Systematic Review and Meta-Analysis

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# The association of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α protein expression with clinicopathological characteristics in papillary thyroid carcinoma

## A meta-analysis

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**Abstract**

**Objective:** To investigate the correlation of hypoxia-inducible factor-1α (HIF-1α) and hypoxia-inducible factor-2α (HIF-2α) protein expression with clinicopathologic characteristics in patients with papillary thyroid carcinoma (PTC) through a meta-analysis.

**Methods:** PubMed, Embase, Web of Science, Cochrane, CNKI, Wanfang, and VIP databases were searched from the establishment of the database to February 2023. The New castle-Ottawa Scale was used to evaluate the quality of the literature. Rev Man 5.3 and Stata14.0 were used to conduct a meta-analysis of the included studies.

**Results:** Twenty-eight articles with 2346 samples were included in the Meta-analysis. Compared with normal thyroid tissues, HIF-1α and HIF-2α proteins were highly expressed in PTC tumor tissues. High expression of HIF-1α protein was associated with tumor size (odds ratio [OR] = 4.50, 95% confidence interval [CI]: 2.88–7.04, $P < .00001$), lymph node metastasis (OR = 4.76, 95% CI: 3.78–5.99, $P < .00001$), TNM stage (OR = 3.67, 95% CI: 2.68–5.03, $P < .00001$), capsular invasion (OR = 2.30, 95% CI: 1.43–3.71, $P = .0006 < .05$), and extrathyroidal extension (OR = 10.96, 95% CI: 4.80–25.02, $P < .00001$). High expression of HIF-2α protein was associated with lymph node metastasis (OR = 4.18, 95% CI: 2.63–6.65, $P < .00001$), TNM stage (OR = 2.56, 95% CI: 1.36–4.82, $P = .004 < .05$), and capsular invasion (OR = 3.84, 95% CI: 1.66–8.88, $P = .002 < .05$). In addition, we concluded for the first time that there was a statistically significant difference in the expression of HIF-1α and HIF-2α in PTC patients (OR = 2.36, 95% CI: 1.26–4.42, $P = .007 < .05$).

**Conclusions:** The high expression of HIF-1α and HIF-2α proteins is closely related to some clinicopathological parameters of PTC, and can provide potential biological indicators for the diagnosis and prognosis of PTC.

**Abbreviations:** 95% CI = 95% confidence interval, EMT = epithelial-to-mesenchymal transition, HIF = hypoxia-inducible factor-1, NOS = New castle-Ottawa Scale, OR = odds ratio, PTC = papillary thyroid carcinoma, TAD = transactivation domains.

**Keywords:** HIF-1α, HIF-2α, meta-analysis, papillary thyroid carcinoma

## 1. Introduction

Thyroid cancer, especially differentiated thyroid cancer, is one of the common malignant tumors in clinic, and its development trend has gradually increased in recent years.[1] Papillary thyroid carcinoma (PTC), as the most common histological type of differentiated thyroid carcinoma, accounts for about 85% to 90% of thyroid cancer, and has the characteristics of high differentiation, low malignancy, slow growth rate and good prognosis.[2,3] At present, there are abundant molecular mechanisms related to the occurrence and development of PTC, such as BRAF-V600, miRNA, IncRAN, MDM2 and other tumor factors, but their specific mechanisms are still unclear.[4]

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Surgical resection is still the main treatment method for PTC, and the clinical effect for patients with distant metastasis is still not significant, and it is not sensitive to radiotherapy and chemotherapy.[5]

Hypoxia-inducible factor (HIF) is a transcription factor that activates the adaptive hypoxic response of the body under hypoxic conditions. It consists of an oxygen-sensitive α subunit and a ubiquitously expressed β subunit,[6] which increases oxygen delivery by inducing angiogenesis and promoting glycolysis-related metabolic pathways. It mainly includes HIF-1 and HIF-2.[7] HIF-1α was the first HIF-α subunit to be identified, which contains a central oxygen-dependent degradation domain that mediates oxygen regulatory stability.[8] In addition, HIF-1α also contains 2 transactivation domains (TAD), TAD-C and TAD-N, in which TAD-C binds to p300/CBP to improve the stability and transcriptional activity of HIF-1α protein, while TAD-N overlaps with oxygen-dependent degradation domain and has the role of continuous protein stabilization.[9] Two other isoforms, HIF-2α and HIF-3α, were subsequently reported.[10] HIF-2α is the functional and active subunit of HIF-2, including 15 exons and 14 introns. The gene length is about 120 kb and the relative molecular weight is $96.5 \times 10^3$.[11] On the 1 hand, HIF-1α and HIF-2α are homologous amino acid sequences to each other, which activate hypoxia-induced gene transcription. On the other hand, each plays its own role. HIF-1α is mainly involved in the adaptive response in the early and acute phase of hypoxia, while HIF-2α is mainly involved in the long-term and chronic hypoxia of tumors.[12] Studies have shown that HIF-1α and HIF-2α are highly expressed in a variety of human cancers, such as oral cancer,[13] gastric cancer,[14] pancreatic cancer,[15] non-small cell lung cancer,[16] breast cancer,[17] cervical cancer,[18] prostate cancer,[19] and glioblastoma,[20] etc.

Thus, HIF-1α and HIF-2α play an important role in the differentiation of benign and malignant tumors and the pathological prognosis of malignant tumors.[21] At present, as a hot issue, it is still controversial whether HIF-α protein expression is correlated with some clinicopathological characteristics of PTC patients. Therefore, this study is the first to conduct a meta-analysis of the correlation of HIF-1α and HIF-2α protein expression with the clinicopathological characteristics of patients with PTC, so as to provide a certain basis for clinical and scientific research.

## 2. Materials and methods

### 2.1. Literature search

PubMed, Embase, Web of Science, Cochrane, CNKI, Wanfang and VIP database were searched by computer from the establishment of the database to February 2023. Using the combination of subject words and free words, the search language is Chinese or English. For example, "Thyroid Neoplasms," "Thyroid Carcinoma," "Thyroid Cancers," "Cancer of the Thyroid," "Thyroid Adenoma," "Papillary thyroid carcinoma," "Thyroid Cancer, Papillary," "Thyroid Carcinomas, Papillary," "hypoxia-inducible factor-α," "Hif-α," "hif-α," "hypoxia-inducible factor-1α," "Hif-1α," "hif-1α," "HIF-1," "hypoxia-inducible" factor-2α ", "HIF-2α," "hif-2α," "HIF-2," etc.

### 2.2. Literature inclusion and exclusion criteria

*Inclusion criteria*: All studies were original; all patients were confirmed to be PTC by postoperative pathological results; sample size and other specific values were provided in all literatures. To investigate the relationship of HIF-1α and HIF-2α expression with the clinicopathological characteristics of PTC patients.

*Exclusion criteria*: Repeated publications; repeated studies of the same patients; reviews, meta-analyses or case reports; the quality of the literature was poor, and the data could not be obtained or collected inappropriately.

### 2.3. Literature screening and data extraction

Two researchers extracted the information of all studies that met the inclusion criteria. The extracted content mainly included: the first author, publication year, country, HIF-α type and detection method, etc. The basic clinicopathological characteristics included like the total number of samples, the expression of HIF-α in PTC patients and normal thyroid patients, tumor size, lymph node metastasis, TNM stage, capsule invasion, and extrathyroidal extension. In addition, the New castle-Ottawa Scale (NOS) standard was used to evaluate the quality of the included literatures,[22] and the NOS score of all literatures was ≥7, indicating that all literatures were of high quality.

### 2.4. Statistical analysis

Rev Man 5.3 and Stata14.0 were used for meta-analysis of the included studies. With $\alpha = 0.05$ as the test level, the pooled odds ratio (OR) and 95% confidence interval (95% CI) were calculated by forest plot as the effect index to describe the correlation between HIF-1α, HIF-2α protein expression and clinicopathological characteristics of patients with PTC. Heterogeneity was tested by $\chi^2$ and $I^2$. When $P > .1$ and $I^2 < 50\%$, the fixed effect model was used for analysis. Otherwise, sensitivity analysis or subgroup analysis was used to determine the source of heterogeneity. Publication bias was analyzed by Begg's and Egger's tests, $P < .05$ indicated publication bias.

## 3. Results

### 3.1. Literature search and screening

A total of 978 literatures were retrieved from the database and 336 literatures were repeated. According to the inclusion and exclusion criteria, the literatures that did not meet the research objectives were excluded. Finally, a total of 28 studies were included in our meta-analysis[23–50] (Fig. 1).

### 3.2. Basic characteristics of the included studies

The literatures published in this study was from 2010 to 2023, and a total of 2346 patients were pathologically diagnosed with PTC. According to the NOS scoring scale, the NOS scores were all ≥7, indicating that the quality of the included studies was reliable.

The basic clinicopathological features of the included literatures are detailed in Table 1. There were 25 literatures that studied the correlation between HIF-1α protein expression and clinicopathological features,[23–47] of which 3 literatures[45–47] involved both HIF-1α and HIF-2α protein expression. A total of 6 articles[45–50] were related to HIF-2α protein expression. There was no statistically significant correlation of HIF-α protein expression with sex, age and Hashimoto's thyroiditis. However, HIF-α protein expression may be correlated with tumor size, lymph node metastasis, tumor stage, capsule invasion, and extrathyroidal extension in PTC patients.

### 3.3. HIF-1α and HIF-2α were differentially expressed in PTC and normal thyroid tissues

***3.3.1. The expression of HIF-1α was different between PTC and normal thyroid tissues.*** Seventeen studies compared the expression of HIF-1α protein in PTC and normal thyroid tissues, and there was slight heterogeneity among the studies ($P = .008 < .1$, $I^2 = 51\% > 50\%$) (Fig. 2A). The results of sensitivity analysis showed that Wang 2013[38] and Xiao et

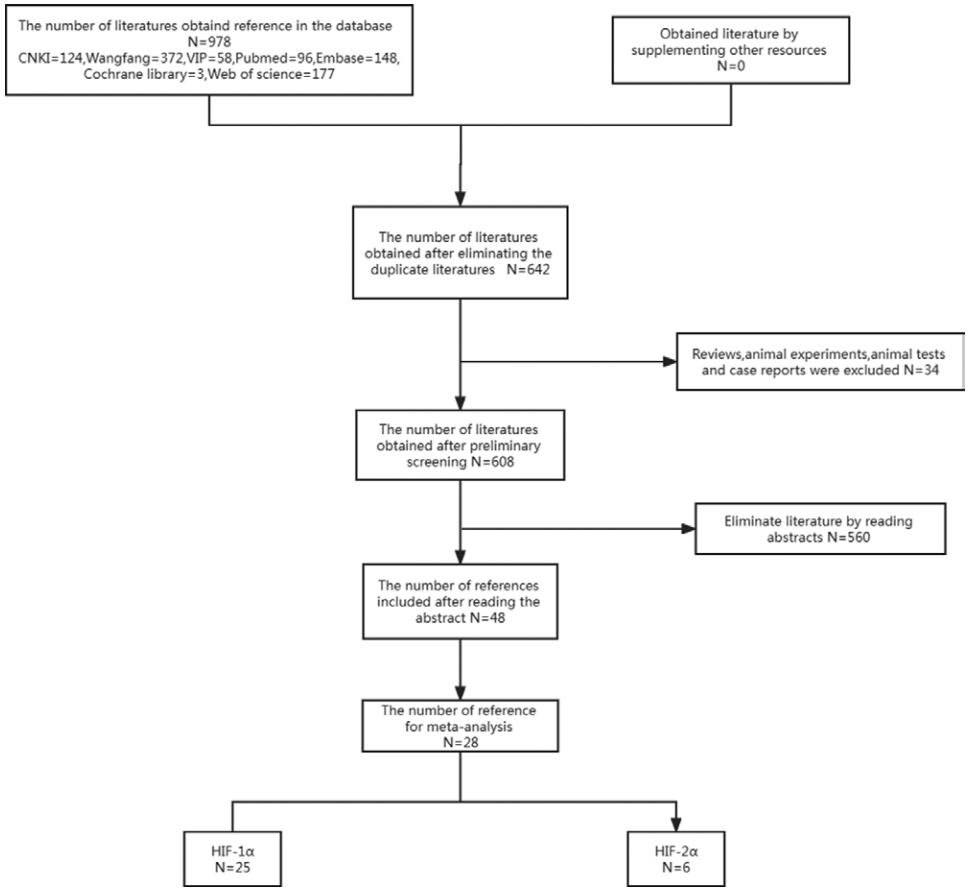


**Figure 1.** The flow diagram of this meta-analysis.

al[26] were the sources of heterogeneity. After excluding these 2 studies, we found that the high expression of HIF-1α protein was significantly correlated with PTC compared with normal thyroid tissues. The difference was statistically significant (OR = 15.82, 95% CI: 11.94–20.96, $P < .00001$) (Fig. 2B).

***3.3.2. The expression of HIF-2α was different between PTC and normal thyroid tissues.*** Five studies compared the expression of HIF-2α protein in PTC and normal thyroid tissues, and there was moderate heterogeneity among the studies ($P = .0004 < .1$, $I^2 = 80\% > 50\%$) (Fig. 2C). Sensitivity analysis showed that Liu et al[45] was the source of heterogeneity. After excluding this study, we found that the high expression of HIF-2α protein was significantly correlated with PTC compared with normal thyroid tissues. The difference was statistically significant (OR = 44.66, 95% CI: 21.36–93.37, $P < .00001$) (Fig. 2D).

### *3.4. The relationship between HIF-1α expression and clinicopathological features*

***3.4.1. Tumor size.*** A total of 5 studies reported the relationship between HIF-1α protein expression and tumor size, and there was slight heterogeneity among the studies ($P = .09 < .1$, $I^2 = 51\% > 50\%$) (Fig. 3A). Chen et al[42] was the main source of heterogeneity according to sensitivity analysis. After excluding this study, we found a statistically significant correlation between high HIF-1α protein expression and tumor size. (OR = 4.50, 95% CI: 2.88–7.04, $P < .00001$) (Fig. 3B).

***3.4.2. Lymph node metastasis.*** There were 21 studies on the relationship between HIF-1α protein expression and lymph node metastasis, and there was no heterogeneity among the studies ($P = .90 > .1$, $I^2 = 0\%$). The results of meta-analysis by fixed effect model showed that the high expression group of HIF-1α was significantly associated with tumor's lymph node metastasis compared with the low expression group. The difference has statistically significant (OR = 4.76, 95% CI: 3.78–5.99, $P < .00001$) (Fig. 3C).

***3.4.3. TNM stage.*** A total of 12 articles reported the relationship between HIF-1α protein expression and TNM stage, and there was slight heterogeneity among the studies ($P = .002 < .1$, $I^2 = 63\% > 50\%$) (Fig. 3D); According to the sensitivity analysis, Qu et al[35] and Wu et al[43] were the main sources of heterogeneity. After excluding these 2 studies, we found a statistical correlation between high HIF-1α protein expression and advanced PTC. (OR = 3.67, 95% CI: 2.68–5.03, $P < .00001$) (Fig. 3E).

***3.4.4. Capsular invasion.*** Six studies discussed the relationship between HIF-1α protein expression and capsular invasion, and there was no heterogeneity among the studies ($P = .54 > .1$, $I^2 = 0\%$). Through the meta-analysis of fixed effect model, we found that when HIF-1α protein was highly expressed, the tumor was more likely to have capsular invasion, and the difference was statistically significant (OR = 2.30, 95% CI: 1.43–3.71, $P = .0006 < .05$) (Fig. 3F).

***3.4.5. Extrathyroidal invasion.*** We included 2 studies on the relationship between the expression of HIF-1α protein and extrathyroidal extension of the tumor. There was no heterogeneity between the studies ($P = .49 > .1$, $I^2 = 0\%$). Meta-analysis using a fixed effect model showed that high expression of HIF-1α protein was statistically associated with extrathyroidal invasion (OR = 10.96, 95% CI: 4.80–25.02, $P < .00001$) (Fig. 3G).

**Table 1**

**Summary showing the comparison of the *P* values of association between HIF-α protein expression and clinicopathological characteristics in papillary thyroid carcinoma.**

| Author | Year | Country | Type | Sample size | Method | Specimen | QS | PTC versus normal tissue | Gender | Age | HT | TS | LNM | TNM | CI | EI | HIF-2α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zhang | 2023 | China | HIF-1α | 59 | IHC | Tissue | 8 | <0.001 | 0.138 | 0.859 | – | – | 0.293 | – | 0.021 | – | – |
| Zhang | 2022 | China | HIF-1α | 70 | IHC | Tissue | 7 | 0.000 | 0.650 | 0.557 | – | – | 0.768 | – | 0.027 | – | – |
| Ma | 2022 | China | HIF-1α | 50 | IHC | Tissue | 8 | 0.000 | 0.562 | 0.403 | – | – | 0.000 | 0.000 | – | – | – |
| Xiao | 2021 | China | HIF-1α | 84 | IHC | Tissue | 7 | <0.010 | 0.391 | 0.103 | – | – | 0.009 | – | – | – | – |
| Fang | 2020 | China | HIF-1α | 60 | IHC | Tissue | 7 | <0.050 | 0.850 | 0.582 | – | 0.214 | 0.004 | 0.003 | – | – | – |
| Gong | 2019 | China | HIF-1α | 120 | IHC | Tissue | 8 | – | 0.278 | 0.387 | – | 0.044 | 0.010 | 0.016 | – | – | – |
| Yang | 2018 | China | HIF-1α | 125 | IHC+WB | Tissue | 8 | 0.000 | 0.857 | 0.381 | – | – | 0.000 | 0.003 | – | 0.000 | – |
| Yu | 2018 | China | HIF-1α | 60 | IHC | Tissue | 7 | <0.050 | 0.112 | 0.426 | – | – | 0.000 | – | – | – | – |
| Tian | 2017 | China | HIF-1α | 88 | IHC | Tissue | 7 | 0.000 | 0.150 | 0.632 | – | 0.620 | | 0.027 | – | – | – |
| Dong | 2017 | China | HIF-1α | 100 | IHC | Tissue | 7 | – | >0.050 | >0.050 | – | – | <0.050 | <0.050 | – | – | – |
| Pan | 2016 | China | HIF-1α | 77 | IHC | Tissue | 7 | 0.000 | – | 0.505 | – | 0.248 | 0.027 | – | – | – | – |
| Cai | 2015 | China | HIF-1α | 120 | IHC | Tissue | 8 | – | 0.570 | 0.528 | – | 0.880 | <0.001 | 0.008 | – | – | – |
| Qu | 2014 | China | HIF-1α | 140 | IHC | Tissue | 7 | <0.050 | 0.111 | 0.177 | – | – | 0.019 | 0.031 | – | – | – |
| Yang | 2014 | China | HIF-1α | 72 | IHC | Tissue | 7 | <0.001 | 0.612 | 0.598 | – | – | 0.029 | – | – | – | – |
| Oskar | 2013 | Austria | HIF-1α | 160 | IHC | Tissue | 7 | 0.018 | – | – | – | <0.001 | <0.001 | – | – | <0.001 | – |
| Wang | 2013 | China | HIF-1α | 129 | IHC | Tissue | 7 | <0.001 | 0.504 | 0.175 | – | – | <0.001 | 0.003 | – | – | – |
| Ilie | 2013 | France | HIF-1α | 114 | IHC | Tissue | 8 | – | 0.317 | – | 0.183 | 0.006 | 0.285 | – | 0.774 | – | – |
| Dong | 2013 | China | HIF-1α | 79 | IHC | Tissue | 7 | – | – | – | – | <0.010 | <0.010 | – | – | – | – |
| Li | 2013 | China | HIF-1α | 58 | IHC | Tissue | 7 | 0.000 | 0.489 | 0.419 | – | – | 0.015 | 0.311 | – | – | – |
| Chen | 2011 | China | HIF-1α | 50 | IHC | Tissue | 8 | – | 0.814 | 0.706 | – | 0.000 | 0.049 | 0.000 | – | – | – |
| Wu | 2011 | China | HIF-1α | 62 | IHC | Tissue | 7 | <0.050 | 0.080 | 0.130 | – | – | 0.000 | 0.046 | – | – | – |
| Choi | 2010 | Korea | HIF-1α | 38 | IHC | Tissue | 7 | – | – | – | – | – | 0.006 | – | 1.000 | – | – |
| Liu | 2016 | China | HIF-1α/HIF-2α | 92 | RT-PCR+IHC | Tissue | 9 | <0.010/<0.010 | 0.223/0.725 | 0.822/0.675 | 0.463/0.256 | –/– | 0.006/0.037 | 0.021/0.009 | 0.012/0.004 | –/– | 0.034/– |
| Liu | 2015 | China | HIF-1α/HIF-2α | 30 | RT-PCR+IHC+WB | Tissue | 9 | <0.010/<0.010 | 1.000/1.000 | 1.000/1.000 | 1.000/0.600 | 0.240/1.000 | 0.020/0.390 | –/– | 0.700/0.220 | –/– | >0.050/– |
| Wang | 2014 | China | HIF-1α/HIF-2α | 70 | IHC | Tissue | 8 | <0.001/<0.001 | 0.811/0.550 | 0.853/0.810 | –/– | –/– | 0.034/0.022 | –/– | –/– | –/– | 0.031/– |
| Wei | 2022 | China | HIF-2α | 60 | IHC | Tissue | 8 | <0.050 | 0.116 | 0.079 | – | 0.795 | | 0.851 | – | – | – |
| Sun | 2016 | China | HIF-2α | 50 | RT-PCR+IHC+WB | Tissue | 7 | – | – | – | – | <0.010 | <0.050 | <0.050 | – | – | – |
| Ni | 2013 | China | HIF-2α | 129 | RT-PCR+IHC | Tissue | 7 | <0.001 | 0.960 | 0.935 | – | – | <0.001 | 0.935 | – | – | – |

CI = capsule invasion, EI = extrathyroidal invasion, HIF-1α = hypoxia-inducible factor-1α, HIF-2α = hypoxia-inducible factor-2α, HT = Hashimoto's thyroiditis, IHC = immunohistochemistry, LNM = lymph node metastasis, QS = quality score, RT-PCR = quantities reverse transcription-polymerase chain reaction, TNM = TNM stage, TS = tumor size, WB = western blotting.

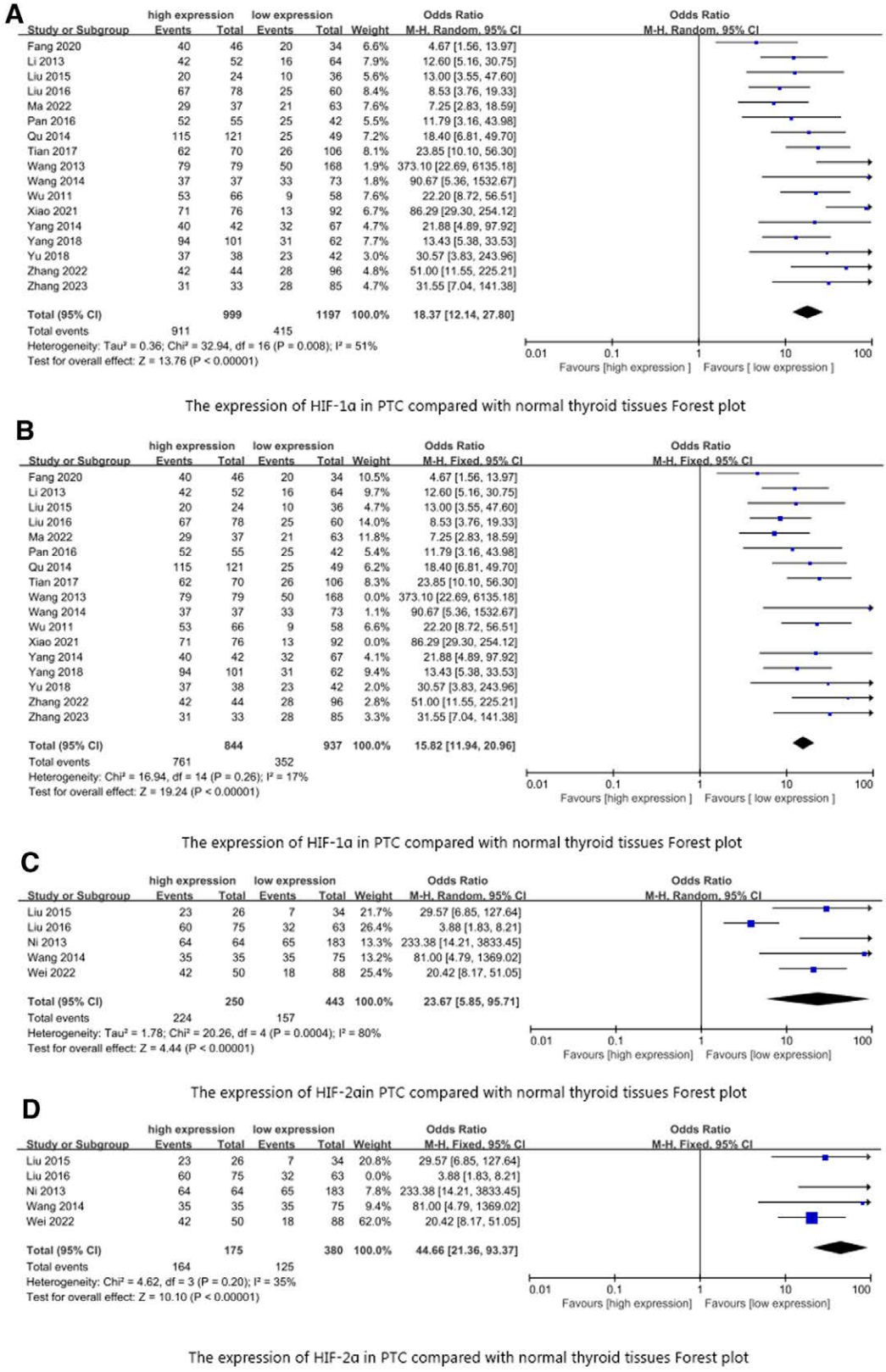


**Figure 2.** (A–D) Forest plot for evaluating the association of HIF-1α and HIF-2α expression in PTC's tissues compare with normal thyroid. HIF-1α = hypoxia-inducible factor-1α, HIF-2α = hypoxia-inducible factor-2α, PTC = papillary thyroid carcinoma.

### *3.5. The relationship between HIF-2α expression and clinicopathological features*

***3.5.1. Tumor size.*** There were 3 studies on the relationship between HIF-2α protein expression and tumor size, and there was slight heterogeneity among the studies ($P = .08 < .1$, $I^2 = 60\% > 50\%$) (Fig. 4A). Through sensitivity analysis, the results suggested that Sun et al[49] was the main source of heterogeneity. After excluding this study, the Meta-analysis

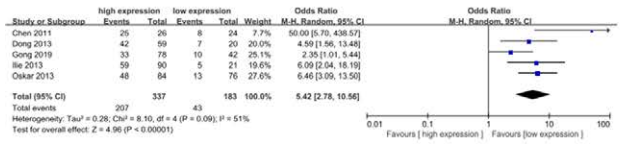


Fig.3A. The association of HIF-1α expression with tumor size forest polt

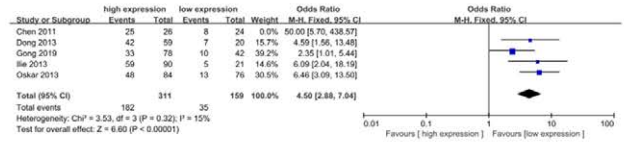


Fig.3B. The association of HIF-1α expression with tumor size forest polt

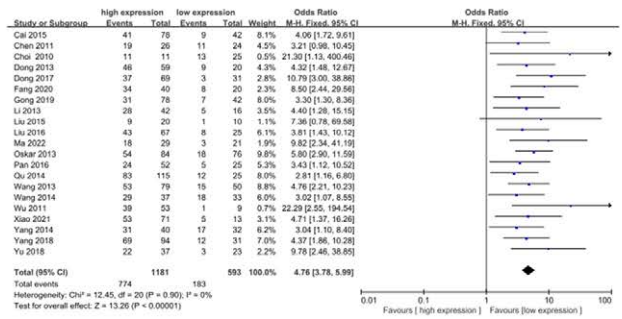


Fig.3C. The association of HIF-1α expression with lymph node metastasis Forest plot

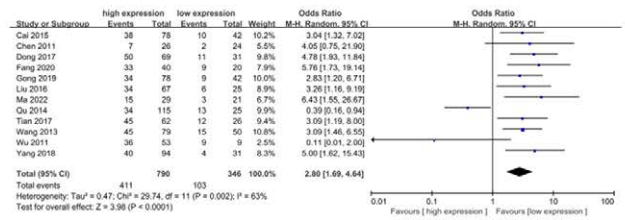


Fig.3D. The association of HIF-1α expression with TNM stage Forest plot

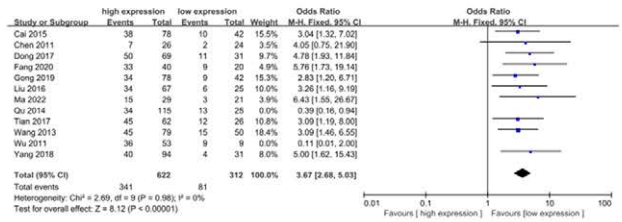


Fig.3E. The association of HIF-1α expression with TNM stage Forest plot

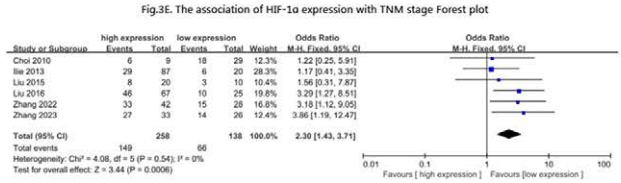


Fig.3F. The association of HIF-1α expression with capsule invasion Forest plot

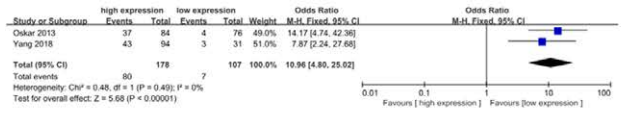


Fig.3G. The association of HIF-1α expression with extrathyroidal invasion Forest plot

**Figure 3.** (A–G) Forest plot for evaluating the relationship between HIF-1α expression and clinicopathological features. HIF-1α = hypoxia-inducible factor-1α

results did not find a significant correlation between HIF-2α expression and tumor size. The difference was not statistically significant (OR = 1.06, 95% CI: 0.44–2.55, $P = .89 > .05$) (Fig. 4B).

***3.5.2. Lymph node metastasis.*** Five studies discussed the relationship between HIF-2α protein expression and lymph node metastasis, and there was no heterogeneity among the studies ($P = .23 > .1$, $I^2 = 29\% < 50\%$). We found that high expression of HIF-2α protein was statistically associated with lymph node metastasis (OR = 4.18, 95% CI: 2.63–6.65, $P < .00001$) (Fig. 4C).

***3.5.3. TNM stage.*** Four studies reported the relationship between HIF-2α protein expression and TNM stage, and there was slight heterogeneity among the studies ($P = .08 < .1$, $I^2 = 56\% > 50\%$) (Fig. 4D). Ni et al[50] was the main source of heterogeneity according to sensitivity analysis. After excluding this study, we found a statistical correlation between high HIF-2α protein expression and advanced PTC (OR = 2.56, 95% CI: 1.36–4.82, $P = .004 < .05$) (Fig. 4E).

***3.5.4. Capsular invasion.*** Two studies discussed the relationship between HIF-2α protein expression and capsular invasion, and there was no heterogeneity among the studies ($P = .86 > .1$, $I^2 = 0\%$). We found that PTC with high HIF-2α expression was more likely to have capsular invasion, and the difference was statistically significant (OR = 3.84, 95% CI: 1.66–8.88, $P = .002 < .05$) (Fig. 4F).

### *3.6. Correlation between HIF-1α and HIF-2α expression*

For the relationship between HIF-1α and HIF-2α protein expression, we included 3 studies, and there was no heterogeneity among the studies ($P = .42 > .1$, $I^2 = 0\%$). Meta-analysis by fixed effect model showed that HIF-1α protein expression was positively correlated with HIF-2α expression in PTC's patients (OR = 2.36, 95% CI: 1.26–4.42, $P = .007 < .05$) (Fig. 4G).

### *3.7. Test for bias*

Begg's test and Egger's test were used to quantify the publication bias of each measurement index (Table 2). The results showed that there was a certain publication shift in the correlation of HIF-1α protein expression with lymph node metastasis and TNM stage (lymph node metastasis: Begg's test $P = .010 < .05$, Egger's test $P = .012 < .05$; TNM stage: Begg's test $P = .020 < .05$, Egger's test $P = .040 < .05$). Figure 5A shows that the newly added 4 points with squares represent the effect sizes that needs to be included in the literature related to lymph node metastasis in the future. As can be seen from Figure 5B, the 2 new points with squares represent the effect sizes of literature related to TNM stage that needs to be included in the future to ensure the symmetry of the funnel plot and eliminate publication bias. In addition, there was no significant publication bias in the other results (Begg's test $P > .05$, Egger's test $P > .05$) (see funnel Fig. 6A–L for details).

## 4. Discussion and conclusions

Hypoxia is a common and typical feature of many solid tumors. With the rapid growth of tumor tissue, angiogenesis is not uniform, and cellular oxygen metabolism is seriously affected. Tumor cells in hypoxic environment can induce the production of HIFs and activate related target genes, then control angiogenesis and change cellular metabolic pathways to adapt to hypoxia, and promote the proliferation, metastasis and invasion of tumor cells.[51] As the protagonist of this process, HIF is composed of both the alpha subunit that regulates oxygen and the beta subunit that makes up the structure. Under aerobic conditions, the 2 proline residues of HIF are hydroxylated by oxygen-dependent prolyl hydroxylase domain proteins.[52] The hydroxylated HIF-α is then combined with the von Hippel-Lindau protein

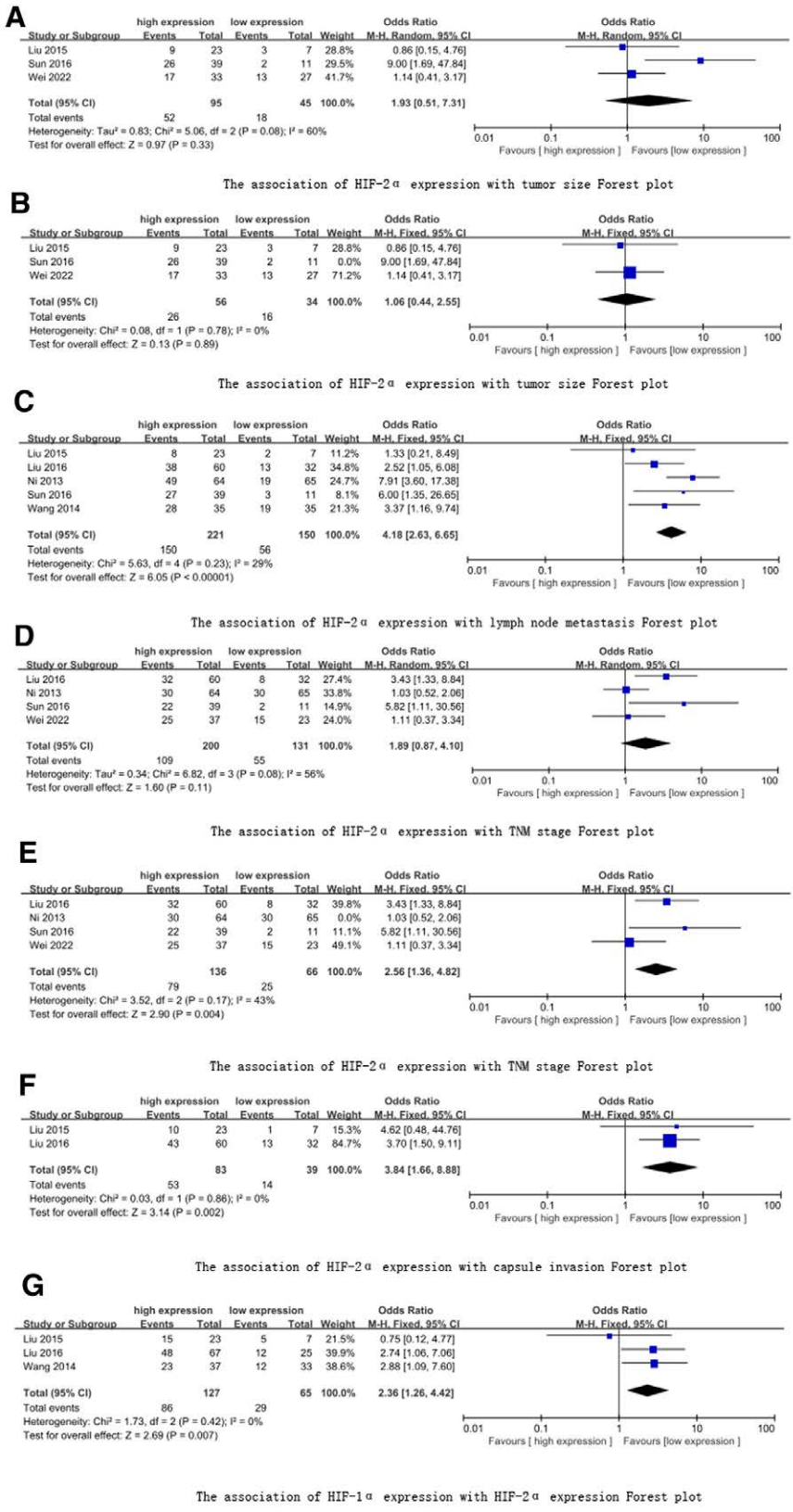


**Figure 4.** (A–G) Forest plot for evaluating the relationship between HIF-2α expression and clinicopathological features. HIF-2α = hypoxia-inducible factor-2α.

(protein) through ubiquitination, and together marks the E3 ubiquitin-ligase complex, which is then rapidly degraded by the proteasome.[53] When hypoxia occurs, oxygen-dependent prolyl hydroxylase domains are inhibited, allowing the accumulated HIF-α to form a heterodimer with HIF-β and induce the activation of HIF-related target genes such as vascular endothelial growth factor.[54] HIF-1α and 2α are the main oxygen sensors that regulate hypoxic adaptation in tumors, they bind to the

**Table 2**

**The *P* values of Begg's test and Egger's test.**

| | | PTC versus normal tissue | Tumor size | LNM | TNM | capsule invasion | Extrathyroidal invasion | HIF-2α expression |
|---|---|---|---|---|---|---|---|---|
| HIF-1α | Begg's | 0.092 | 1.000 | 0.010 | 0.020 | 0.707 | 1.000 | 1.000 |
| | Egger's | 0.070 | 0.996 | 0.012 | 0.040 | 0.363 | – | 0.060 |
| HIF-2α | Begg's | 0.308 | 1.000 | 0.806 | 1.000 | 1.000 | – | – |
| | Egger's | 0.053 | – | 0.440 | 0.785 | – | – | – |

HIF-1α = hypoxia-inducible factor-1α, HIF-2α = hypoxia-inducible factor-2α, LNM = lymph node metastasis, PTC = papillary thyroid carcinoma, TNM = TNM stage.

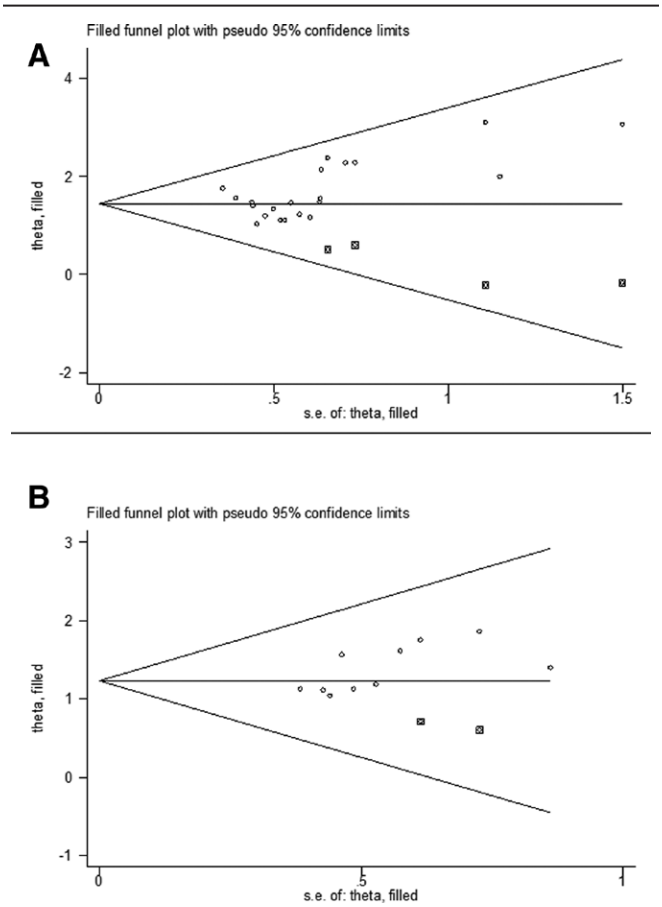


**Figure 5.** (A and B) Funnel plot after using the trim and fill method.

same hypoxia response elements and act on different transcriptional targets. Finally, increasing the migration, invasion and metastasis of tumors by promoting epithelial-to-mesenchymal transition (EMT) in tumor cells.[55] EMT is a cell biological program that naturally occurs in all developmental stages of cells and tissues in the body. Under physiological conditions, the EMT program is rarely activated, but in tumor tissues, the activated EMT program transforms epithelial cells into mesenchymal cells and causes tumor cells to break through the basement membrane, which is closely related to the occurrence, development, metastasis and spread of tumor cells.[56] Several studies have shown that HIF-1α and HIF-2α adjust LncRNA expression under hypoxic conditions, and then regulating EMT transcription factors and signaling pathways to promote EMT progression in tumors.[57,58]

Although patients with PTC have a good prognosis, with a 5-year survival rate of 95%, the incidence of PTC is increasing year by year due to various complex factors and potential pathogenesis, and the rapid progress of PTC is inseparable from the tumor microenvironment.[59] Therefore, it is important to study the growth and metastasis of PTC's tumor cells in hypoxic microenvironment. The specific mechanism of HIF-1α and HIF-2α expression in PTC is still unclear. Song et al[60] proposed a new molecular biological mechanism of the HIF-1α/TERT axis, which showed that HIF-1α acts as an activator by binding to telomerase reverse transcriptase promoter-related sequences in a hypoxic environment. Inhibiting the activity of mammalian target of rapamycin induces the activation of autophagy to promote the growth and metastasis of PTC's tumor cells. Regarding the relationship between HIF-2α and PTC, it has also been demonstrated for the first time that HIF-2α, TWIST and CXCR4 mRNA levels were significantly higher in PTC tissues than in normal thyroid. There is a significant positive correlation among the 3 mRNA. TWIST is a direct target of HIF-2α, and HIF-2α can promote the invasion and migration of tumor cells by simultaneously up-regulating TWIST and CXCR4, which has also been confirmed in subsequent related studies.[50]

In fact, as for the expression of HIF-1α and HIF-2α proteins in PTC, the specific molecular mechanism is still unclear, mainly in the macro aspects. Du et al[61] showed that HIF-1α could significantly activate the expression of angiogenic factor genes under hypoxia conditions, which was closely related to PTC lymph node metastasis. HIF-1α influences related clinicopathologic features by participating in many important tumor biologic behaviors of PTC, including stimulating vasogenesis, promoting tumor cell metastasis, and inhibiting tumor cell apoptosis. The study of Hui et al[62] showed that the positive expression rate of HIF-1α in thyroid papillary carcinoma reached 86.11%, mainly by nuclear staining. It was considered that the tumorigenesis might be related to the hypoxia of thyroid papillary carcinoma cells. HIF-2α can participate in the infiltration and metastasis of thyroid papillary carcinoma cells. The higher the level of HIF-2α, the higher the degree of malignancy of the tumor, which may be related to the metastasis caused by the rapid growth of solid tumor caused by hypoxia in the tumor.[48] Sun et al[49] concluded that the mechanism of HIF-2α on PTC is related to the up-regulation of CITED2 and PTPRZ1 protein expression, and HIF-2α may promote the occurrence, development, invasion and metastasis of PTC through the high expression of CITED2 and PTPRZ1.

In terms of the effect of HIF-1α and HIF-2α protein expression on the clinicopathological characteristics of PTC, we included a total of 28 studies.[23–50] Among them, 25 studies[23–47] were related to the expression of HIF-1α, 6 studies[45–50] were related to HIF-2α, and among these studies, 3 studies[45–47] talked about the expression of HIF-1α and HIF-2α at the same time. Consistent with most previous findings, our study found that HIF-1α and HIF-2α proteins were highly expressed in PTC's tumor tissues compared with normal thyroid. In addition, some studies also mentioned that HIF-α had a certain correlation with tumor tissues' calcification[37] and multifocality[28] in some patients with PTC. However, due to the limited number of literatures, meta-analysis could not be performed, and we hope that more relevant studies will be added in the future. For the patients with PTC, BRAFV600E mutation is highly likely

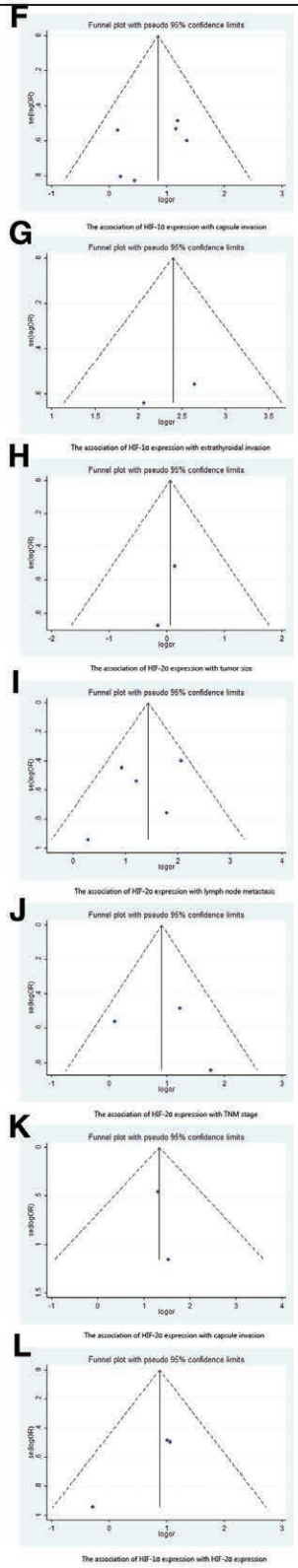


Figure 6. (A–L) Funnel plot for evaluating the test of bias.

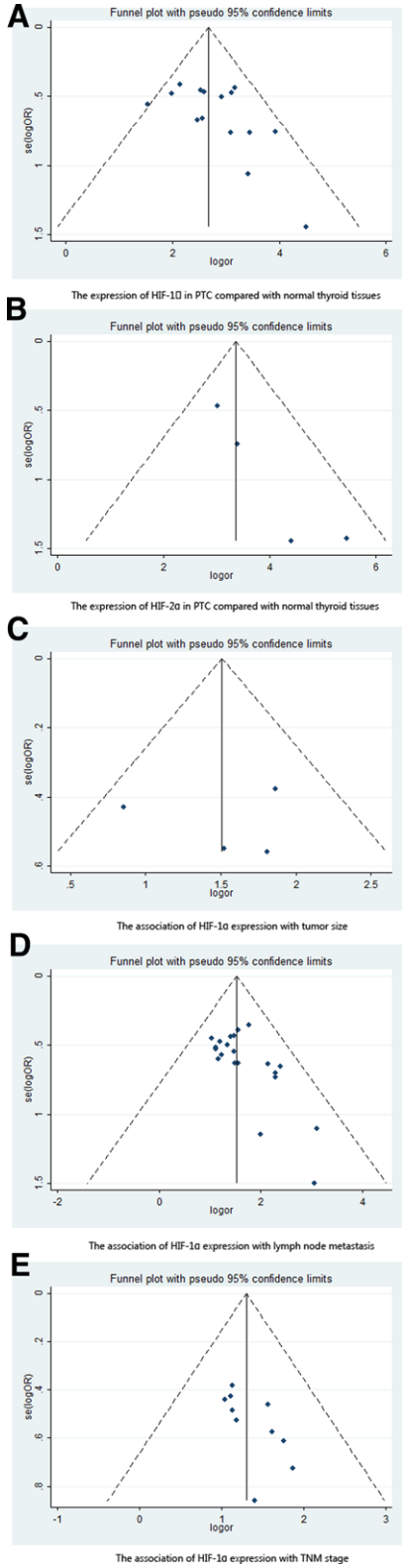


Figure 6. Continued

to occur, which is a poor prognostic sign of PTC' progression and is closely related to the expression of molecular markers in the primary tumors and metastatic tissues.[63] According to the study of Ilie et al,[39] there is a correlation between HIF-1α expression and BRAFV600E mutation. However, because the correlation of this factor has not been reported in other literatures, meta-analysis is not performed. For other clinicopathological features, meta-analysis results showed that HIF-1α protein expression was significantly correlated with tumor size, lymph node metastasis, TNM stage, capsular invasion and extrathyroidal extension. HIF-2α expression was significantly correlated with lymph node metastasis, TNM stage and capsular invasion. Contrary to the study results of Sun et al,[49] we have not found a clear correlation between tumor

size and HIF-2α expression. Because HIF-1α and HIF-2α are homologous to each other, we considered that there may be a positive correlation between the expression of HIF-1α and HIF-2α. After analyzing 3 literatures of Liu et al,[46] Liu et al,[45] and Wang et al,[47] we concluded for the first time that there is a statistical significance between the expression of HIF-1α and HIF-2α in the patients with PTC. However, so far no study has been conducted to explain the correlation between the expression of HIF-1α and HIF-2α in PTC, and the mechanism behind of them needs to be further explored.

Of course, our study also has some limitations: literatures were mainly included in Chinese and English, and most of them were from China. The correlation between HIF-1α and HIF-2α expression and clinicopathological characteristics of PTC patients worldwide needs to be further investigated; the number and sample size of the included studies were small, and some results showed mild statistical heterogeneity; there were some differences in the experimental methods of some literatures, which led to some bias in the results; at present, the relevant research is mainly focused on the tissue level, and more in-depth research on the mechanism from the cellular and molecular levels is needed in the future. So far, the correlation between some clinicopathological features of patients with PTC and HIF-1α has been widely studied, however, the number of studies on its correlation with HIF-2α is still small. Therefore, more high-quality, multi-center and large-sample studies are strongly recommended to be carried out in the future.

In conclusion, the high expression of HIF-1α and HIF-2α proteins is closely related to some clinicopathological parameters of PTC, which can provide potential biological indicators for the diagnosis and prognosis of the patients with PTC.

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