Prognostic role of snail in lung cancer
Protocol for a systematic review

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Abstract

Background: Increasing studies were performed to explore the prognostic value of snail in lung cancer (LC), however, with inconsistent results. Hence, this systematic review is aimed to evaluate the prognostic role of snail in patients with LC.

Methods: A comprehensive literature search in the PubMed, Embase, the Cochrane Library, and Web of Science databases will be conducted to identify eligible studies. Language is limited as English. We will employ hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the correlations between snail expression and overall survival (OS), progression-free survival (PFS), recurrence-free survival (RFS), disease-free survival (DFS), and clinicopathological features. Meta-analysis will be performed using STATA 14.0 software.

Results: This study will provide a high-quality synthesis of current evidence of the correlations between snail expression and OS, PFS/RFS/DFS, and clinicopathological features.

Conclusion: The study will provide updated evidence to assess whether the expression of snail is in association with poor prognosis in patients with LC.

Ethics and dissemination: It is not necessary for ethical approval because individuals cannot be identified. The protocol will be disseminated in a peer-reviewed journal or presented at a relevant conference.

PROSPERO registration number: This systematic review protocol has been registered in the PROSPERO network (No. CRD42018095191).

Abbreviations: 95% CIs = 95% confidence intervals, DFS = disease-free survival, EMT = epithelial to mesenchymal transition, HRs = hazard ratios, LC = Lung cancer, NOS = Newcastle–Ottawa Quality Assessment Scale, NSCLC = non-small-cell lung cancer, OS = overall survival, PFS = progression-free survival, PRISMA-P = preferred reporting items for systematic reviews and meta-analyses protocols, RFS = recurrence-free survival, SCLC = small-cell lung cancer, Snai1 = snail homolog 1.

Keywords: lung cancer, prognosis, protocol, snail, systematic review.

1. Introduction

Lung cancer (LC) is currently a leading cause of cancer-related mortality.[1] Histologically, it can be classified as non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC)—the former mainly consists of adenocarcinoma, squamous cell carcinoma, and large cell carcinoma and represents 85% of all cases of LC.[2] Advances for LC have been slow, for which the 5-year relative survival is currently 18%. While more than one-half of cases are diagnosed at a distant stage, leading to an extremely poor 5-year survival rate of 4% among patients with stage IV LC.[3] The high recurrence and metastasis largely contribute to the poor overall prognosis.[4] Therefore, it is crucial to identify reliable therapeutic and prognosis biomarkers for LC.

The prognosis is highly associated with the metastatic behavior of the tumor.[5] The epithelial to mesenchymal transition (EMT) seems to play a crucial role. It is a complicated process, during which, epithelial cells lose their epithelial features and gain mesenchymal phenotypes, and epithelial cells become migratory and invasive.[6,7] As one of transcription factors, snail homolog 1 (Snai1) is considered as one of the primary drivers of EMT and has been implicated in the EMT associated with tumor progression.[6,8] The expression of snail has been disclosed to associate with invasion and metastasis as well as poor prognosis of many malignancies, such as gastric cancer,[9] breast cancer,[10] hepatocellular carcinoma,[11] colorectal cancer,[12] and lung cancer.[13] In recent years, a number of studies analyze
the relationship between the snail expression and prognosis of patients with LC, but due to differences in research method, sample size and the study population, the findings of a single study are difficult to extend to the entire population and the obtained conclusions are inconsistent. The study conducted by Merikallio et al[14] supported that the expression of snail was strongest in small cell lung cancer. Whereas, the prognostic role of snail is contradictory in NSCLC. Yanagawa et al[15] indicated that lung adenocarcinoma patients with elevated snail expression had a significant reduction of survival time, while the high expression of snail was not a prognostic biomarker in lung squamous cell carcinoma. However, the study conducted by Sun et al[16] indicated that lung adenocarcinoma patients with elevated snail expression had a significant reduction of survival time, while the high expression of snail was not a prognostic biomarker in lung squamous cell carcinoma. Given that the clinical results are controversial, we aim to systematically evaluate the prognostic role of snail in lung cancer patients.

2. Methods

2.1. Study registration

The protocol for this systematic review was registered on PROSPERO with registration number: CRD42018095191. This protocol follows the guidelines according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) statement guidelines.[17]

2.1.1. Data sources and search strategy. This study will perform a complete computer-based search of the PubMed, Embase, Web of Science, and the Cochrane Library databases for clinical trials up to the date of April 28, 2018. The strategy is created based on a discussion among all reviewers according to the Cochrane handbook guidelines. The following search terms will be used: lung neoplasm(s), lung cancer(s), lung tumor(s), lung carcinoma(s), pulmonary cancer(s), snail family transcription factors, snail, snail 1, prognosis, outcome, prognostic value, survival, and prognostic biomarker(s). The example search strategy in Table 1 will be used for PubMed. This search strategy will be modified and used for the other databases. The other strategies for Embase, Web of Science, and the Cochrane Library databases are shown in Supplement Tables 1–3, http://links.lww.com/MD/C341. Besides, we will examine reference lists of all retrieved articles that may fulfill our eligibility requirements in order to avoid missing relevant studies.

2.2. Inclusion and exclusion criteria

The main inclusion criteria are as follows: Retrospective or prospective studies evaluating the prognostic relationship between the expression of snail and LC; the expression of snail in tissues detected by immunohistochemistry analysis; providing sufficient data to estimate the hazard ratios (HRs) for overall survival (OS) and progression-free survival (PFS)/relapse-free survival (RFS)/disease-free survival (DFS), along with their 95% confidence intervals (CIs) or p values; published in English.

The main exclusion criteria are as follows: Reviews, case reports, conference abstracts, specialist experience, comments and cell or animal studies. studies without enough data to pool the HRs; studies not focusing on the role of the snail expression on the clinicopathological features or prognoses in LC; not published in English.

2.3. Data extraction and quality assessment

2.3.1. Selection of studies. All review authors have received training to ensure a good understanding of the purpose and process of the review. All identified studies will combine together in a single reference manager file created by Endnote X8 and duplicate studies will be removed using this software. The selection process will be conducted by 2 reviewers (MS and GD) independently. Initially, we will screen the titles, abstracts, and keywords of all retrieved records. The articles meeting inclusion criteria will be reviewed comprehensively by reading the full text. A table named “Reasons for excluded studies” will be used for recording excluded studies. We will resolve disagreements by consensus between the 2 reviews or by involving a third review author (ML). Using the PRISMA-compliant flowchart (http://www.prisma-statement.org), the screening and selection process of this study will be documented and summarized. The primary selection process is shown in a PRISMA flowchart (Fig. 1).

2.3.2. Data extraction and management. Data extraction of the included studies will be performed by 2 independent authors (MS and GD). We will use a data extraction form (Excel) designed by all the authors in consensus to conduct the data extraction. The following information are extracted: name of the first author, publication time, country, number of patients, recruitment time, follow-up duration, analysis method, tumor type, clinicopathological features, antibody epitope, antibody company, method and score for its evaluation, cut-off value of snail overexpression, positive expression rate, HRs, and their 95% CIs. If HRs and their 95% CIs are not reported, we will extract them from Kaplan–Meier curves using the methods proposed by Tierney et al.[18] Any disagreement will be solved by consensus or an arbiter (ML).

2.3.3. Assessment of quality in included studies. The Newcastle–Ottawa Quality Assessment Scale (NOS) is employed to assess the quality of the selected studies. The NOS included 3 main aspects: selection, comparability, and outcome.[19] A study with a score of at least 5 will be considered of high quality. Two independent authors (MS and DL) will use the criteria outlined in the NOS. Any disagreement will be resolved by discussion or by involving an arbiter (ML).

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Table 1

| Search Strategy Used in PubMed. |
|--------------------------------|
| **Search items**             |
| #1 (((((...)“lung neoplasms” [MeSH Terms] OR “lung neoplasms” [Title/Abstract]) OR “lung neoplasm” [Title/Abstract]) OR “lung cancer” [Title/Abstract]) OR “lung cancers” [Title/Abstract]) OR “lung tumor” [Title/Abstract]) OR “lung tumors” [Title/Abstract]) OR “lung carcinoma” [Title/Abstract]) OR “lung carcinomas” [Title/Abstract]) OR “pulmonary cancers” [Title/Abstract]) OR “pulmonary cancer” [Title/Abstract])
| #2 (((())“Snail Family Transcription Factors” [MeSH Terms] OR “Snail Family Transcription Factors” [Title/Abstract]) OR “snail” [Title/Abstract]) OR “snail 1” [Title/Abstract])
| #3 (((())“prognosis” [MeSH Terms]) OR “prognosis” [Title/Abstract]) OR “outcome” [Title/Abstract]) OR “prognostic value” [Title/Abstract]) OR “survival” [Title/Abstract]) OR “prognostic biomarker” [Title/Abstract]) OR “prognostic biomarkers” [Title/Abstract])
| #4 #1 and #2 and #3 and #4 |
2.3.4. Measures of prognosis. For prognostic outcomes including OS, PFS, RFS, DFS, data will be expressed as the HRs along with their 95% CIs or p values.

2.3.5. Management of missing data. In several studies which some data are missing, we will consider why the data are missing (missing at random or not). We will try to contact the authors to request any inadequate and missing data of the included studies. If the data are still incomplete, available case analysis will be performed. And we will address the potential impact of missing data on the findings of the review in Section 3.

2.3.6. Assessment of heterogeneity. The heterogeneity was assessed across all studies by Cochran’s Q test and Higgins I^2 method. When the result of a Q-test (I^2 ≥ 50% or P < .05) indicating substantial heterogeneity, while I^2<50% will be taken as evidence of no heterogeneity. In cases of substantial heterogeneity, we will explore the possible causes by sensitivity analysis and subgroup analyses.

2.3.7. Assessment of publication biases. We will assess the publication bias with a funnel plot if the number of included studies is more than 10. Visual inspection of funnel plot, Egger’s test, Begger’s test are used to evaluate publication bias (P < .01 is considered statistically significant).

2.3.8. Data synthesis. Meta-analysis will be performed using STATA software (version 14.0; Stata Corp, College Station, TX). The prognosis outcomes are explored using the HRs and the corresponding 95% CIs. If I^2≥50%, the random-effect model will be used for data analysis, otherwise a fixed-effect model will be used for data analysis. Besides, the sensitivity analysis and subgroup analysis will be employed for exploring the causes of
heterogeneity. All the p values are 2-side and \( P < .05 \) is considered statistically significant.

### 2.3.9. Subgroup analysis

The prognosis outcomes mainly contain the OS and PFS/DFS/RFS. In cases of high heterogeneity, we will perform subgroup meta-analyses to determine the possible factors that may influence the results. The following subgroup analyses will be considered:

1. Different nationalities
2. Histology type of LC
3. Different statistical analyses.

### 2.3.10. Sensitivity analysis

The sensitivity analysis will conduct using the “metanint” STATA command (sequential exclusion of each individual study then pooled HRs) to examine the robustness of the pooled results.

### 3. Discussion

Cancer metastasis is the major cause for the poor survival of LC patients. The metastasis of LC is extremely complex processes, where multiple steps are involved. EMT is considered to be one of the major molecular mechanisms inducing tumor invasion, metastasis, and postoperative recurrence. It is an important one of the major molecular mechanisms inducing tumor invasion, motility of tumor cells.

The first step of EMT process is that epithelial cells lose cell–cell junctions and the epithelial marker E-cadherin. E-cadherin acts as a tumor suppressor inhibiting invasion and metastasis. While, downregulation of E-cadherin expression increases tumor cell motility and promotes invasion. The snail, which is zinc finger transcription factors, is over-expressed in epithelial cell lines and mainly repress expression of E-cadherin to reduce cell adhesion during the EMT.

Therefore, snail is an important factor in regulating EMT and its high expression is related to the enhancement of LC invasion, metastasis, and progression.

Several studies were conducted to explore the prognostic significance of snail in LC. Unfortunately, the results of these researches were controversial. A number of studies have shown that EMT transcription factor snail is closely related to the prognosis of patients with LC, but some individual studies show that there is no clear relationship between snail and the prognosis of patients with LC. Hence, we hope this review will provide more accurate and objective evidences of the relationship between the snail and the prognosis of patients with LC.

While, there are several limitations that need to be addressed in this review. Firstly, only studies published in English will be included, which may increase the bias of our study. Secondly, different nationalities, histology type of LC and different statistical analyses may run risk of heterogeneity. In addition, the methods and cut-off definitions for evaluating snail expression may be different.

The PRISMA-P checklist of the protocol is supplied in PRISMA-P checklist.

### Author contributions

Data curation: Meiling Shi, Guangtong Dong, Daorui Li.
Supervision: Peitong Zhang, Kaiwen Hu.
Writing – original draft: Meng Li, Xing Zhang.
Writing – review & editing: Meng Li.

### References

1. Torre L, Bray F, Siegel R, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
2. Kowal A, Winniewski A, Kusnierczyk P, et al. Human leukocyte antigen (HLA)-G gene polymorphism in patients with non-small cell lung cancer. Thorac Cancer 2015;6:613–9.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
4. Park E, Park SY, Sun PL, et al. Prognostic significance of stem cell–related marker expression and its correlation with histologic subtypes in lung adenocarcinoma. Oncotarget 2016;7:42502–12.
5. Atmaca A, Wirtz RW, Werner D, et al. SNAI2/SLUG and estrogen receptor mRNA expression are inversely correlated and prognostic of patient outcome in metastatic non-small cell lung cancer. BMC Cancer 2015;15:300.
6. Chen S, Jiang S, Hu F, et al. Foxk2 inhibits non-small cell lung cancer epithelial-mesenchymal transition and proliferation through the repression of different key target genes. Oncol Rep 2017;37:2353–47.
7. Lin TY, Hsu HY, Ling Zhi–8 reduces lung cancer mobility and metastasis through disruption of focal adhesion and induction of MDM2-mediated slug degradation. Cancer Lett 2016;375:340–8.
8. Choudhary R, Li H, Winn RA, et al. Peroxisome proliferator-activated receptor-γ inhibits transformed growth of non-small cell lung cancer cells through selective suppression of snail. Neoplasia 2010;12:224–7.
9. Muqbil I, Wu J, Aboukameel A, et al. Snail nuclear transport: the gateways regulating epithelial-to-mesenchymal transition? Semin Cancer Biol 2014;27:39–45.
10. Chen X, Li J, Hu L, et al. The clinical significance of snail protein expression in gastric cancer: a meta-analysis. Hum Genomics 2016;10:22.
11. Wu J, He Z, Yang XM, et al. RCD1 depletion attenuates TGF-beta-induced EMT and cell migration by stabilizing cytoskeletal microtubules in NSCLC cells. Cancer Lett 2017;400:18–29.
12. Wang X, Shi W, Shi H, et al. TRIM11 overexpression promotes proliferation, migration and invasion of lung cancer cells. J Exp Clin Cancer Res 2016;35:100.
13. Kwon CH, Park HJ, Choi JH, et al. Snail and serpinA1 promote tumor progression and predict prognosis in colorectal cancer. Oncotarget 2015;6:20312–26.
14. Merkillaio H, Turppenniemi-Hujanen T, Paakko P, et al. Snail promotes an invasive phenotype in lung carcinoma. Resp Res 2012;13:104.
15. Yanagawa J, Walser TC, Zuo LX, et al. Snail promotes CXCR2 ligand-dependent tumor progression in non-small cell lung carcinoma. Clin Cancer Res 2009;15:6820–9.
16. Sun BS, Pei BX, Zhang K, et al. Significance of interstitial tumor-associated macrophages in the progression of lung adenocarcinoma. Oncol Lett 2016;12:4467–76.
17. Shamsier L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015;350:7647.
18. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
19. Maxwell L, Santesso N, Tugwell PS, et al. Method guidelines for Cochrane Musculoskeletal Group systematic reviews. J Rheumatol 2006;33:2304–11.
[26] Xie D, Gore C, Liu J. Role of DAB2IP in modulating epithelial-tomesenchymal transition and prostate cancer metastasis. Proc Natl Acad Sci U S A 2010;107:2485–90.

[27] Lim WC, Kim H, Kim Y-J. Dioscin suppresses TGF-β1-induced epithelial-mesenchymal transition and suppresses A549 lung cancer migration and invasion. Bioorg Med Chem Lett 2017;27:3342–8.

[28] Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 2008; 14:818–29.

[29] Zhang K, Li XY, Wang ZM, et al. MiR-22 inhibits lung cancer cell EMT and invasion through targeting snail. Eur Rev Med Pharmacol Sci 2017;21:3398–604.