Evidence-Based Medicine

Prognostic value of wingless-type proteins in non-small cell lung cancer patients: a meta-analysis

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Background: Wingless-type protein (Wnt) signaling pathway plays a crucial role in the development of human malignancies, such as epithelial-to-mesenchymal transition (EMT) and the maintenance of cancer stem cells (CSCs). Several studies have shown that the expression levels of Wnt proteins, ligands of Wnt signaling pathway, are related to clinical outcomes of non-small cell lung cancer (NSCLC) patients. This meta-analysis aimed to assess the prognostic value of Wnts proteins in patients with NSCLC.

Methods: A multiple electronic literature search was conducted to identify all articles referring to the prognostic value of Wnt proteins in patients of NSCLC up to July 2016. Eligible studies were included in a meta-analysis in order to summarize the extracted data in terms of pooled hazard ratios (HRs) and their 95% confidence intervals (95% CIs).

Results: Ten studies published between 2005 and 2015 were eligible for this meta-analysis. The total number of patients included was 1,805. The combined HR for all eligible studies evaluating the overall survival (OS) of NSCLC patients with positive Wnt expression was 1.60 (95% CI: 1.39–1.84). The subgroup analysis showed both Wnt1 and Wnt5a are associated with clinical outcome of NSCLC patients.

Conclusions: Overexpression of Wnt proteins, as well as Wnt1 or Wnt5a alone, was markedly associated with adverse OS in lung cancer patients, suggesting that Wnts may act as a prognostic marker among NSCLCs.

Keywords: Meta-analysis; non-small cell lung cancer (NSCLC); precision medicine; prognostic significance; Wingless-type protein (Wnt)

Submitted Jul 26, 2016. Accepted for publication Aug 18, 2016.
doi: 10.21037/tlcr.2016.08.08
View this article at: http://dx.doi.org/ 10.21037/tlcr.2016.08.08

Introduction

Lung cancer is a highly aggressive cancer. It represents the leading cause of cancer-related deaths worldwide, and non-small cell lung cancer (NSCLC) accounts for 80–85% of all lung cancers (1). Despite recent advancements in the diagnosis and treatment of NSCLC (2), the overall 5-year survival rate remains approximately 15 (3,4). In clinical practice, disease stage and performance status are important and independent prognostic factors. However, there is an urgent need for the development of useful and potential prognostic biological markers (5).

The Wingless-type protein (Wnt) pathway plays a key role in the regulation of signaling pathway regulating cell proliferation, differentiation, apoptosis, and migration. Dysfunction of this pathway can contributes to and tumorgenesis (6,7). The Wnt protein family consists of at least 19 secreted cysteine-rich glycoproteins, overexpression of which will ultimately leads to expression of target genes associated with tumorgenesis (8-11) and tumor progression (12). Several previous studies have investigated the clinical significance of Wnt proteins. Xu *et al*. (13) found that Wnt1-positive expression in patients with NSCLC was closely associated with short overall survival (OS). Yao *et al*. (14) showed that high level of Wnt5a expression was also related to poor outcomes among NSCLC patients.

Aiming to investigate the prognostic value of Wnt protein overexpression on survival of patients with NSCLC we performed a systematic review of the current literature and in order to summarize the available the available data and a meta-analysis of the eligible studies. A further attempt to investigate the variable results between different centers was also performed by assessing the heterogeneity among studies and the potential publication bias.

Methods

Search strategy

A multiple electronic health database search on all articles on the prognostic value of Wnt protein overexpression on survival of patients with NSCLC published up to July 2016 was performed and reference lists were thereafter manually searched for relevant articles. Mesh terminology used was: (((((survival[Title/Abstract]) OR prognosis[Title/Abstract]) OR outcome[Title/Abstract])) AND ((non-small cell lung cancer[Title/Abstract]) OR NSCLC[Title/Abstract])) AND ((wnt[Title/Abstract]) OR Wingless-type protein[Title/Abstract]). We used the "Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)" guidelines, the recent extension of the PRISMA statement for network meta-analysis for the current meta-analysis (15).

Inclusion criteria

Studies were reviewed by two authors (Jiajia Jin, Ping Zhan) independently. Studies included in the current meta-analysis had to meet the following criteria: (I) must evaluate Wnt expression level in NSCLC patients by immunohistochemistry (IHC); (II) must involve NSCLC patients have been be diagnosed by the golden standard of histopathologic examinations; and (III) must concern connection between Wnt proteins and survival data with regard to lung cancer.

An extensive effort was made to minimize the impact of covert duplicate or metachronous republication from the same groups on the patient sample size; in these cases, only the latest report was included. Case reports, letters, review articles, or laboratory articles, were excluded of the current analysis.

Data extraction and quality assessment

The eligible studies were evaluated by two reviewers (Jiajia Jin and Ping Zhan) independently using the "Newcastle-Ottawa Scale" (NOS) to examine their quality (16,17). All of the studies included were considered to be of high quality with a score more than five each one based on the NOS. Data retrieved from each report included the first author's name, publication year, country, histology, metastasis (TNM) stages, the number of patients, test methods, the percent of Wnt positivity, hazard ratio (HR) and 95% confidence interval (CI) survival rates and Wnt type (*Table 1*). Patient outcome was defined as OS, calculated from the date of operation to the date of death or date of last follow-up.

Statistical methods

The HRs and their 95% CIs were combined as the pooled effective value. If HRs and their 95% CIs were not given explicitly, they were extracted from Kaplan-Meier curves as Tierney described previously (26). A statistical test for heterogeneity using the I square test was performed (27). The I square value lies between 0% to 100% and it presented with a 95% CI; I square value over 50% is considered

Table 1 Main characteristics and results of the eligible studies

First author, year (ref.)	Patients source	Histology	Stage	N pts	Method	Primary antibody	Positive (%)	HR (95% CI)	Survival results	Wnt type
Huang 2005 (18)	Japan	NSCLC	I–III	123	IHC	Goat polyclonal antibody Santa Cruz	56.7	2.451 (1.2–5.1)	Poor	Wnt5a
Nakashima 2008 (19)	Japan	NSCLC	I–III	151	IHC	Rabbit polyclonal antibody Santa Cruz	40.4	1.983 (1.216–3.236)	Poor	Wnt1
Huang 2008 (20)	Japan	NSCLC	I–III	216	IHC	Rabbit polyclonal antibody Santa Cruz	49.1	1.784 (1.132–2.813)	Poor	Wnt1
Nakashima 2010 (21)	Japan	NSCLC	I–III	122	IHC	Rabbit polyclonal antibody Santa Cruz	53.3	1.45 (0.63–3.34)	Negative	Wnt1
Wang 2010 (22)	China	NSCLC	I–IV	115	IHC	Rabbit monoclonal antibody Abcam	62.6	1.41 (0.84–2.36)	Negative	Wnt1
Xu 2011 (13)	Korea	NSCLC	I–IV	262	IHC	Rabbit polyclonal antibody Santa Cruz	36.6	1.699 (1.039–2.778)	Poor	Wnt1
Nakashima 2012 (23)	Japan	NSCLC	I–III	128	qPCR IHC	mouse monoclonal antibody Zymed Laboratories	18.8	2.226 (1.082–4.577)	Poor	Wnt3
Yao 2014 (14)	China	NSCLC	I–IV	205	IHC	Abcam	61.95	1.47 (1.04–2.06)	Poor	Wnt5a
Lu 2015 (24)	China	NSCLC	I–IV	219	IHC	Abcam	35.6	1.774 (1.221–2.576)	Poor	Wnt5a
Huang 2015 (25)	China	NSCLC	NM	264	IHC	Rabbit monoclonal antibody Abcam	34.4	1.319 (1.001–1.739)	Poor	Wnt2

IHC, immunohistochemistry; RT-PCR, reverse transcriptase PCR; NSCLC, non-small-cell lung cancer; AC, adenocarcinoma; HR, hazard ratio; CI, confidence interval; N pts, number of patients; NM, not mentioned; Wnt, Wingless-type protein.

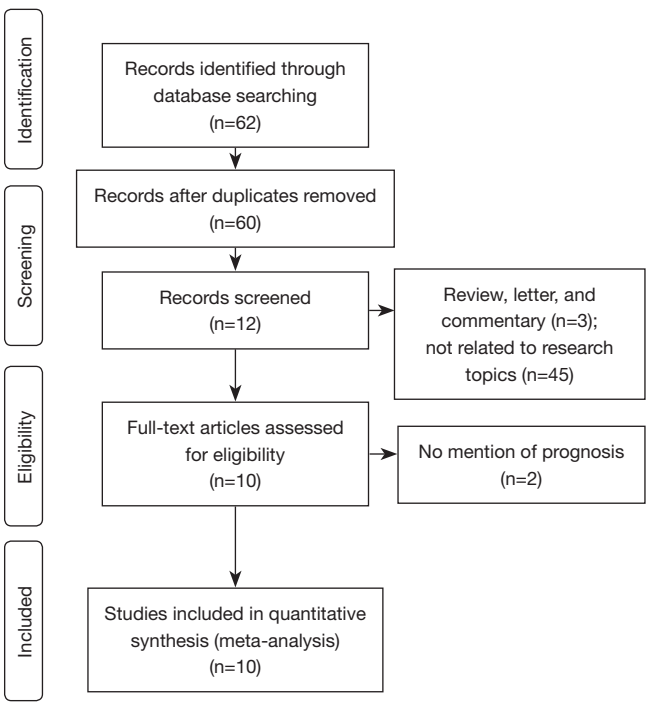

Figure 1 Flow diagram of the study selection in our meta-analysis.

to indicate significant heterogeneity. The heterogeneity and robustness of pooled proportions were explored by conducing sensitivity analyses. However, because there are no published randomized clinical trials on this topic and all the reported studies are retrospective or prospective case series, the sensitivity analysis was limited to the exclusion of extreme studies as identified by the construction of funnel plots. The subgroup analysis was performed by Wnt type. Moreover, we assessed publication bias by Begg's funnel plots. Meta-analysis was conducted using STATA version 12.0 (Stata Corporation, College Station, TX, USA) statistical software.

Results

Study selection and characteristics

The electronic literature search yielded 112 citations, 10 of which fulfilled the inclusion criteria and were reviewed in the present study. Flow diagram of the study selection in our meta-analysis is shown in *Figure 1*. Ten observational studies published between 2005 and 2015 were eligible for final analysis. The total number of patients included

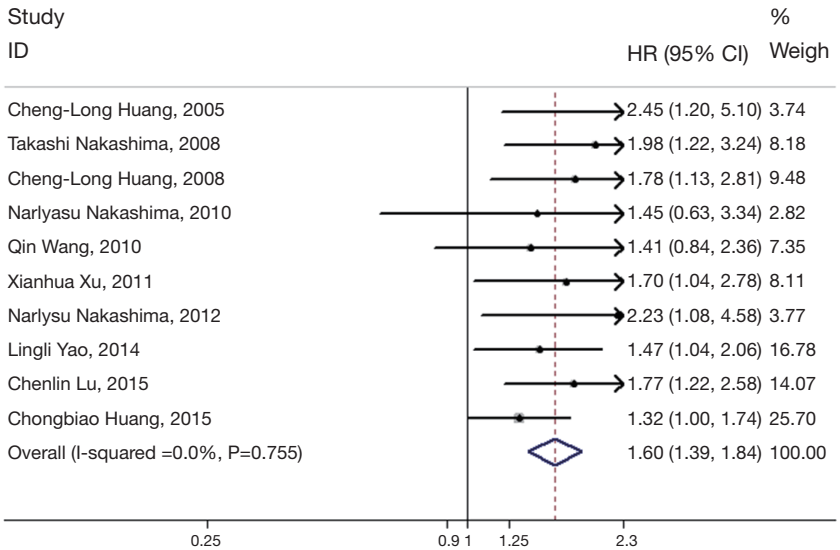

Figure 2 Meta-analysis of the association between positive Wnt expression and overall survival (OS) in patients with non-small cell lung cancer (NSCLC). Forest plot of hazard ratios (HRs) and 95% confidence intervals (CIs) from each study were shown.

was 1,805. The major characteristics of the ten eligible publications are reported in *Table 1*. The Wnt proteins investigated, including Wnt1, Wnt2, Wnt3 and Wnt5a, were all detected by cytoplasmic staining. The subgroup analysis was performed by Wnt protein type. Of the ten studies, five studies referred to the relation between Wnt1 positive expression and OS (13,19-22), and three studies on Wnt5a (14,18,24), and two studies the role of Wnt2 (25) and Wnt3 (23) respectively.

Meta-analysis

Overall, the pooled HR for the eligible studies evaluating the relation between Wnt proteins and OS was 1.60 (95% CI: 1.39–1.84) using the fixed effects model (*Figure 2*). The heterogeneity among the studies was not significant (I^2=0.0%, P=0.755). The subgroup analysis was performed by Wnt protein type. Five studies were found to investigate the role of Wnt1 (13,19-22), two studies the role of Wnt2 (25) and Wnt3 (23) respectively and three studies the role of Wnt5a (14,18,24). Higher Wnt1 expression and Wnt5a expression were associated with poorer OS, as the pooled HR for OS was 1.69 (95% CI: 1.34–2.14) and 1.68 (95% CI: 1.32–2.13), respectively (*Figure 3*). Meanwhile, the heterogeneity among the studies was not significant (I^2=0.0%, P=0.900 and I^2=0.0%, P=0.424, respectively).

There was no publication bias, as evidence by the symmetrical pattern of the funnel plots (*Figure 4*).

Discussion

The present meta-analysis revealed that NSCLC patients with a positive Wnt expression showed a shorter OS than patients with a negative Wnt expression. The subgroup analysis still showed that high Wnt1 and Wnt5a expression was markedly related to poor OS among patients. Thus, our results imply that Wnt proteins may play a role as prognostic biomarkers for predicting outcomes of NSCLC patients. There was no significant heterogeneity among the studies, whereas the potential publication bias was also low.

Some limitations of our meta-analysis should be noted. Firstly, the total sample size of the present meta-analysis (n=1,805) is limited by the available studies included, which constrains the statistical power of the analysis. The available studies were also all derived from Asian populations, and whether the present trends in this study can be applicable across all continents remains to be established. Secondly, some potential bias existed when HR was extracted from Engage Digitizer instead of obtained directly from published statistics. As such, data is estimated from the provided Kaplan-Meire curve for some studies which may yield some inaccuracies. Thirdly, antibody clones used were not uniform and methods to detect Wnt protein expression level were not unified. Despite the limitations mentioned above, to our knowledge, this is the first meta-analysis concentrated on the correlation of Wnt expression with prognosis of lung cancer.

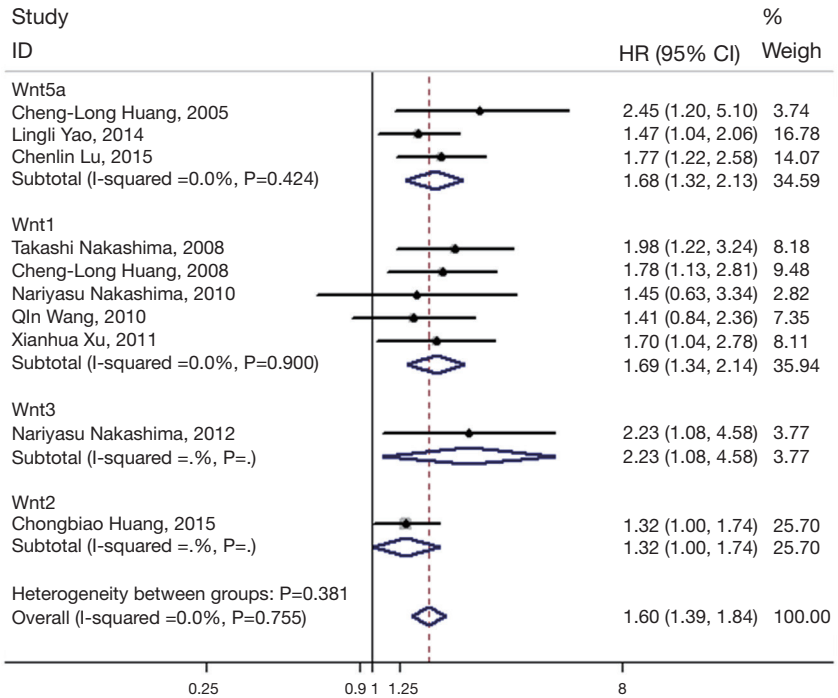

Figure 3 Subgroup analysis of the association between overall survival (OS) of non-small cell lung cancers (NSCLCs) and positive Wnt1 expression or Wnt5 expression. Forest plot of hazard ratios (HRs) and 95% confidence intervals (CIs) from each study were shown.

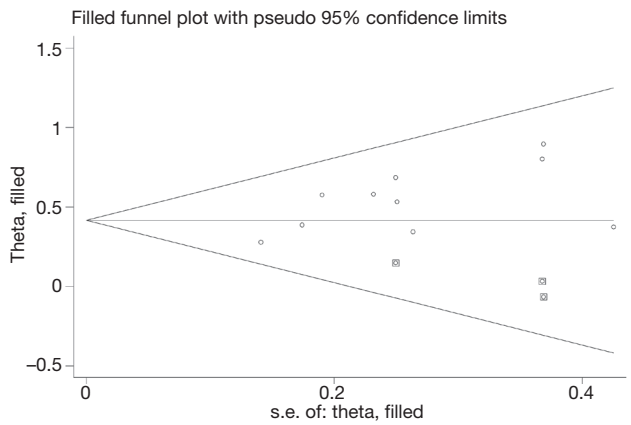

Figure 4 Begg's funnel plots for assessing potential publication bias on overall survival.

Wnt signals are transduced to the canonical and non-canonical cascades in a cellular context-dependent manner (28,29). The most known Wnt signaling pathway is the canonical Wnt pathway, which signals through β-catenin. Other Wnt signaling pathways include the Wnt/Ca^{2+} flux pathway (30,31), and the protein kinase A pathway (30), cJun N-terminal kinase (31), and the small GTPases Rho, Rac, and Cdc 42 (31). Among 19 Wnt family members in of mammalian cells, Wnt1 and Wnt3 preferentially activate the canonical Wnt signaling cascade, whereas Wnt5a preferentially activates non-canonical Wnt signaling cascades (28-31). Canonical Wnt signaling activation via the Frizzled family of receptors will finally contribute to the promotion of tumorigenesis through the expression of its target genes, such as Cyclin D1 and c-Myc (31,32). Xu *et al.* (13) showed that Wnt1 expression was significantly associated with those of β-catenin and c-Myc in NSCLC; Nakashima *et al.* (23) found that Wnt3 expression in NSCLC was associated with c-Myc expression and tumor progression; Yao *et al.* (14) reported that Wnt5a contributes to NSCLC progression through the promotion of angiogenesis. Together these facts indicate that Wnt1, Wnt3 and Wnt5a are able to promote lung carcinogenesis.

Therapeutics targeting *ALK* fusions and *EGFR* mutations are in the clinical practice for NSCLC patients, and therapeutics targeting *RET* fusions, *ROS1* fusions and *FGFR1* amplification are in clinical trials for NSCLC patients in the world (33-36). However, recurrence after targeted therapies is inevitable owing to acquired mutations in the drug-target genes, activation of by-pass signaling

pathways and epithelial-to-mesenchymal transition (EMT). Because the WNT signaling cascades are involved in the regulation of drug resistance and cell motility in human cancers (12,37,38), upregulation of Wnt proteins might contribute to the poor prognosis of NSCLC patients through the mechanisms related to the maintenance of cancer stem cells (CSCs) and promotion of invasion and metastasis.

In summary, our meta-analysis reveals that the positive Wnt protein is significantly associated with poor OS in patients with NSCLC. The early detection of Wnt may be of great value in evaluating the clinical outcomes of NSCLC patients.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Jin J, Zhan P, Qian H, Wang X, Katoh M, Phan K, Chung JH, Lv T, Song Y; Written on behalf of the AME Lung Cancer Collaborative Group. Prognostic value of wingless-type proteins in non-small cell lung cancer patients: a meta-analysis. Transl Lung Cancer Res 2016;5(4):436-442. doi: 10.21037/tlcr.2016.08.08

