Correlation between epidermal growth factor receptor mutations and nuclear expression of female hormone receptors in non-small cell lung cancer: a meta-analysis

Qihua He[1,2], Mingzhe Zhang[3], Jianrong Zhang[1,2], Ying Chen[1,2], Jiaxi He[1,2], Jianfei Shen[1,2], Yang Liu[1,2], Shengyi Zhong[1,2], Long Jiang[1,2], Chenglin Yang[1,2], Yuan Zeng[1,2], Minzhang Guo[1,2], Xuewei Chen[1,2], Jianxing He[1,2], Wenhua Liang[1,2]

[1]Department of Thoracic Surgery and Oncology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease & National Clinical Research Center for Respiratory Disease, Guangzhou 510120, China; [3]Department of Cardiology, Xiamen Cardiovascular Hospital, Xiamen 361004, China

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Correspondence to: Wenhua Liang and Jianxing He. Department of Thoracic Surgery and Oncology, the First Affiliated Hospital of Guangzhou Medical University; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: liangwh@gird.cn; drjianxing.he@gmail.com.

Background: Compared with male, female non-small cell lung cancer (NSCLC) patients have better response when treated with epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs), suggesting a potential association between female hormones and EGFR mutation. However, the results provided by previous studies were inconclusive and controversial. We sought to examine the link between the expression of nuclear female hormone receptors and EGFR mutations in NSCLC.

Methods: Electronic databases were used to search the relevant articles. The involved hormone receptors included estrogen receptor (ER) and progesterone receptor (PR). The primary endpoint was the occurrence of ER/PR expression and EGFR mutation in NSCLC patients.

Results: Five studies fulfilled the criteria and were included in our analysis. Patients with high ER-β expression had higher positive EGFR mutation than low ER-β patients (44.2% *vs*. 23.7%), and there was a significant difference between the two groups [odds radio (OR) 3.44, 95% confidence interval (CI): 2.40-4.93, Z=6.72, P<0.001]. However, there is no significant correlation between EGFR mutations and ER-α (when included ER-α3, OR 1.20, 95% CI: 0.62-2.33, Z=0.55, P=0.58; and when included ER-α4, OR 1.18, 95% CI: 0.62-2.25, Z=0.51, P=0.61) or PR (OR 1.29, 95% CI: 0.40-4.10, Z=0.43, P=0.67). No significant publication bias was observed.

Conclusions: High nuclear expression of ER-β, but not ER-α or PR is correlated with EGFR mutations in NSCLC. The underlying mechanism and potential translational relevance warrant further investigation.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR) mutation; estrogen receptor (ER); progesterone receptor (PR); meta-analysis

Introduction

Lung cancer, predominantly non-small cell lung cancer (NSCLC), is the leading cause of cancer-related mortality in males and the second cause of cancer-related mortality in females (1). Despite smoking is the predominant risk factor for lung cancer, tobacco can explain only 75% of its incidence (2). A large proportion of lung cancer patients are never-smokers and this phenomenon is more frequent occurred in females and the adenocarcinoma cell type (3).

Extensive reports confirmed that estrogen not only impacts normal lung cell differentiation and maturation, but also affect the growth of lung cancer. It has been reported that estrogen receptor α (ER-α) expressed in the nucleus (0-45%) and cytoplasm (0-73%) of malignant lung cancer cells (4-7). And estrogen receptor β (ER-β) seems to be more coincident that it express only in nuclear in 46% to 60% of NSCLC cases (4-9). Estrogen not only stimulates the transcription of estrogen-responsive genes of lung cells directly but also transactivates the epidermal growth factor receptor (EGFR) pathway (7).

Compared with male, female NSCLC patients respond better when treated with EGFR inhibitors such as gefitinib. EGFR mutation constitutes a larger proportion of lung cancers in female than male, in East Asians than other ethnic and associated with a longer period of fertility (10). EGFR mutation occurs more frequently in female patients and it is the hormone that distinguishes male and female. It seems that a potential connection between female hormone and EGFR mutation. Some studies implied that estrogen interacts with the development of lung cancer. A post hoc analysis suggested that the lung cancer patients may increase the risk of death when treat with hormone replacement therapy (11). Postoperative breast cancer patients treat with tamoxifen will increase the risk of lung cancer (12). In addition, data from the present study showing that EGFR protein expression is down-regulated in response to estrogens and up-regulated in response to antiestrogens (13). In clinic, we observed that significant rash and masculinization occur in patients who had good responds to EGFR-tyrosine kinase inhibitor (TKI). We hypothesize that there is an interaction between estrogen level and EGFR mutated tumors. These studies suggest that ER pathway might be associated with the EGFR pathway.

ER and progesterone receptor (PR) played a pivotal role in estrogen pathway. Previous studies provided controversial results and were limited by small sample size. A more comprehensive analysis was warranted. Therefore, we performed this meta-analysis to synthesize current evidence.

Materials and methods

Literature search

All relevant articles were retrieved by searching PubMed, Embase and the Central Registry of Controlled Trials of the Cochrane Library using a combination of the terms: "ER" or "estrogen receptor" or "PR" or "progesterone receptor" and "lung". No restriction by language or year was set in the search. The last research time was October 23, 2014.

Data collection

Eligible studies should meet the following criteria: (I) studies which evaluated the association between nuclear ER or PR and EGFR mutation in NSCLC; (II) studies published in English regardless of publication time; (III) the original papers containing enough data. Studies failed to meet the inclusion criteria will be excluded.

Statistical analysis

The results were reported as pooled odds radios (ORs) with the corresponding 95% confidence interval (CI). The pooled OR and its 95% CIs were calculated by the methods proposed by Mantel and Haenszel. Statistical heterogeneity across studies was assessed with a forest plot and the inconsistency statistic (I^2). In consideration of any potential heterogeneity, we conducted this meta-analysis with a random-effect model in order to avoid any potential heterogeneity. Subgroup and sensitivity analysis were stratified for predisposed factors when available. Statistical significance was considered at P<0.05. All calculations were performed using Review Manager (version 5.0 for Windows; the Cochrane Collaboration, Oxford, UK)

Publication bias

An extensive search strategy was made to minimize the potential for publication bias. Graphical funnel plots were generated to visually assess a publication bias (14). The statistical methods to detect funnel plot asymmetry were the rank correlation test of Begg and Mazumdar and the regression asymmetry test of Egger (14,15).
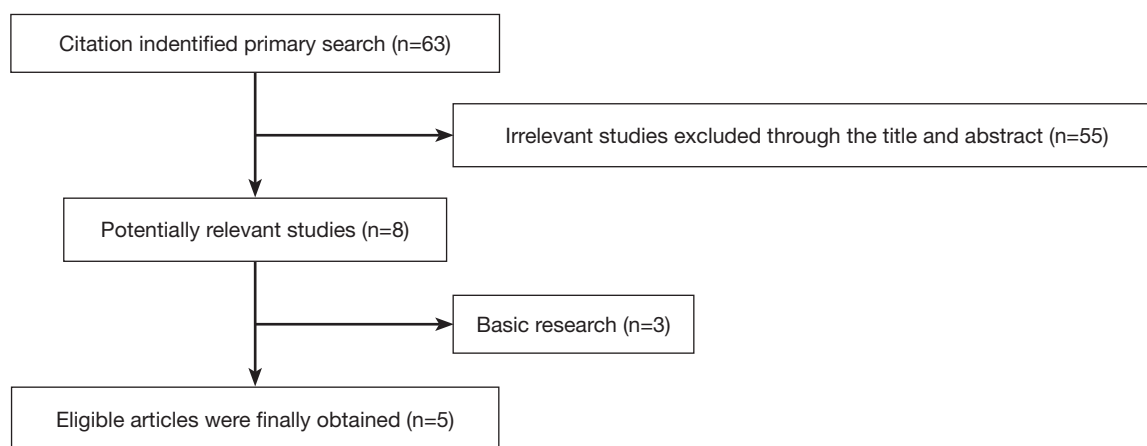
Figure 1 Profile summarizing the trial flow.

Results

Eligible studies

We identified 63 potentially relevant records through the search strategy. Fifty-five studies was excluded after checking the title and abstract, for it was very clear that their research contents didn't meet our inclusion criteria. Then the full texts of eight articles were carefully screened, three studies were excluded as basic research. At last, a total of five studies were eligible for the final analysis. *Figure 1* summarized the flow chart. Among these studies, the relationship between ER-α and EGFR mutation were estimated in five studies, and there are four results for ER-α in Raso's article (including ER-α3 and ER-α4 respectively expression in nucleus and cytoplasm) only ER-α3 and ER-α4 expression in nucleus were kept in the results. And there are three studies estimated the relationship between ER-β and EGFR. *Table 1* summarized the characteristics of all involved studies.

Relationship between EGFR mutation and expression of ER and PR

According to all studies with useful data, patients with high ER-β had positive EGFR mutation than low ER-β patients (44.2% *vs.* 23.7%), and there was a significant difference between the two groups (OR 3.44, 95% CI: 2.40-4.93, test for overall effect: Z=6.72, P<0.001; heterogeneity: χ^2=0.62, P=0.73, I^2=0%; *Figure 2*). No significant difference of EGFR mutation can be found between patients with high and low PR expression (OR 1.29, 95% CI: 0.40-4.10, test for overall effect: Z=0.43, P=0.67; heterogeneity: χ^2=3.12,

P=0.08, I^2=68%). Similar results were observed in the analysis of high ER-α and EGFR mutation (here are two results for ER-α in Raso's article, when included ER-α3, OR 1.20, 95% CI: 0.62-2.33, test for overall effect: Z=0.55, P=0.58; heterogeneity: χ^2=14.92, P=0.005, I^2=73%; and when included ER-α4, OR 1.18, 95% CI: 0.62-2.25, test for overall effect: Z=0.51, P=0.61; heterogeneity: χ^2=15.47, P=0.004, I^2=74%). No significant publication bias was observed by graph (*Figure 3*) or by Begg's or Egger's tests.

Discussion

The association of ER and PR expression with the EGFR mutation was controversial based on previous small-size reports. A meta-analysis is needed to incorporate all available results in order to give further insights on this conflicting issue. In the current literature, we found that high ER-β and PR expression was significantly higher, while ER-α to be high expression but the difference did not to be significance.

Adenocarcinoma is more likely to develop in women and young patients, which directs to the importance of estrogen in lung cancer. The preceding studies reported that an interaction of EGFR mutation and ER expression (including ER-α and nuclear ER-β), suggesting that some shared signaling pathway may exist between them (13). As acknowledged, there are several mechanisms to activate EGFR in lung cancer cells, including increased ligands and/or receptor, and mutation of the EGFR tyrosine kinase domain (2). It has been reported that the EGFR and mitogen-activated protein kinase 1 growth pathways will be activated when the rapidly released EGFR ligands in

Table 1 Characteristics of eligible studies evaluating female hormone receptors level and EGFR mutation status

| Lead author, year (references)	No. of patients	Gender (male/female)	Receptor	Expression	Evaluation ER	Evaluation EGFR	Antibody	EGFR+ ER high/EGFR− ER high	EGFR+ ER low/EGFR− ER low	EGFR+ PR high/EGFR− PR high	EGFR+ PR low/EGFR− PR low
Sun, 2011 (10)	316	210/106	ER-α	Nucleus	Petcentage	PCR tional analysis	Anti-ER-antibody (HC-20, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-PR antibody (SP2, Lab Vision Corporation, Fremont, CA)	84/30	162/40	120/22	126/48
Nose, 2009 (16)	447	280/167	ER-α	Cytoplasm	Score	Sequence	Rabbit polyclonal anti-ERα antibody (Santa Cruz Biotechnology, Santa Cruz, CA)	75/146	94/132		
			ER-β	Nucleus			Rabbit polyclonal anti-ERβ antibody (Santa Cruz Biotechnology)	113/104	56/174		
Raso, 2009 (17)	174	150/163	ERα-3	Nucleus	Score	PCR	Estrogen receptor α-3, clone HC20, Santa Cruz Biotechnology, Inc.	7/9	21/137		
				Cytoplasm				21/68	6/78		
			ERα-4	Nucleus			Estrogen receptor α-4, clone 1D5, Lab Vision Corporation	18/50	9/93		
				Cytoplasm				13/20	14/123		
			ERβ-1	Nucleus			Estrogen receptor β-1, clone H150, Santa Cruz Biotechnology	22/70	5/75		
Toh, 2010 (18)	93	65/44	ER-α		Score	Chromatography	Mouse antihuman ER-α (1D5; 1:500 dilution)	4/8	33/48	31/50	6/6
			ER-β				Monoclonal mouse antihuman ER-β (PPG5/10; 1:100) from Dako	6/4	33/54		
Shimizu, 2012 (19)	97	23/28	ER-α	Membrane	Score	PCR	Clone HC-20, Santa Cruz Biotechnology, Santa Cruz, CA, 1/500 dilution	15/9	11/16		
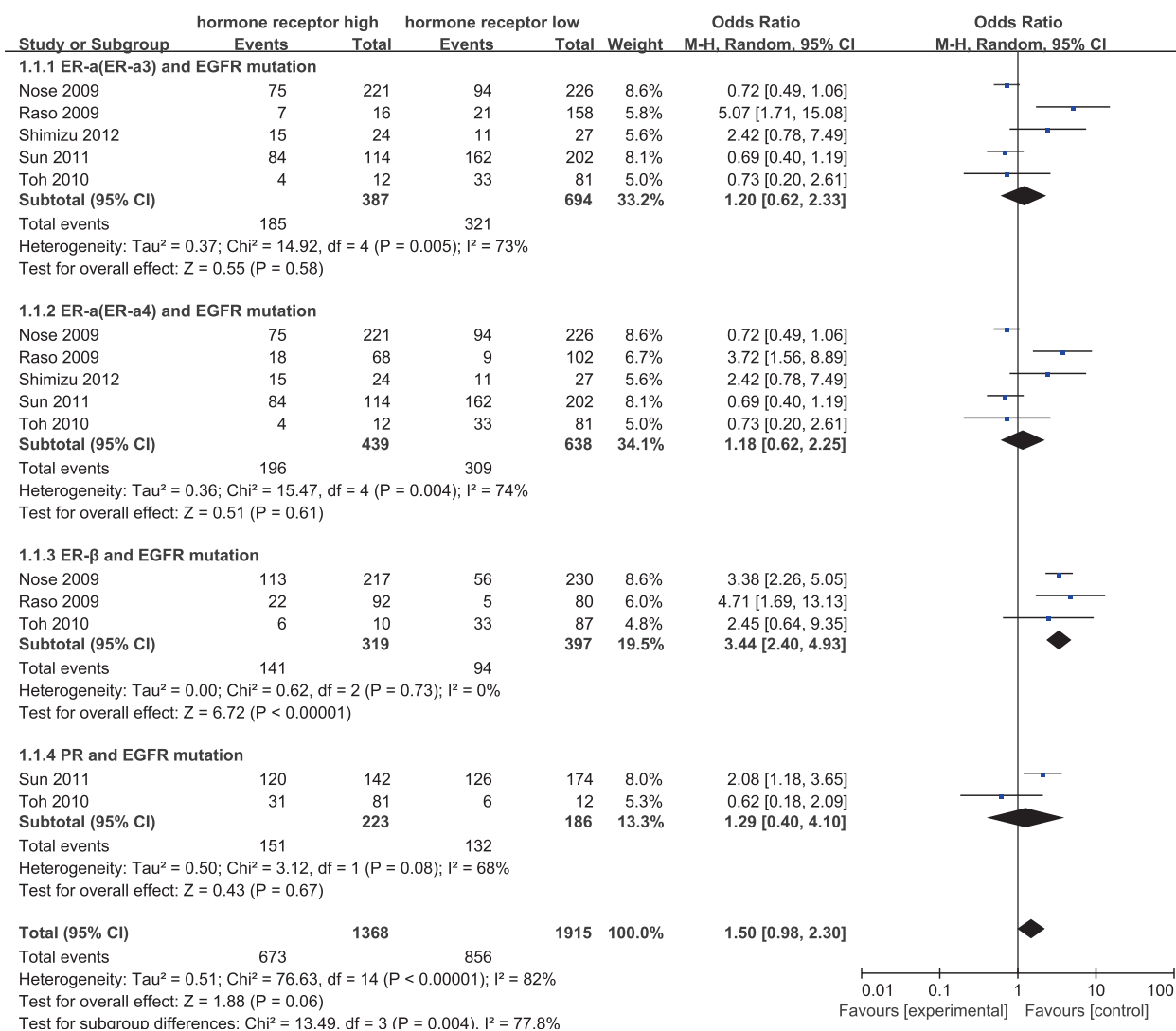
Figure 2 Meta-analysis on female hormone receptor expression and EGFR mutation status. CI, confidence interval; ER, estrogen receptor; EGFR, epidermal growth factor receptor; I², inconsistency statistic; PR, progesterone receptor.

estrogen stimulation of lung cancer cells (13). Therefore, estrogenic signaling increased growth that depends on the EGFR pathway is rational.

In NSCLC cells, aromatase produces estrogen which stimulates the ER signaling pathway leading the development and progression of the tumor (13,20-24). It's worth noting that the concentration of estradiol in NSCLC cells has a positively relationship with aromatase mRNA expression. Kohno and colleagues reported that cell line which has an EGFR mutation with a high aromatase mRNA expression was more sensitive to exemestane alone and cell

growth was more significantly inhibited by the combination of exemestane and erlotinib than which has high aromatase expressions without EGFR mutations (20).

The previous studies have reported that estrogen down modulator could enhance antitumor activity in NSCLCs no matter alone or combined with EGFR-TKI. In our current study, high ER-α expression with EGFR mutation has no significant statistical difference with the control group. The reasons may be as follow. Firstly, different criterion exists in different studies: (I) antibodies and dilutions; (II) the subcellular location of ER-α; (III) scoring systems for
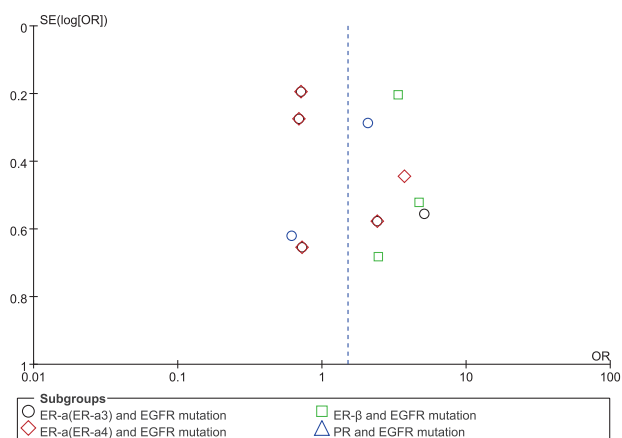
Figure 3 Funnel plot of all measures. OR, odds radio; ER, estrogen receptor; EGFR, epidermal growth factor receptor; PR, progesterone receptor.

staining. According to the report, ER-β only affect the lung adenocarcinomas with EGFR mutation suggesting that hormonal and EGFR pathways have a synergy in the progression of lung adenocarcinoma. In our study, the outcome of PR was controversial. It may associate with the small sample sizes. It was reported that PR was associated with better clinicopathologic features. For another, although ER-α showed 95% homologous identity to ER-β, immunohistochemical results show that ER-α mainly located in cytoplasm, while ER-β mainly located in nucleus in lung cancer cell and study showed that estrogen induce the proliferation mainly by ER-β.

This is the first study to comprehensively answer the interaction between EGFR mutation and ER. However, there are several limitations. First, exons identified as mutants were heterogeneous among included articles but we were unable to assess whether 19 or 21 exon alterations. In addition, we cannot assessment the progression-free survival (PFS) or overall survival (OS) for patients which have an EGFR mutation with a high estrogen expression.

In conclusion, high ER-β expression is correlated with EGFR mutations in NSCLC. The underlying mechanism and potential translational relevance warrant further investigation.

Acknowledgements

Footnote

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

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