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Original Article

Expression and significance of TWIST, a zinc finger transcription factor, in laryngeal carcinoma among Chinese population: a meta-analysis

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Abstract: TWIST, an epithelial-mesenchymal transition inducer, has been implicated in the progression of a variety of cancers. Published evidence concerning the association of TWIST expression with laryngeal carcinoma risk has generated inconclusive results. Thus, we aimed to conduct a meta-analysis to address this controversy. A meta-analysis assessing the expression of TWIST in laryngeal cancer was conducted. Eligible studies for the period up to Jun 2015 were identified. After rigorous screening, a total of four studies met the inclusion criteria and they happened to be conducted on Chinese population. The results showed that TWIST positive expression rate in laryngeal cancer tissues was higher than that in normal tissues (OR=8.91, 95% CI=4.55-17.46). TWIST expression might have a correlation with low differentiation (OR=5.40, 95% CI=2.02-14.44), advanced clinical stage (OR=6.52, 95% CI=3.61-11.78), presence of lymph node metastasis (OR=9.10, 95% CI=4.26-19.41) and distant metastasis (OR=8.12, 95% CI=2.10-31.42), but not age and gender. The data suggested that TWIST might play critical roles in the development of laryngeal carcinoma. Further well-designed studies are warranted to get a more conclusive result.

Keywords: TWIST, expression, laryngeal carcinoma, epithelial-mesenchymal transition, meta-analysis

Introduction

Laryngeal carcinoma is the most frequent malignancy of the upper respiratory tract that severely affects the life quality of patients, with compromise of ability to talk, breathe, and swallow [1]. Complex interactions between many genetic and environmental factors might contribute to laryngeal cancer risk. Previously, cigarette smoking, alcohol drinking and human papilloma virus infection had been indicated to be associated with developing laryngeal carcinoma [2]. Thus, the etiological factors for this cancer are complicated. To find new biomarkers for predicting the prognosis of laryngeal cancer patients is needed.

Previously, epithelial-mesenchymal transition (EMT) has been shown to be correlated closely with development and progression of tumors [3]. Aberrant activation of EMT in epithelial tumors usually has been shown to indicate cancer development. Some factors might play critical roles during EMT. TWIST, a basic helix-loop-helix transcription factor, has been regarded as an important EMT inducer [4].

Reports showed that over-expression of TWIST might contribute to the genesis and progression of cancers such as thyroid cancer [5] and oral cancer [6]. In addition, TWIST act as a useful predictor of unfavorable prognosis for lung cancer [7]. Thus, TWIST was involved in both early events and advanced phases of a number of cancers.

A number of studies have been devoted to the roles of TWIST in laryngeal carcinoma. However, the results were inconclusive. In a recent study, we found that TWIST expression might confer progression of oral cancer by performing a meta-analysis [8]. In this study, we aimed to conduct a quantitative meta-analysis including published data with respect to laryngeal cancer up to Jun 2015 that increases statistical power to address this problem.

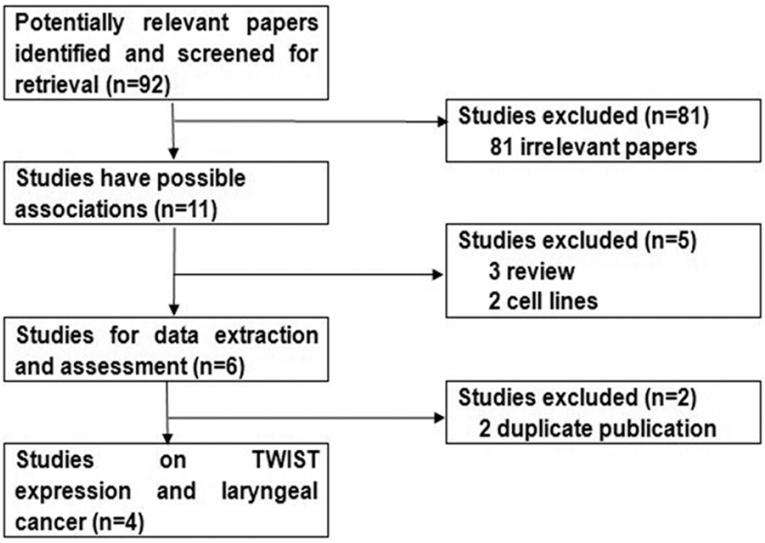

Figure 1. The flow diagram of included/excluded studies.

Materials and methods

Literature search strategy

A systematic search was carried out in the databases such as Medline, EMBASE, and CNKI (China National Knowledge Infrastructure) without a language limitation. Papers published up to Jun 2015 were considered. A combination of the following keywords was used for literature searching: TWIST, EMT, neoplasm, tumor, cancer, head and neck, and larynx. All searched studies were retrieved and the bibliographies were checked for other relevant publications.

Inclusion criteria

Several criteria were used for the literature selection: first, studies focused on the correlation between TWIST expression in primary laryngeal cancer tissues and pathological features; second, papers stated detailed clinical data of cancer cases who were not subjected to radiotherapy or chemotherapy prior to inclusion; third, immunohistochemistry (IHC) was used as the major detection method. In addition, Patients should be informed of the investigational nature of the research and each provided written informed consent prior to recruitment. Accordingly, the exclusion criteria were used as follows: first, papers showed an inconsistent judgment standard for positive TWIST expression or TWIST expression in non-primary tumor tissues including those detected from the blood circulation of patients; second, studies concerned animal experiments or cell line cultures; third, reviews and duplicate publications. After rigorous searching, we reviewed all papers in accordance with the criteria defined above for further analysis.

Data extraction

All eligible publications were reviewed independently by two of the authors according to the inclusion criteria and necessary information was carefully extracted from the selected papers. If the extracted data were conflicting, a discussion was made in order to reach an agreement. After this process, if a consensus was not reached, another author was consulted to resolve the dispute, and then a final decision was made by the majority of the votes. A database was established, in which the extracted data were listed.

Quality evaluation

The Newcastle-Ottawa Scale (NOS) was adopted to evaluate the quality of the included studies [9]. Three dimensions including selection, comparability and exposure were involved in the NOS evaluation, which contained four, one and three items, respectively. A star system was utilized to semi-quantitatively determine the quality of the studies, with a grade of more than or equal to 7 indicating high quality, a grade of 3-6 indicating intermediate-quality, and a grade of less than 3 indicating low-quality. Studies with low-quality should be excluded from the list.

Statistical analysis

The association of TWIST expression with clinical features was evaluated by the pooled odd ratio (ORs) and their 95 confidence interval (CIs). Hazard ratio (HRs) and their 95% CIs were used to evaluate the correlation between TWIST expression and the prognosis of patients with laryngeal carcinoma. A chi-square based Q statistic test was performed to assess heterogeneity. In particular, a $P>0.05$ for a given Q-test suggested a lack of heterogeneity among

Table 1. Characteristics of studies included in the present meta-analysis

First Author	Year	Number of Patients			Measurement method	Cut-off of IHC	Method of quantification	Outcome	Study type	Study location	Study quality points	Language
		total	TWIST negative or low	TWIST positive or high								
Huang	2011	80	27	53	IHC	≥5%	Percentage of staining	A B C D E F G	RC	Hebei	6	Chinese
Lu	2011	66	22	44	IHC	Not defined	Percentage of staining	B D F G	RC	Shandong	5	English
Hu	2013	60	19	41	IHC	≥10%	Percentage of staining	A B C D E F G	RC	Jiangsu	5	Chinese
Zhu	2014	49	19	30	IHC	4-12	Sum of percentage and intensity	A B C D G	RC	Hebei	4	Chinese

A. The control benign tissue; B. Clinical stage; C. Differentiation; D. Lymph node metastasis; E. Distant metastasis. F. Gender; G. Age; RC. Retrospective cohort.

Table 2. Main results of the meta-analysis

Clinical features	Overall OR (95% CI)	P	Heterogeneity test (Q, P)	Model
TWIST expression (Cancer vs. Normal)	8.91 (4.55-17.46)	<0.05	1.68, 0.432	Fixed-effect
Age (≥60 vs. <60)	0.90 (0.54-1.52)	>0.05	3.27, 0.532	Fixed-effect
Gender (Male vs. Female)	0.71 (0.34-1.48)	>0.05	1.40, 0.497	Fixed-effect
Clinical stage (III + IV vs. I + II)	6.52 (3.61-11.78)	<0.05	3.24, 0.356	Fixed-effect
Lymph node metastasis (Yes vs. No)	9.10 (4.26-19.41)	<0.05	1.66, 0.646	Fixed-effect
Differentiation (Low vs. Moderate + High)	5.40 (2.02-14.44)	<0.05	1.82, 0.403	Fixed-effect
Distant metastasis (Yes vs. No)	8.12 (2.10-31.42)	<0.05	0.40, 0.529	Fixed-effect

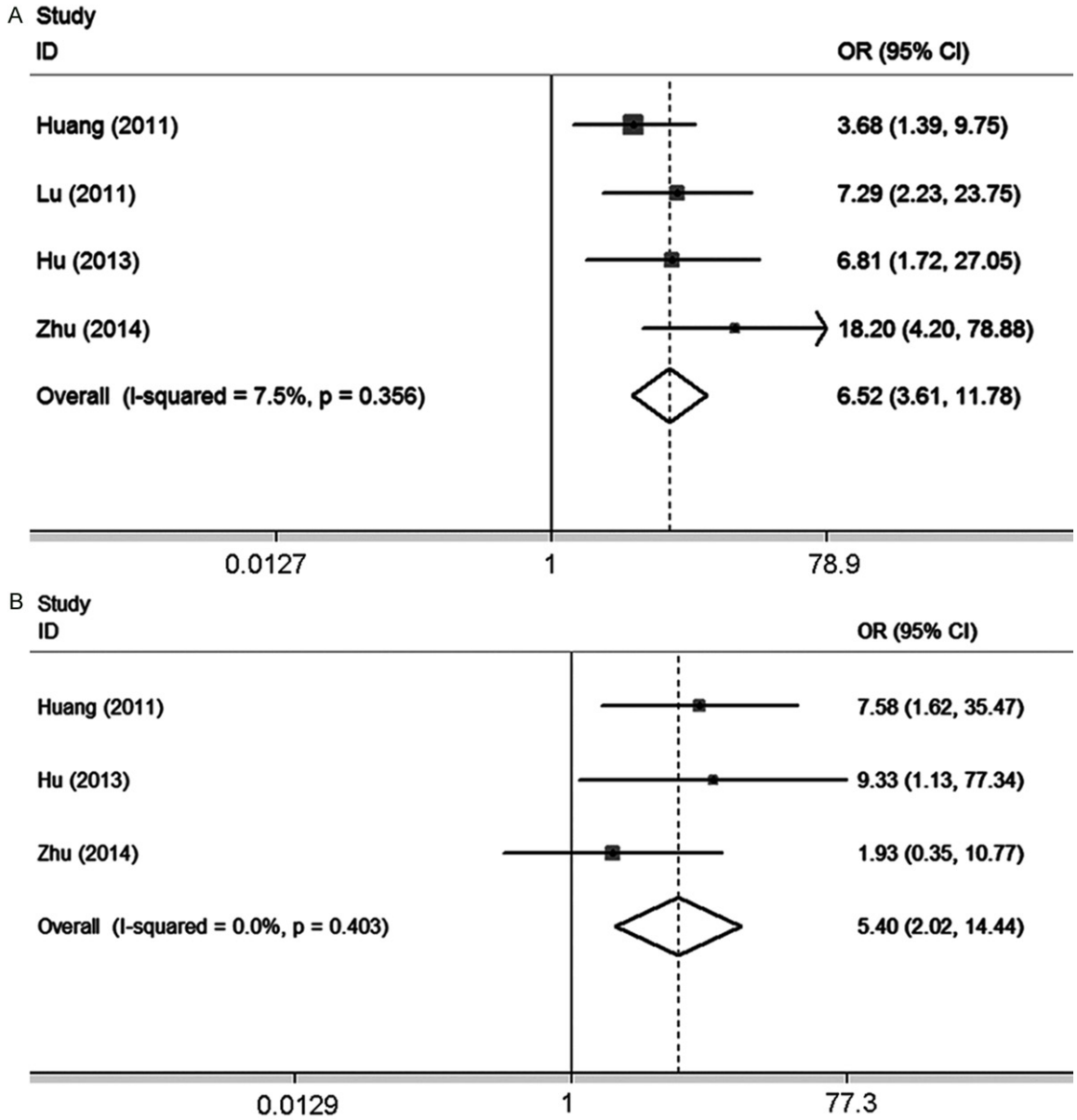

Figure 2. Forest plots showed that TWIST expression was associated with clinical stage (A) and differentiation (B).

the studies, and in this case ORs were pooled according to a fixed-effect model (Mantel-Haenszel) [10]; otherwise, a random-effect model (DerSimonian and laird) was used [11]. Sta-

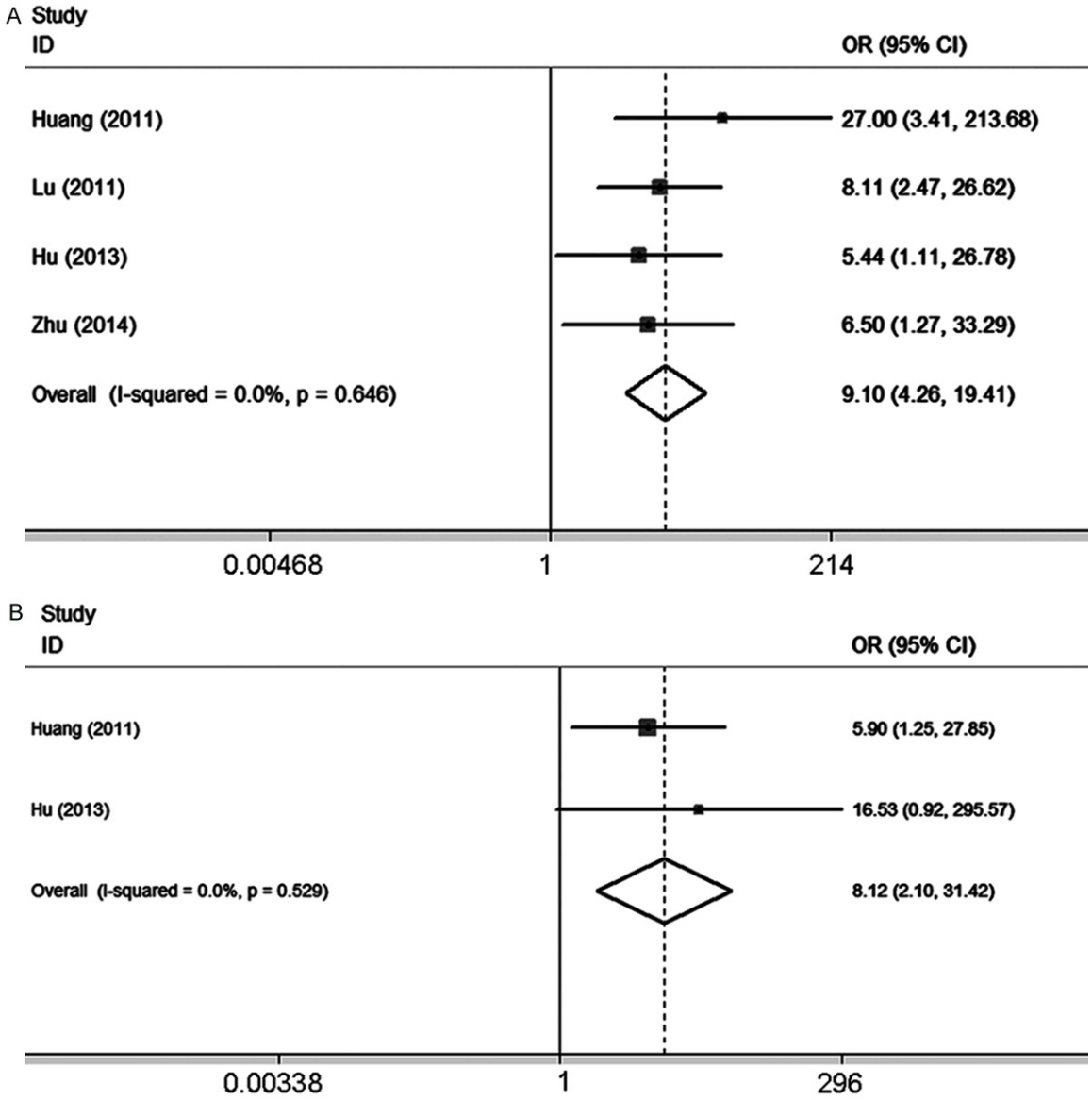

Figure 3. Forest plots showed that TWIST expression was associated with lymph node metastasis (A) and distant metastasis (B).

tistical analysis was undertaken using the program STATA 11.0 software (Stata Corporation, Texas, USA).

Results

Study characteristics

Eligible publications were retrieved and screened in our searching process. A total of ninety-two publications were searched, of which eighty-one irrelevant papers were excluded. Eleven publications were preliminary eligible. Then, three review articles [12-14] and two studies concerning laryngeal cancer cell line [15, 16] were excluded. Thus, six studies [17-22] were selected for data extraction. Afterwards, two papers [18, 21] were further excluded because they reported duplicate data that were involved in a selected study [20]. Lastly, four studies were selected for information extraction and assessment [17, 19, 20, 22] (**Figure 1**).

Of the four studies, one was written in English [17] and the other three were in Chinese. Coincidently, all the studies were conducted on Chinese population. No study on other ethnicities such as Caucasian was included because relevant reports could not be searched in the databases. The relevant information was listed

in **Table 1**. According to the lists, basic characteristics of the studies such as the first author and the number of cases for each study were presented. Notably, information about survival time was not available due to a lack of relevant data in the primary papers. Therefore, the prognosis of patients could not be assessed in the present meta-analysis. The quality assessment indicated that the qualities of the included studies were intermediate.

Meta-analysis results

The main results of the meta-analysis were presented in **Table 2**. The data pooling were made by using fixed-effect models because the *P* values for Q-tests were more than 0.05 for each comparison. Positive expression of TWIST in laryngeal cancer tissues were significantly higher than that in the normal tissues (OR=8.91, 95% CI=4.55-17.46). No correlation was found between TWIST expression and some clinicopathological features, such as age and gender. Nevertheless, positive TWIST expression was correlated with clinical stage (III + IV vs. I + II, OR=6.52, 95% CI=3.61-11.78) and differentiation (Low vs. Moderate + High, OR=5.40, 95% CI=2.02-14.44), indicating that TWIST might have an association with the elevated levels of malignancy (**Figure 2**). In addition, TWIST expression might make a contribution to lymph node metastasis (Yes vs. No, OR=9.10, 95% CI=4.26-19.41), and distant metastasis (Yes vs. No, OR=8.12, 95% CI=2.10-31.42), suggesting that TWIST might increase the ability of cancer cells to migrate and thus facilitate cancer metastasis (**Figure 3**).

Sensitivity analysis and bias diagnostics

To test the stability of the results, we used one-way sensitivity analysis [23] to assess the overall data. As a result, the results was not statistically overturned when any one study was removed from the whole line (data not shown) each time, confirming that the results were credible. Publication bias was not assessed because the number of the included studies was limited.

Discussion

In the present study, the results showed that TWIST expression might have a correlation with low differentiation, advanced clinical stage, presence of lymph node metastasis and distant metastasis, indicating that TWIST expression might contribute to the development and progression of laryngeal carcinoma.

Laryngeal carcinoma can strongly affect the life quality of patients because this type of cancer may directly influence speaking, breathing and eating on account of its specific site. The mechanisms of laryngeal cancer development are not fully understood. Recently, EMT has attracted much attention because this term depicts a process in which cells lose epithelial and gain mesenchymal characteristics, which is accompanied by a loss of cell-cell cohesiveness, leading to enhanced cell migratory capacity [24].

In the present meta-analysis, over-expression of TWIST was shown in cancer tissues rather than normal tissues, indicating that TWIST might have a correlation with the genesis of laryngeal cancer. The results further indicated that TWIST might have a correlation with cancer cell metastasis. However, the mechanisms of TWIST in cancer progression remain unclear. Evidence suggested that TWIST might act as a transcriptional factor that reduces the cell adhesion by modulating gene expression, and as a consequence, an alteration in cytoskeletal dynamics and cell shapes followed, and then a conversion from epithelial features to the mesenchymal phenotype emerged [25]. Also, TWIST may help cells obtain cancer stem cell-like stemness properties that are characterized by limitless proliferative potential and high migration capacity [26]. Thus, the cancer cells obtained elevated migratory abilities. Moreover, TWIST can stimulate angiogenesis by enriching and activating stromal macrophages that have been believed to directly or indirectly modulate tumor microenvironment [27, 28]. The macrophages might secrete proteases such as MMPs [29] and cathepsins [30] that might degrade the surrounding extracellular matrix and thus facilitate metastasis of cancer cells. In addition, TWIST could promote lymphatic metastasis by increasing expression of VEGF-C [31]. Therefore, TWIST might contribute to cell migration and metastasis through multi-steps and complicated pathways.

For all comparisons, between-study heterogeneity was not observed. Thus, fixed-effect models were used. Nevertheless, publication bias was not assessed because the number of the selected publications was limited. Thus, there might exist any publication bias and therefore the results should be interpreted with caution.

Several limitations should be addressed. First, in this meta-analysis, only mainstream databases in Chinese and English were used. Papers written in other languages were missed in our search. Therefore, the selection bias might exist. Second, the cut-off definition of TWIST appeared to be different in each study. Also, the normalized standards of the experiment and relevant positive or negative references are needed. Third, prognostic value of TWIST would be of great value for laryngeal cancer patients. However, information about prognosis could not be found in the primary literature. Hence, future investigations on this aspect are required. Furthermore, only studies focusing on Chinese population were included in the present meta-analysis, though we did not restrict the search items to Chinese during the literature searching process, owing to a lack of data on other countries and ethnicities. Therefore, the results could only represent the characteristics regarding Chinese population.

Despite the limitations, the data of the present meta-analysis showed a marked association of TWIST expression with low differentiation, advanced clinical stages, lymph node and distant metastasis, suggesting that TWIST might play critical roles in the development of laryngeal carcinoma.

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Disclosure of conflict of interest

None.

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