

# Prognostic value of the expression of estrogen receptor $\beta$ in patients with non-small cell lung cancer: a meta-analysis

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**Background:** Lung cancer remains the leading cause of cancer-related deaths in men and the second leading cause in women worldwide. It is becoming increasingly clear that estrogen and estrogen receptors are involved in the pathogenesis and development of lung cancer. However, observational studies on the prognostic role of estrogen receptor  $\beta$  (ER $\beta$ ) in non-small cell lung cancer (NSCLC) are controversial.

**Methods:** To clarify the impact of ER $\beta$  in NSCLC survival, we performed this meta-analysis that included eligible studies. The combined hazard ratios (HR) and their corresponding 95% confidence intervals (CIs) were calculated in terms of overall survival.

**Results:** A total of eleven studies with 3,300 patients were evaluable for this meta-analysis. Our results suggested that ER $\beta$  overexpression had no relationship on survival of patients with NSCLC, the HR (95% CI) was 1.000 (0.954–1.047) overall. Moreover, there was no heterogeneity between the studies.

**Conclusions:** ER $\beta$  overexpression indicates no relationship of prognosis for patients with NSCLC.

**Keywords:** Estrogen receptor  $\beta$  (ER $\beta$ ); prognosis; lung cancer; meta-analysis

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## Introduction

Lung cancer is the leading cause of death in both men and women throughout the world (1). The cause of high mortality of lung cancer is mainly caused by the diagnosis of patients with advanced stage of cancer, at this time the tumor cells have already occurred in the local or distant invasion and metastasis, and another important aspect is the lack of effective treatment. In addition to the traditional tumor radiotherapy, chemotherapy, gene mutation detection technology has become another popular therapeutic tool. For the majority advanced non-small cell lung cancer (NSCLC) patients, especially who with adenocarcinoma, targeted therapy became increasingly important in the treatment (2). Through a large number of data contrast

tests (3–6), it can be seen that the treatment of molecular mutation has got a certain effect (2). In addition to EGFR, ALK etc. the estrogen receptor is another promising targeting gene (7).

Recently, it has been suggested that estrogen plays an important role in the occurrence, development, and metastasis of lung cancer. In lung cancer, similar to breast cancer, aromatase is a crucial enzyme in estrogen synthesis (7), and enzymatic activity in lung cancer cell lines and normal lung tissue was exhibited (8). Although tobacco smoking remains a significant risk factor for adenocarcinoma, approximately 20% of women with lung cancer are never smokers (9). This phenomenon maybe suggests that estrogen is associated with lung cancer in the other hand.

**Table 1** Main characteristics and results of the eligible studies

First author	Year	Patients source	SCC/ADC/others	Stage	No. of Pts	Method	Cutoff	HR estimation	HR (95% CI)
Aresti	2014	Spain	42/41/13	I-IV	96	RT-PCR/ELISA	NA	HR	0.70 (0.38–1.29)
Monica	2012	Italy	34/57/15	III-IV	106	IHC	NA	HR	1.75 (1.00–3.03)
Navaratnam	2012	Canada	NA	I-IV	79	IHC	>60 score	HR	0.41 (0.21–0.80)
Verma	2012	Japan	36/129/4	I-IV	169	IHC	>10%	HR	0.721 (0.30–0.98)
Karlsson	2011	Sweden	0/68/0	I-V	68	IHC	>30%	HR	0.47 (0.22–1.01)
Mah	2011	American	93/226/58	I-IV	377	IHC	≥3 score	HR	1.6 (0.84–2.09)
Stabile	2011	American	62/103/18	I-IV	183	IHC	≥7 score	HR	1.05 (1.00–1.10)
Mauro	2010	Argentina	18/33/6	I	58	IHC	≥10%	HR	0.57 (0.13–0.80)
Skov	2008	Denmark	56/40/8	I-III	104	IHC	≥10%	HR	0.63 (0.41–0.97)
Schwartz	2005	American	13/231/35	NA	278	IHC	≥10%	HR	1.01 (0.65–1.57)
Kawai	2005	Japan	28/102/2	I-IV	132	IHC	≥5 score	HR	1.49 (0.76–2.93)

SCC, squamous cell carcinoma; ADC, adenocarcinoma; No. of Pts, number of patients; CI, confidence interval; RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme linked immunosorbent assay; HR, hazard ratios; IHC, immunohistochemistry.

In view of the fact that there is an association between ER overexpression and human lung cancer, most studies reported so far are limited in their sample size and discrete outcomes. We therefore carried out a meta-analysis of data from published studies to quantitatively review the effect of ER overexpression in tumor tissue on survival in patients with NSCLC.

## Methods

### Search strategy and study selection

The selected publications were identified by using up-to-date electronic databases, including PubMed, Web of Science, China National Knowledge Infrastructure (CNKI). An upper date limit of August 31, 2015 was applied; we used no lower date limit. Searches included the terms “estrogen receptor”, “ER”, “ER $\beta$ ” and “prognosis”. We also reviewed the Cochrane Library for relevant articles. The references reported in the identified studies were also used to complete the search.

Studies eligible for inclusion in this meta-analysis met the following criteria: (I) measure estrogen receptor  $\beta$  (ER $\beta$ ) expression in the primary lung cancer tissue with immunohistochemistry (IHC) or enzyme linked immunosorbent assay (ELISA)/reverse transcription-polymerase chain reaction (RT-PCR); (II) provide information on survival (studies investigating response rates only were excluded); (III) studies that reported a hazard ratio (HR) and confidence interval (CI) or could be

calculated from the sufficient data; and (IV) when the same author reported results obtained from the same patient population in more than one publication, only the most recent report, or the most complete one, was included in the analysis. Two reviewers (P Zhan and L Ma) independently determined study eligibility. Disagreements were resolved by consensus.

### Data extraction and quality assessment

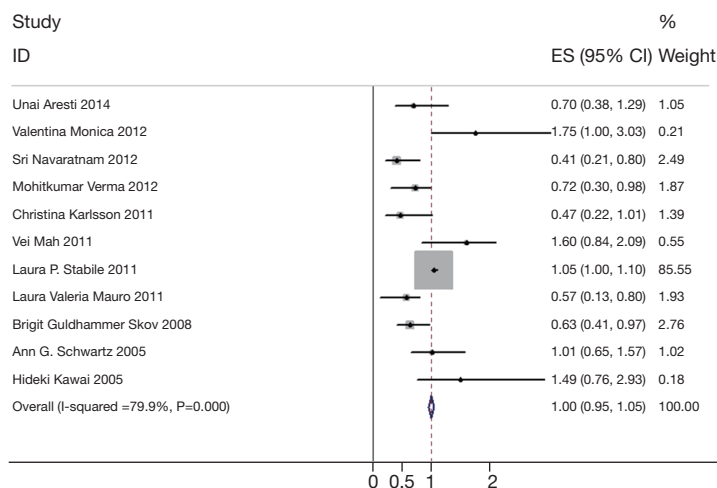
The final articles included were assessed independently by two reviewers (P Zhan and L Ma). Data retrieved from the reports included first author, publication year, patient source, histology, disease stage, test method, cut-off value, and HR with 95% CI (Table 1).

If data from any of the above categories were not reported in the primary study, items were treated as “not applicable”. We did not contact the author of the primary study to request the information.

### Statistical methods

For the quantitative aggregation of the survival results, hazard ratios (HR) and their 95% CIs were combined to give the effective value. When these statistical variables were not given explicitly in an article, they were calculated from available numerical data using methods reported by Parmar *et al.* (10).

Heterogeneity of the individual HRs was calculated with Chi-squared tests according to Peto’s method (11).



**Figure 1** Meta-analysis (forest plot) of the eleven evaluable studies assessing ER $\beta$  expression in NSCLC stratified by ethnic source. ER $\beta$ , estrogen receptor $\beta$ ; NSCLC, non-small cell lung cancer.

Meanwhile, heterogeneity test with  $I^2$  statistic and Q statistic was performed. All the studies included were categorized by patient race, histology, disease stage. Individual meta-analysis was conducted in each subgroup. If HRs were found to have fine homogeneity, a fixed effect model was used for secondary analysis; if not, a random effect model was used. In this meta-analysis, DerSimonian-Laird random effects analysis (12) was used to estimate the effect of estrogen receptor overexpression on survival. By convention, an observed HR  $>1$  implies worse survival for the group with estrogen receptor overexpression. The impact of estrogen receptor on survival was considered to be statistically significant if the 95% CI did not overlap with 1. Horizontal lines represent 95% CIs. Each box represents the HR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (HR =1.0).

Evidence of publication bias was sought using the methods of Egger *et al.* (13) and of Begg *et al.* (14). Moreover, contour-enhanced funnel plot (15) was performed to aid in interpreting the funnel plot. If studies appear to be missing in areas of low statistical significance, then it is possible that the asymmetry is due to publication bias. If studies appear to be missing in areas of high statistical significance, then publication bias is a less likely cause of the funnel asymmetry. Intercept significance was determined by the *t*-test suggested by Egger ( $P<0.05$  was considered

representative of statistically significant publication bias). All calculations were performed using STATA version 11.0 (Stata Corporation, College Station, TX, USA).

## Results

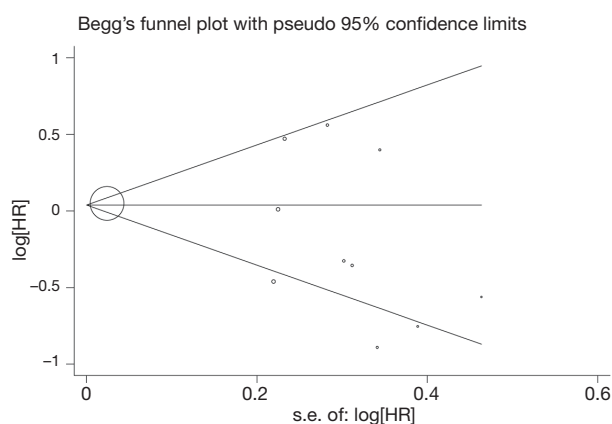
### Study selection and characteristics

Eleven studies (16-26) published between 2005 and 2014 were eligible for this systematic review with meta-analysis. All reported the prognostic value of ER $\beta$  status for survival in NSCLC patients. The total number of patients included was 1,650 ranging from 58 to 377 patients per study (median 218). The major characteristics of the eleven eligible publications are reported in *Table 1*.

These publications followed several different patient cohorts. The NSCLC studies considered either all lung cancer subtypes ( $n=4$ ) and adenocarcinomas ( $n=1$ ). All eleven studies used IHC or RT-PCR/ELISA to evaluate ER $\beta$  expression in NSCLC.

### Meta-analysis

The results of the meta-analysis are reported in *Figure 1*. Overall, the combined HR for all eligible studies evaluated ER $\beta$  expression in NSCLC was 1.000 (95% CI: 0.954–1.047), indicating that ER $\beta$  overexpression was an indicator of negative results for NSCLC patients. Meanwhile, no significant heterogeneity was detected among these studies ( $I^2=79.9\%$ ,  $P=0.000$ ) (*Figure 1*).



**Figure 2** Funnel plot of the eleven evaluable studies assessing ER $\beta$  in NSCLC. ER $\beta$ , estrogen receptor $\beta$ ; NSCLC, non-small cell lung cancer.

### Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All five eligible studies investigating NSCLC patients yielded a Begg's test score of  $P=0.274$  and an Egger's test score of  $P=0.183$ , meanwhile according to the contour-enhanced funnel plot (Figure 2), the absence of publication bias was found in all eleven studies. These results suggest that there is no publication bias.

### Discussion

The development of lung cancer is a complex process, which is regulated by many kinds of cytokines, chemokines, tumor inflammatory microenvironment, vascular normalization, gene pathway and so on. Some studies indicate that ER is playing an important role in this process. In the recent years, with the continuous development of estrogen receptor researches, the understanding of the mechanism of estrogen receptors in cancer is deepening, but the main mechanism is not clear in lung cancer. They exert their functions through two different specific pathways, genomic and non-genomic (27,28), with the same biological effects: proliferation, growth, apoptosis, differentiation and angiogenesis.

The non-genomic pathway is characterized by the rapid initiation of signaling molecules such as MAPK (29), Akt, etc., and the related signal molecules are phosphorylated, which play an important role in the process. Genomic pathway is mainly mediated by classical pathway (ERE) and AP-1 (30), which is composed of the non-classical pathway

mediated by transcription factors (27). All in all, a large number of studies on the mechanism of estrogen receptor action still need to be proved.

Stabile's research proposed that in an in vivo lung tumor xenograft model, the drug combination of gefitinib (an EGFR receptor tyrosine kinase inhibitor) and fulvestrant (an ER antagonist) treatment decreased tumor volume (31). In addition, the clinical trials of estrogen receptor antagonists in the treatment of lung cancer are also in the exploration (32-35). This result above all suggested that ER $\beta$  could play a more important role in biological behavior of NSCLC (36). In contrast, in the present meta-analysis, we have combined eleven published studies including 3,300 patients with NSCLC to yield summary statistics that indicate that ER $\beta$  overexpression has a significant correlation with negative result of survival in NSCLC patients.

Although we performed a meta-analysis of the overall survival and ER $\beta$  expression, there were several limitations that should be considered. First, different countries' studies were included in this analysis, which may cause language bias. Second, the risks calculated in our meta-analysis may be an overestimate due to publication bias. Some eligible studies were excluded from this meta-analysis because they lacked sufficient data on survival; negative or small-sample studies may be less likely to be published. Third, the method used to extrapolate the HR was different, which was extracted from the data included directly or which was calculated from the survival curves. Fourth, it could be explained by the fact that differences in antibodies used for staining, different criteria for defining stain positivity, and different patient demographics such as tumor stage, histological subtype, and adjuvant treatment. Nevertheless, no publication bias was detected using Begg's test, indicating that the statistics obtained approximate the actual results. All that analyses suggest that our results were statistically reliable.

At first, the ER is deeply researched in breast cancer. Selective estrogen receptor modulators (SERMs) are ER ligands that in some tissues (i.e., bone and cardiovascular system) act like estrogens but block estrogen action in others (37). Tamoxifen and raloxifene have emerged effect on breast cancer treatments. Some studies have examined how estrogen signaling can influence NSCLC growth and how interactions with growth factors signaling pathways promote lung cancer. Further, new findings are presented to show that selective targeting of these complexes, intersecting pathways can effectively inhibit the growth of human NSCLC. Sequential or combined use of ER

antagonists (fulvestrant or tamoxifen) and EGFR TKIs can block the growth of NSCLC tumor (38). These results also confirmed that estrogen receptors are associated with lung cancer.

In the recent years, scholars have been concerned about the status of estrogen receptor in lung cancer, and they are trying to understand the relationship between the occurrence, development and prognosis of lung cancer. They would like to find a new way for the treatment of lung cancer. But there are a lot of differences between domestic and foreign literature reports, and even contradictory results. So far, the intrinsic link between ER and lung cancer, and the exact mechanism are not clear yet. In the future, large prospective studies are needed to confirm the clinical utility of ER $\beta$  as an independent prognostic marker.

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### Footnote

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

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