the heterogeneity between primary studies, we meta-analyzed the data in a random-effects model. Five studies were included in this meta-analysis to assess the association of HIF-2 $\alpha$  expression with OS (Figure 2). The pooled HR was 1.640, but 95% CI was 0.648 to 4.151. Then we randomly removed one of the studies and combined the HR. The results showed that the HRs ranged from 1.374 (95% CI: 0.495-3.815) to 2.129 (95% CI: 0.693-6.544), which suggests that the difference between expression of HIF-2 $\alpha$  and OS in patients with HCC was not statistically significant. There was no evidence of publication bias on OS using Begg's test ( $p=0.881$ ).

**2. Correlation of HIF-2 $\alpha$  expression with clinicopathological features.** We also assessed the correlation between expression of HIF-2 $\alpha$  and clinicopathological features in HCC. Overexpression of HIF-2 $\alpha$  was significantly associated with capsule infiltration (OR=2.738, 95% CI: 1.709-4.386) (Figure 3A), vein invasion (OR=2.458, 95% CI: 1.053-5.734) (Figure 3B), and histological grade (OR=0.172, 95% CI: 0.042-0.713) in HCC (Figure 3C). We also

found that there was no statistical significance between HIF-2 $\alpha$  expression and other tumor features, including tumor size, necrosis, and liver cirrhosis in HCC. All of the results are summarized in Table 2.



**Figure 2** - The association of hypoxic inducible factor-2alpha expression with overall survival in hepatocellular carcinoma. HR - hazard ratio



**Figure 3** - The correlation between expression of hypoxic inducible factor-2alpha and capsule infiltration in HCC (A), vein invasion (B), histological grade (C) in HCC. HCC - Hepatocellular carcinoma

**Table 2** - Correlation of hypoxic inducible factor-2alpha expression with clinicopathological features.

Clinicopathological features	Study number	Sample size	OR	Pooled data 95% CIs	P-value	Heterogeneity I <sup>2</sup> (%)	P-value	Model used
Tumor size <sup>10-14</sup>	5	709	2.173	0.553-8.533	0.226	91.1	0.000	REM
Capsule infiltration <sup>10-13</sup>	4	605	2.738	1.709-4.386	0.000	61.0	0.542	FEM
Vein invasion <sup>11-13</sup>	3	538	2.458	1.053-5.734	0.038	69.4	0.038	REM
Liver cirrhosis <sup>11-14</sup>	4	642	1.179	0.525-2.647	0.690	76.1	0.006	REM
Histological grade <sup>10-14</sup>	5	709	0.172	0.042-0.713	0.015	33.2	0.000	FEM
Necrosis <sup>10,11,13</sup>	3	479	2.362	0.472-11.815	0.295	89.9	0.000	REM

OR - odd ratio, FEM - fixed effects model, REM - random effects model, CI - confidence interval, I<sup>2</sup> - I-square test



**Discussion.** Due to rapid cell proliferation and the formation of aberrant blood vessels, hypoxia has been proven to be present in solid tumors,<sup>16</sup> similarly in HCC, even though it is one of the most hyper-vascularized tumors. Intrahepatic hypoxia has been shown to be correlated with tumor progression, invasiveness, resistance to chemotherapy, and metastasis.<sup>17</sup> In addition, HIFs play a central role in hypoxia response in HCC. Recently, numerous immunochemical analyses have demonstrated that overexpression of HIFs was detected in a number of primary and metastatic human cancers. Some of them mentioned that overexpression of HIF-2 $\alpha$  was associated with tumor progression, metastasis, and poor outcome in HCC.

Our meta-analysis aimed to examine the association between HIF-2 $\alpha$  expression and the prognosis and clinicopathologic features of HCC. We combined the outcome of 902 patients with HCC from the 5 studies, and result showed that the 95% CI overlapped 1, which means no statistically significant, even removed one of the studies. They all suggested that the overexpression of HIF-2 $\alpha$  was not relevant to poor outcome in HCC by statistical analysis, but was correlated with capsule infiltration, vein invasion, and histological grade. This indicates that HIF-2 $\alpha$  may play an important role in tumor angiogenesis, metastasis, and recurrence in HCC, but not in prognosis.

It was reported<sup>17</sup> that HIF-2, which was the heterodimerization of HIF-2 $\alpha$  and HIF $\beta$  (ARNT) can activate TGF- $\alpha$  and cyclinD1 in promoting cell proliferation in numbers of tumors, and can promote metastasis through regulating the factors involved in controlling metastasis such as CXCR4, TWIST, and E-cadherin. As we know, CXCR4 and its ligand SDF1 are highly expressed under hypoxia in a number of cell types.<sup>18</sup> It was reported that<sup>19</sup> HIF-1 can promote the regulation of TWIST in tumor metastasis and E-cadherin, which were involved in EMT (epithelial-mesenchymal transition), while HIF-2 $\alpha$  seemed to repress this expression.<sup>20</sup> Moreover, HIF-2 can activate tumor suppressor genes such as SCGB3A1 to repress the growth of a tumor.<sup>21</sup>

Conditional inactivation of HIF-2 $\alpha$  suppressed the development of VHL-associated liver hemangiomas, suggesting that liver angiogenesis was predominantly regulated by HIF-2 $\alpha$ .<sup>22</sup> The expression of HIF-2 $\alpha$  was positively correlated with VEGF in HCC, and it can directly activate the expression of genes encoding the pro-angiogenic factor, erythropoietin and angiopoietin. All of them provide evidence for the role of HIF-2 $\alpha$  in tumor angiogenesis in HCC.

The conduction of higher quality, larger sample size, and more controlled trials is required to verify the hypothesis. Limitations of our meta-analysis include small sample size and regional limitations (all the patients of HCC were derived from China). We used a random effects models to analyze the data of OS, and the heterogeneity was detected ( $I^2=90.6\%$   $p=0.000$ ). The limitation of restricted exclusion may explain the heterogeneity, and different methods of extracting HRs may also lead to bias in results. Direct analysis of variance may be more reliable than calculating from data or extracting from survival curves.

In summary, HIF-2 $\alpha$  was correlated with the progression of HCC but not with poor prognosis. However, the conclusion was restricted by the limitations of the included studies. More studies evaluating the significance of HIF-2 $\alpha$  expression in HCC are necessary in the future.

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