High Expression of Bcl-2 Protein Predicts Favorable Outcome in Non-small Cell Lung Cancer: Evidence from a Systematic Review and Meta-analysis

Xian-Da Zhao1&*, Yu-Yu He1&, Jun Gao2, Chen Zhao1, Ling-Lin Zhang1, Jing-Yuan Tian1, Hong-Lei Chen1*

Abstract

Background: The prognostic value of Bcl-2 protein expression in non-small cell lung cancer (NSCLC) is under debate. We therefore systematically reviewed the evidence for Bcl-2 protein effects on NSCLC survival to elucidate this issue. Materials and Methods: An electronic search in Pubmed and Embase complemented by manual searches in article references were conducted to identify eligible studies to evaluate the association between Bcl-2 protein expression and overall survival (OS) as well as disease free survival (DFS) of NSCLC patients. Combined hazard ratios (HRs) with corresponding 95% confidence intervals (95%CIs) were pooled using the random-effects model. Results: A total of 50 trials (including 52 cohorts) encompassing 7,765 patients were pooled in the meta-analysis regarding Bcl-2 expression and OS of NSCLC patients. High expression of Bcl-2 protein had a favorable impact (HR=0.76, 95% CI=0.67-0.86). In the group of Bcl-2 expression and DFS, 11 studies including 2,634 patients were included. The synthesized result indicated high expression of Bcl-2 protein might predict good DFS (HR=0.85, 95% CI=0.75-0.95). Conclusions: Our present meta-analysis demonstrated favorable prognostic values of Bcl-2 expression in patients with NSCLC. Further prospective trials are welcomed to validate the utility of assessing Bcl-2 in NSCLC patient management.

Keywords: Bcl-2 - non-small cell lung cancer - prognosis - survival - systematic review - meta-analysis

Introduction

Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer death in males around the global and its incidence is steadily increasing in females (Jemal et al., 2011). Despite diagnostic and therapeutic improvements, the survival of lung cancer patients is still severe, with only 15% patients in US survive more than five years after diagnosis (Dela Cruz et al., 2011; Kumar, 2012). Among the pathological classification spectrum, the non-small cell lung cancer (NSCLC) which typically includes lung adenocarcinoma, lung squamous cell carcinoma and large cell lung carcinoma occupies more than 70% of new incidences (Collins et al., 2007). Investigating on the diagnosis, treatment and management of NSCLC, therefore, is a steady hot-spot that will relieve the harmess of NSCLC.

Prognostic markers are biological markers or molecular biomarkers which are used alone or in combination to predict clinical outcomes at the time of diagnosis. To generate useful prognostic markers for NSCLC, one way is analyzing basic clinicopathological features by basic laboratory methods (Kaya et al., 2013) and another way is characterization of proteins and genes involve in tumor initiation and progression process at molecular level (Coate et al., 2009). Base on biological pathways, NSCLC prognostic markers are divided into several categories, including oncogenes or proto-oncogenes (e.g. RAS), tumor suppressor genes (e.g. P53, BRCA1, RRM1, ERCC1), markers of over-proliferation (e.g. EGFR) and markers of aggressive characteristics, such as angiogenesis (e.g. VEGF) (Mitsudomi et al., 2000; Mascaux et al., 2005; Brennes et al., 2006; Rosell et al., 2007; Zheng et al., 2007; Coate et al., 2009; Pirker et al., 2012). Even though these prognostic markers are identified, their effects remain controversial in different cohorts and clinical utilities are rather limited. More prognostic markers evaluation will be helpful in promoting the translation of laboratory findings to clinical practices, strengthening and optimizing current personalized treatment strategies and thus is a dynamic research subject in NSCLC.

Apoptosis is a pathway in which cells activate enzymes that degrade the cells’ own nuclear and cytoplasm to eliminate cells that are no longer needed and genetically altered or injured beyond repair, such as cancer cells. Apoptosis results from the activation of either the...
death receptor (extrinsic) pathway or the mitochondrial (intrinsic) pathway. The powers of death antagonists (e.g. Bcl-2, Bcl-XL, Bcl-W, Mcl-1) and agonists (e.g. Bax, Bak, Bcl-Xs, Bad, Bid) determine the fate of cells (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). The Bcl-2 proto-oncogene is a 230kb gene that is originally discovered in a follicular B-cell lymphoma and now confirmed in various tumors. Its product, Bcl-2 protein, is located in the inner mitochondrial membrane and inhibits apoptosis to prolong cell survival by arresting cells in the G0/G1 phase of the cell cycle (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). Envision of apoptosis is one of the most obvious hallmarks of cancers (Kumar, 2012). In several kinds of tumors, the biological functions of Bcl-2 protein have been linked with protecting tumor cells from apoptosis and drug induced death (Zhang and Zhang, 2013). As the consequence, Bcl-2 protein was evaluated in various cancers to investigate their prognostic and predictive significances, including NSCLC (Gascoyne et al., 1997; Anagnostou et al., 2010; Abd El-Hafez et al., 2013).

Although a large number of studies regarding Bcl-2 expression in predicting the survival of NSCLC patients emerged, its definite role remained controversial (Anagnostou et al., 2010; Graziano et al., 2010; Gao et al., 2012). To reconcile the contradiction, a systematic review and meta-analysis that synthesizes current original trials are urgently needed. In present study, we performed this work to assess the prognostic values of Bcl-2 expression in NSCLC in an objective and impartial way.

Materials and Methods

Publication search strategy and selection criteria

Here, we reported the study following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). To be eligible for inclusion, original studies must meet the following criteria: (i) hazard ratio (HR) and 95% confidence interval (CI) for overall survival (OS) or disease free survival (DFS) of NSCLC patients according to Bcl-2 expression (protein, DNA or RNA) dichotomic status (i.e. Bcl-2 positive vs Bcl-2 negative) either was reported or could be computed from the data presented; (ii) dealt with primary NSCLC only (not in metastatic tissue or tissue adjacent to tumor) and included more than 40 patients; (iii) when more than one study was confirmed to report results obtained from the same patient cohort, only the most informative one was included; (iv) full-length papers in English.

An electronic search on Pubmed and Embase, using the strategy in Table 1, complemented by manual search in articles identified by electronic search, was conducted to select the original studies. The search conducted on October 3rd, 2013, and no chronological search criteria were used. Moreover, a review of European and American “grey literature” databases (National Technical Information Service and System for Information on Grey Literature in Europe) was conducted as well. The eligibility assessment was performed by two authors (XD Zhao and YY He) independently via two steps. The reviewers firstly screened titles and abstracts to determine possible eligible studies and then read the text for further validation. All reviewers were trained under the same standard and practiced using five articles for calibration. Disagreements between the two authors were resolved by consensus.

Data extraction

Data extraction was conducted by three authors (XD Zhao, YY He and HL Chen) via carefully reading the full-texts. The three authors got agreement via discussion when the extracted data were not uniform with each author. The extracted information included: first author’s name; country; year of publication; inclusion and exclusion criteria; number of patient; age; gender; NSCLC stage; detection method; primary antibody information; cut-off value of dichotomous status; Bcl-2 positive rate; follow-up interval; survival data analysis method and HR and 95%CI of OS and DFS.

Methodological Assessments

To assess methodology, three investigators (XD Zhao, YY He and HL Chen) read each publication and scored them independently according to Newcastle-Ottawa scale (NOS) (Wells GA SB, 2000). Each item of the NOS scoring system was assessed using an ordinal scale (possible values 2, 1, 0 for item 5 and 1, 0 for other items of the NOS evaluation system). Final scores were reached in a meeting attended by all three evaluators via a consulting manner. Along with evaluation of original trials, the systematic review and meta-analysis itself was assessed. The widely used “Assessment of Multiple Systematic Reviews” (AMSTAR) checklist was performed for evaluating the current research (Shea et al., 2007).

Statistical analysis

In this systematic review and meta-analysis, a study was classed as “positive (+)” when high Bcl-2 expression level was a favorable OS/DFS predictor. Other situations, Table 1. Search Strategy (up to October 3rd, 2013)}

<table>
<thead>
<tr>
<th>Search step</th>
<th>Search terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Bcl-2[Title/Abstract]</td>
</tr>
<tr>
<td>#2</td>
<td>Bcl2[Title/Abstract]</td>
</tr>
<tr>
<td>#3</td>
<td>Bcl 2[Title/Abstract]</td>
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<td>B-cell leukemia-2[Title/Abstract]</td>
</tr>
<tr>
<td>#5</td>
<td>B-cell lymphoma-2[Title/Abstract]</td>
</tr>
<tr>
<td>#6 OR #7 OR #8 OR #9 OR #10 OR #11</td>
<td>#6 OR #7 OR #8 OR #9 OR #10 OR #11</td>
</tr>
<tr>
<td>#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23</td>
<td>#24 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 #25=#6 AND #12 AND #24</td>
</tr>
</tbody>
</table>

| #13 | NSCLC[Title/Abstract] |
| #14 | NSCLCs[Title/Abstract] |
| #15 | Non-Small-Cell Lung Carcinoma*[Title/Abstract] |
| #16 | Non-Small-Cell Lung Cancer*[Title/Abstract] |
| #17 | Non Small Cell Lung Carcinoma*[Title/Abstract] |
| #18 | Non Small Cell Lung Cancer*[Title/Abstract] |
| #19 | Nonsmall Cell Lung Carcinoma*[Title/Abstract] |
| #20 | Nonsmall Cell Lung Cancer*[Title/Abstract] |
| #21 | Lung Adenocarcinoma[Title/Abstract] |
| #22 | Lung Squamous cell carcinoma[Title/Abstract] |
| #23 | Large Cell Lung Carcinoma[Title/Abstract] |

including the situation where high Bcl-2 expression level predicted poor OS/DFS or failed to predict OS/DFS were called “negative (-)”. For the quantitative aggregation of the survival results, HRs and corresponding 95% CIs were used. We extracted the HR and 95% CI from each trial based on the results provided in the publication. We preferred to include data from multivariable Cox hazard regression analysis if available as they were most accurate. Otherwise, we extracted data from univariate analysis instead. In cases where HRs and 95% CIs (Bcl-2 positive group vs Bcl-2 negative group) were not directly reported, we estimated them via loge hazard ratio (logHR) and standard error (SE) (logHR) and survival curves using the methods developed by Parmar et al. (1998), Williamson et al. (2002) and Tierney et al. (2007). The software used for calculating these values was designed by Tierney and his colleagues, published in Trials, 2007 (Tierney et al., 2007). If not available, we tried to connect with authors for unreported data.

Meta-analysis was performed using STATA 12.0 (Stata Corporation, College Station, TX, USA). In order to choose correct statistical model to summarize effect sizes of selected studies, we combined the consideration of heterogeneity testing (p-value and I²-value), the differences between original trials and premises of statistic models (Borenstein M, 2009). When homogeneity testing showed significant heterogeneity (p<0.1 and I²>50%), random-effect model was chosen. Considering the obvious differences among original trials, such as different regions, disease stages, primary antibodies and inconsonant cut-off points, we preferred the random-effect model even homogeneity testing was not significant (p≥0.1 or I²≤50%). Forest plots were used to illustrate the HR and corresponding 95% CI of each included study and the synthesized results. An observed HR<1 indicated a better outcome for the high expression group and was considered statistically significant if corresponding 95% CI did not overlap 1. For subgroup analysis, five stratifying variables including publication year, cohort region, number of patients, disease stage and quality score were selected based on our review of original studies. We didn’t use more than “study number/10” stratifying variables to avoid excessive data mining. Publication bias was assessed by visually evaluating the symmetry of funnel plot and formally with the Begg’s tests, p>0.05 indicated no potential publication bias. Sensitivity analysis was performed by extraction of each single study to investigate the stability of the results.

Results

Study selection and quality assessment

Through the database search, a total of 542 articles were identified for initial evaluation after removing duplicates (Figure 1). Among the first round excluded articles, 360 articles belong to one of the following: basic studies in cell lines or animal models; review articles; articles not in English and abstracts. In the second round, 128 articles were excluded because of sample size lower than 40 patients and failing to provide enough survival data for extracting HRs and corresponding 95% CIs. After the two rounds of exclusion, 54 articles evaluating the prognostic value for OS or DFS of Bcl-2 levels in NSCLC were remained for further quality assessment in detail and data pooling.

The main characteristics of the 54 studies (56 independent cohorts) eligible for the systematic review and meta-analysis were showed in Table 2. A total of 31 studies reported HRs and corresponding 95% CIs. In the remaining 23 studies, we evaluated their HRs and corresponding 95% CIs based on reported data. The total study sample size of 54 studies was 8522 with a mean of 158 (range, 45-535 patients). 23 cohorts (Pezzella et al., 1993; Fontanini et al., 1995; Fontanini et al., 1996; O’Neill et al., 1996; Apolinario et al., 1997; Koukourakis et al., 1997; Pastorino et al., 1997; Fontanini et al., 1998; Cox et al., 2000; Moldvay et al., 2000; Nguyen et al., 2000; Cox et al., 2001; Laudanski et al., 2001; Rigau et al., 2002; Swinson et al., 2002; Grossi et al., 2003; Swinson et al., 2004; Fokkema et al., 2006; Yaren et al., 2006; Ludovini et al., 2008; Anagnostou et al., 2010; Griminger et al., 2010; Karpathiou et al., 2013) evaluated patients from Europe, 12 cohorts (Ritter et al., 1995; Anton et al., 1997; Kwiatkowski et al., 1998; D’Amico et al., 1999; Mehdi et al., 1999; Carvalho et al., 2000; Han et al., 2002; Huang et al., 2003; Poleri et al., 2003; Renouf et al., 2009; Anagnostou et al., 2010; Graziano et al., 2010) from America and 21 cohorts (Ohsaki et al., 1996; Higashiyama et al., 1997; Ishida et al., 1997; Kim et al., 1998; Dosaka-Akita et al., 1999; Huang et al., 1999; Hwang et al., 2001; Hanaoka et al., 2002; Lai et al., 2002; Tomita et al., 2003; Shibata et al., 2004; Yoo et al., 2007; Liu et al., 2008; Lee et al., 2009; Ma, 2009; Shim et al., 2009; Zhu et al., 2009; Shi et al., 2011; Gao et al., 2012; Ko et al., 2013) from Asia. Methodological score of each selected study using NOS evaluation system was listed in Table 2. NOS scores of 1-3, 4-6 and 7-9 were defined as low, intermediate and high quality studies, respectively. All the included 54 studies had a median overall score of 7 (range 5 to 9), indicating the high quality of included original studies. The AMSTAR evaluation system was

![Figure 1. PRIMA Flow Diagram of Study Inclusion](image)
performed to assess the quality of this systematic review and meta-analysis and our research fulfilled more than 9 of 11 items (except item 1 and 5) in AMSTAR evaluation system, indicating a good quality was reached.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Region</th>
<th>Sample Number</th>
<th>Stage III&amp;IV%</th>
<th>Follow-up Range (Month)</th>
<th>Follow-up Median (Month)</th>
<th>Method</th>
<th>Primary Antibody (Dilution)</th>
<th>Cut-off Value</th>
<th>Positive Rate%</th>
<th>Conclusion Score</th>
<th>Quality Score</th>
</tr>
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<td>UK</td>
<td>115</td>
<td>0</td>
<td>1.7-76</td>
<td>34</td>
<td>IHC</td>
<td>Clone100</td>
<td>PPC</td>
<td>21.7 + 8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Fontanini-1</td>
<td>1995</td>
<td>Italy</td>
<td>89</td>
<td>0.2-41</td>
<td>25%</td>
<td>IHC</td>
<td>Clone124 (1:20)</td>
<td>PPC</td>
<td>1%</td>
<td>67 + 7</td>
<td></td>
<td>5</td>
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<td>USA</td>
<td>126</td>
<td>1-91</td>
<td>39</td>
<td>IHC</td>
<td>Clone124 (1:80)</td>
<td>PPC</td>
<td>5%</td>
<td>37 - 6</td>
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<td>6</td>
</tr>
<tr>
<td>A.J.O’neill</td>
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<td>Ireland</td>
<td>66</td>
<td>1-47</td>
<td>NR</td>
<td>IHC</td>
<td>Clone124 (1:50)</td>
<td>PPC</td>
<td>1%</td>
<td>25* - 7</td>
<td></td>
<td>7</td>
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<tr>
<td>Obiashi</td>
<td>1996</td>
<td>Japan</td>
<td>96</td>
<td>53.5%</td>
<td>NR</td>
<td>IHC</td>
<td>Clone124 (1:60)</td>
<td>PPC</td>
<td>20%</td>
<td>19.2* + 8</td>
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<td>8</td>
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<td>32.5%</td>
<td>32.5% (Mean)</td>
<td>IHC</td>
<td>Clone124 (1:20)</td>
<td>PPC</td>
<td>1%</td>
<td>60 + 7</td>
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<tr>
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<td>1997</td>
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<td>21.1</td>
<td>3-80</td>
<td>IHC</td>
<td>Clone124 (1:50)</td>
<td>PPC</td>
<td>10%</td>
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<td>1.75%</td>
<td>IHC</td>
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<td>15%</td>
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<tr>
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<td>45</td>
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<td>Clone100 (1:25)</td>
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<td>IHC</td>
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<tr>
<td>Anton</td>
<td>1997</td>
<td>USA</td>
<td>427</td>
<td>18.9</td>
<td>3-185</td>
<td>IHC</td>
<td>Clone124 (1:60)</td>
<td>PPC</td>
<td>46.8 - 5</td>
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<td>7</td>
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<td>Italy</td>
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<td>NR</td>
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<td>IHC</td>
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<td>21.7 + 8</td>
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<td>32</td>
<td>18.2-65.4</td>
<td>IHC</td>
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<td>PPC</td>
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<td>IHC</td>
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<td>PPC</td>
<td>34 + 7</td>
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<td>Mehdi</td>
<td>1999</td>
<td>USA</td>
<td>241</td>
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<td>PPC</td>
<td>34 + 7</td>
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<tr>
<td>D’Amico</td>
<td>1999</td>
<td>USA</td>
<td>408</td>
<td>UB&gt;60</td>
<td>NR</td>
<td>IHC</td>
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<td>PPC</td>
<td>20%</td>
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<td>Cox-1</td>
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<td>23.6</td>
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<td>PPC</td>
<td>20%</td>
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<td>France</td>
<td>227</td>
<td>44.1</td>
<td>1-80</td>
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<td>PPC</td>
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<td>Brazil</td>
<td>45</td>
<td>44</td>
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<td>NR</td>
<td>IHC</td>
<td>Clone124 (1:50)</td>
<td>PPC</td>
<td>34 + 7</td>
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<td>18.9</td>
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<td>Poland</td>
<td>100</td>
<td>52.9%</td>
<td>6.9-42.7</td>
<td>IHC</td>
<td>Clone124 (1:100)</td>
<td>PPC</td>
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<td>PPC</td>
<td>27.9 + 8</td>
<td></td>
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<td>7</td>
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</tbody>
</table>

*UB: Upper Bound; LB: Lower Bound; IHC: Immunohistochemistry; WB: Western-blot; RT-PCR: Reverse Transcription-polymerase Chain Reaction; PPC: Present Positive Cell; AQUA: Automated Quantitative Analysis; # indicates the data is from whole patients cohort which includes patients in survival analysis
High Bcl-2 Expression Predicts Good Outcome in NSCLC Cases

Totally, 50 studies (52 independent cohorts) that included 7765 patients reported the OS predictive value of Bcl-2 in NSCLC. Figure 2A demonstrated the forest plot of individual HR and corresponding 95% CI and results from the meta-analysis regarding Bcl-2 expression and OS of NSCLC patients. Certain degree of heterogeneity was observed in this group (I²=56.2%, p=0.00). Overall, the pooled HR and 95% CI for all studies showed a significant decreased risk of death in NSCLC patients with high Bcl-2 expression level (HR=0.76, 95% CI=0.67-0.86, random-effect model). Sensitivity analysis via omitting original investigations in order to validated stability of overall analysis result (Figure 3A). The funnel plot (Figure 4A) for publication bias indicated a good degree of symmetry, demonstrating no obvious publication bias existed. Begg's test showed no significant publication bias as well (p=0.84).

Subgroup analysis by publication year, cohort region, number of patients, disease stage and quality score was performed and the results are summarized in Table 3. Particularly, very good homogeneity was observed in subsets of American studies and studies contain only early stage patients (Both I²=0.00%).

Figure 2. Meta-analysis of Impact of Bcl-2 Expression on OS (A) and DFS (B) of Patients with NSCLC. Results are presented as individual and pooled HR, and corresponding 95% CI

Figure 3. Result of Sensitivity Analysis of Aggregated Result of Bcl-2 Expression on OS (A) and DFS (B) of Patients with NSCLC

Figure 4. Funnel Plots of Included Trails Reporting Prognostic Value of Bcl-2 Expression in OS (A) and DFS (B) of Patients with NSCLC
pooled results supported a favorable predictive value of Bcl-2 expression excepted subsets of studies in Asia (HR=0.95, 95%CI=0.73-1.23, random-effect model), studies contain only late stage patients (HR=0.93, 95%CI=0.71-1.24, random-effect model; HR=1.05, 95%CI=0.68-1.61, random-effect model) and studies with low quality score (HR=0.87, 95%CI=0.67-1.14, random-effect model).

Effects of Bcl-2 expression on DFS of NSCLC
As indicated in Figure 2B, 11 studies including 2634 patients reported the DFS predictive value of Bcl-2 in NSCLC. The pooled result reached statistical significance that high Bcl-2 expression level predicted good DFS (HR=0.85, 95%CI=0.75-0.95, random-effect model). Obvious heterogeneity was observed in this group (p=0.02 or I²=52.2%). Sensitivity analysis via omitting individual investigation order indicated a certain degree of unstable of the aggregated result when some studies were deleted (Figure 3B). Funnel plot and Begg’s test were performed to detect publication bias. The result of Begg’s test didn’t reach significant (p=0.592), however, the funnel plot indicated a certain degree of asymmetry (Figure 4B).

Discussion
Lung cancer, with a high incidence and mortality rate, is regarded as a serious health threat around the global and NSCLC, is the most common pathological type (Collins et al., 2007; Jemal et al., 2011). Even though a few prognostic and predictive markers have been validated, such as EGFR, identifying more established markers possessing the predictive value for survival of NSCLC patients remains a topic for exploration. Bcl-2, an inhibitor of intrinsic cell apoptosis, has important functions in tumor initiation and progression (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). In past twenty years, increasing number of studies were performed to evaluate prognostic value of Bcl-2 expression in NSCLC, however, the controversy still retain. A systematic review and meta-analysis is an effective way to reconcile the contradiction and lead to a relative confirmed conclusion. We thus conducted this work to aggregate current original results to elucidate the outcome predictive values of Bcl-2 in NSCLC.

Our systematic review and meta-analysis based on the outcomes of 7765 patients from 50 individual trails (52 independent cohorts), revealing that high expression of Bcl-2 protein is a favorable OS predictive marker in patients with NSCLC (HR=0.76, 95%CI=0.67-0.86). This result keeps stable in sensitivity analysis, indicating the real and steady effect of Bcl-2 expression on NSCLC OS prediction. Similarly, the pooled data based on 2634 patients, suggested high expression of Bcl-2 protein predicted good DFS in patients with NSCLC (HR=0.85, 95%CI=0.75-0.95). Unfortunately, the current conclusion regarding DFS is not stable enough as showed in Figure 3B.

Although we strived to validate our aggregated results by several inclusion/exclusion criteria and analysis methods, our approach didn’t eliminate all potential biases. Funnel plot and Begg’s test didn’t present significant publication bias; however, the potential publication bias in our study couldn’t be totally excluded. To utmost make sure that we can get full information in original studies, our systematic review and meta-analysis only took into account fully published studies. Though this inclusion criterion ensured us to get sufficient information about each trials and analyzing heterogeneities in different trials, it increased the risk of false positive results as well. As we know, studies that do not possess statistically significant results are less frequently published in full papers, but probably in abstracts that were ruled out of our analysis. It should be also note that our analysis only searched original articles published in English. As Egger M et al. (1997) indicated, positive results are more frequently published in English, while those negative ones tend to be more often published in native languages. This limitation will potentially lead to favor of positive original studies in our analysis and influence the reliability of synthesized conclusions.

Another potential source of bias derived from the method of extracting HRs and corresponding 95%CIs. If the original articles didn’t report the HR and 95%CI, we had to estimate them based on data available in the article and the survival curve. Since the method established by Parmar et al. (1998), Williamson et al. (2002) and Tierney et al. (2007) cannot thoroughly restore all primary data for calculating HRs and 95%CIs, random errors existed in this process without doubt. Besides, the use of same cohort of patients for different publications couldn’t be totally excluded. If the patient number was not totally the
same in two papers, we assumed that the authors were honest enough not to re-report the results from the same cohort of patients. Thus, we couldn’t rule out same cohorts were included twice or more in this meta-analysis, that would give higher weighting to a particular positive or negative trend.

The heterogeneity within different studies is of concern when interpreting the clinical utility of the current conclusion that high expression of Bcl-2 predicts good survival in patients with NSCLC. As showed in Figure 2A, heterogeneity testing detected certain degree of inter study heterogeneity. Information in Table 2 visually displayed the difference between original trails, such as the primary anti-body dilution, cut-off point of positive and negative (high and low) and survival data analysis method. Therefore, more well-designed retrospective and prospective trails that aim to promote its clinical utilities via validating the most suitable disease stage, best cut-off point and so on are still highly welcomed.

According to our aggregated result, patients with Bcl-2-positive tumors had significantly better survival than those with Bcl-2-negative tumors. It seems that this conclusion is controversial with biological functions of Bcl-2 protein. Originally, the high expression of Bcl-2 gene product is implicated in tumorigenesis because of its ability to prolong cell survival through the inhibition of apoptosis (Hockenbery et al., 1990). The process of apoptosis involves both the anti-apoptotic proteins (such as Bcl-2, Bcl-X, and Bfl-1) and the pro-apoptotic proteins (such as Bax, Bak and Bad), which can interact collaboratively or antagonistically to regulate cellular apoptosis (Kroemer, 1997; Chipuk et al., 2010; Hardwick and Soane, 2013). Thus, the study of only one apoptotic protein leads to a partial appraisal of apoptosis and this may partly reconcile the above paradox. Here, we may suppose that evaluating the anti-apoptotic and pro-apoptotic proteins in combination in NSCLC tissues is possibly an interesting and worthwhile research topic. Furthermore, the cell cycle entry inhibition role and carcinogenesis inhibition phenomenon of Bcl-2 as demonstrated in some articles (Pierce et al., 2002; Kirkin and Soane, 2013) also suggested the possibility and rationality of high Bcl-2 expression is a good prognosticator. Of course, more direct biological evidences are highly needed to illustrate the molecular mechanisms and support the current conclusion.

To sum up, this meta-analysis got a rather safe conclusion that high expression of Bcl-2 protein predicted good OS in NSCLC patients. However, as inter study heterogeneity exists in current trails, the fittest cut-off value, disease stage and other clinical practice relevant parameters remain undetermined. For further research, more high quality prospective clinical trials and high quality retrospective cohort studies are worthwhile to be performed and highly needed.

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