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# Ephrin Receptor B2 Expression May Be a Prognostic Marker for Patients With Cancer: A Meta-analysis

HYUN MIN KOH[1], CHANG LIM HYUN[2,3], BO GUN JANG[2,3] and HYUN JU LEE[4,5]

[1]*Department of Pathology, Gyeongsang National University Changwon Hospital, Changwon, Republic of Korea;*
[2]*Department of Pathology, Jeju National University School of Medicine, Jeju, Republic of Korea;*
[3]*Department of Pathology, Jeju National University Hospital, Jeju, Republic of Korea;*
[4]*Department of Pathology, Soonchunhyang University College of Medicine, Cheonan, Republic of Korea;*
[5]*Department of Pathology, Soonchunhyang University Cheonan Hospital, Cheonan, Republic of Korea*

**Abstract.** *Background: Recent studies have revealed that ephrin receptor (EPH) is implicated in important signal transduction of cancer development and progression. EPHB2 is expressed in human cancer, and reported to be related to the prognosis of colorectal, gastric and breast cancer. This meta-analysis was systematically assessed the prognostic roles of EPHB2 expression in patients with cancer. Patients and Methods: PubMed, Embase and Cochrane library were searched for eligible studies up to May 2020. Pooled hazard ratio (HR) and 95% confidence interval (CI) were calculated to evaluate the relationship of EPHB2 expression with overall and disease-free survival in patients with cancer. Results: The pooled HRs for low expression of EPHB2 were 1.65 (95% CI=1.30-2.09, p<0.001) and 1.63 (95% CI=1.33-1.99, p<0.001) for overall and disease-free survival, respectively. Low expression of EPHB2 was significantly correlated with higher tumor grade and stage [odds ratio (OR)=3.04, 95% CI=1.70-5.42, p<0.001; and OR=1.82, 95% CI=1.11-2.99, p=0.018], lymph node metastasis (OR=2.13, 95% CI=1.64-2.77, p<0.001), and higher overall stage (OR=2.14, 95% CI=1.71-2.69, p<0.001). Conclusion: EPHB2 expression may be a valuable prognostic marker for patients with cancer.*

Ephrin receptor (EPH) is a member of the largest family of receptor tyrosine kinases, and is of two types, EPHA and EPHB according to sequence homology and binding affinity for their ligands (1, 2). EPH is regarded as a candidate for anticancer treatment because it is involved in pathological as well as physiological processes (2). Recent studies have revealed that EPH is implicated in valuable signal transduction of cancer development and progression (3). In particular, EPHB2 expression has been reported to be associated with survival of patients with cancer (3, 4). However, the prognostic roles of EPHB2 expression have not been analyzed systematically. Here, we investigated the prognostic roles of EPHB2 expression in cancer for an integrated understanding.

*Correspondence to:* Hyun Ju Lee, MD, Ph.D., Department of Pathology, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, 31 Soonchunhyang 6 gil, Dongnam-gu, Cheonan-si, Chungcheongnam-do, 330-721, Republic of Korea. Tel: +82 415701499, Fax: +82 415703580, e-mail: drlhjmd@daum.net

*Key Words:* Cancer, ephrin receptor B2, meta-analysis, prognosis.

## Patients and Methods

*Search strategy.* Eligible studies were found by searching PubMed, Embase and Cochrane library up to May 2020 using the following terms: (EPHB2 or ephrin receptor B2) and (cancer or tumor or carcinoma or neoplasm or malignancy) and (prognostic or predict or prognosis or survival or outcome). A manual search was also carried out.

*Inclusion and exclusion criteria.* Eligible studies had to meet the following criteria: (i) EPHB2 expression was identified in the human cancer tissue; (ii) EPHB2 expression was assessed by immunohistochemistry and the relationship between EPHB2 expression and survival was evaluated; (iii) hazard ratio (HR) and 95% confidence interval (CI) were directly presented. The following were excluded: (i) Duplicate studies; (ii) conference abstracts, reviews, letters, and non-English articles.

*Data extraction and quality assessment.* The following information was collected separately by two Authors: First author, publication year, country, cancer type, sample size, gender and median or mean age of patients, study period, follow-up duration, and cut-off value of EPHB2 expression. The quality of included studies was evaluated using the Newcastle-Ottawa Scale.

*Statistical analysis.* The prognostic significance of EPHB2 expression was estimated using pooled HR and 95% CI. Heterogeneity among the included studies was evaluated by $I^2$ value. Heterogeneity between the included studies was assessed by subgroup analysis. Publication bias was assessed by funnel plot and Egger's test. A sensitivity analysis was performed to investigate the consistency of

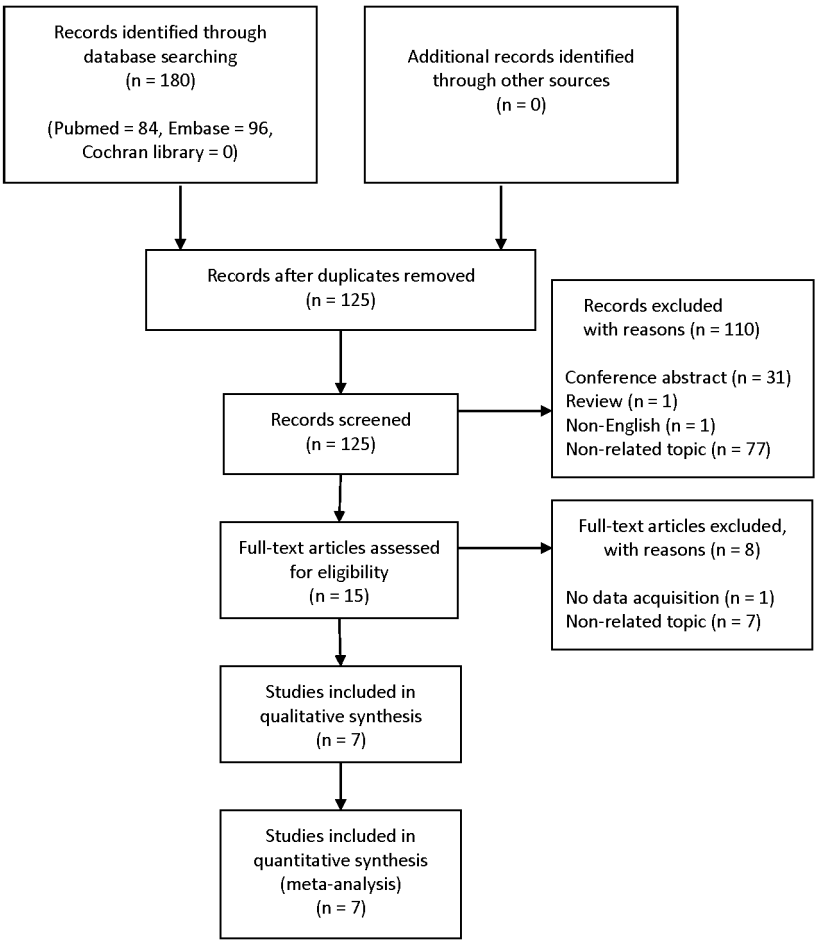


Figure 1. *Flow diagram of study selection process.*

the pooled results. All statistical analyses were conducted using StataSE 12 (Stata, College Station, TX, USA). Statistical significance was determined only when the *p*-value was less than 0.05.

## Results

*Search results and study information*. The process of study selection is shown in Figure 1. After duplicates were removed, 125 articles were screened for eligibility. Finally, seven studies including 2,436 patients with cancer were analyzed.

The basic information of included studies is presented in Table I. The included studies consisted of three types of cancer, colorectal (CRC) (n=5), gastric (n=1), and breast (n=1). The quality of the included studies was rated as good quality, with seven or more points.

*Relationship between low expression of EPHB2 and overall survival (OS)*. This analysis included seven studies with 2,436 patients with cancer and disease-specific or cancer-specific survival was considered as OS.

The heterogeneity between these studies was considerable ($I^2$=58.6%, $p$=0.025). The pooled HR for OS was 1.65 (95% CI=1.30-2.09, $p$<0.001) using the random-effects model, implying that low expression of EPHB2 was related to poor OS (Figure 2). In the subgroup analysis, there was significant intergroup heterogeneity ($p$=0.012), and the results revealed that low expression of EPHB2 was significantly associated with unfavorable OS in patients with CRC (HR=1.41, 95% CI=1.15-1.73, $p$=0.001) (Figure 3).

*Relationship between low expression of EPHB2 and disease-free survival (DFS)*. In this analysis, recurrence-free or metastasis-free survival was considered as DFS and included four studies with 1,050 patients.

The heterogeneity between these studies was very low ($I^2$=0.0%, $p$=0.684). The pooled HR was 1.63 (95% CI=1.33-1.99, $p$<0.001) using the fixed-effects model (Figure 4), indicating low expression of EPHB2 to be

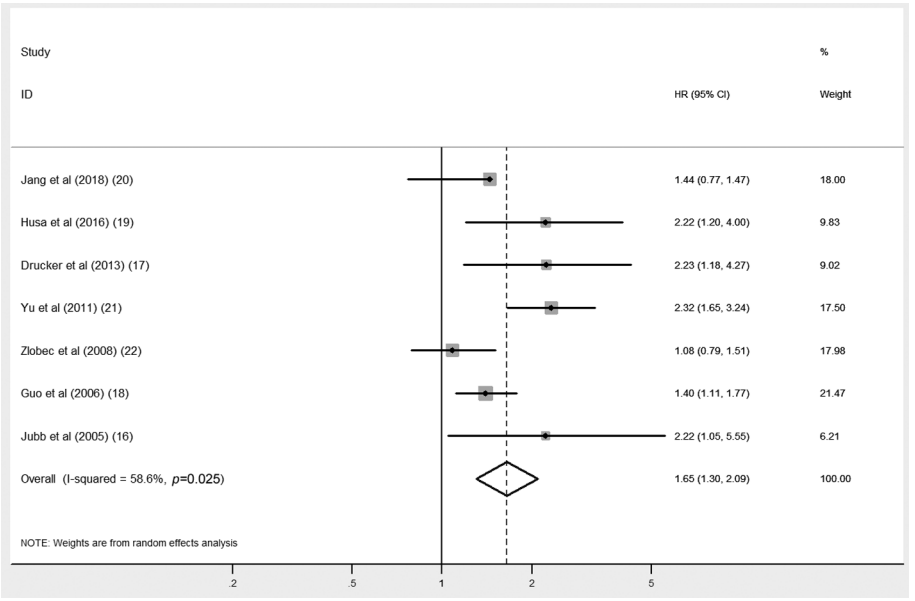


Figure 2. *Forest plot for the relationship between ephrin receptor B2 expression and overall survival. CI: Confidence interval; HR: hazard ratio.*

Table I. *The basic information of included studies.*

| Study (Ref) | Country | Cancer type | Sample size | M/F | Age (years) | Study period | Follow-up (months) | Clinical outcome | EPHB2 IHC cut-off | Survival analysis | NOS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Jang *et al*. (2018) (20) | Korea | Colorectal cancer | 567 | 343/224 | NA | 2004-2006 | NA | OS | H-score: Intensity score × percentage of positive tumor cells (<40, median value) | MVA | 7 |
| Husa *et al*. (2016) (19) | Sweden | Breast cancer | 216 | - | NA | 1976-1990 | NA | DSS, MFS | The membrane staining intensity (<2, negative and weak) | MVA | 7 |
| Drucker *et al*. (2013) (17) | Canada | Colon cancer | 159 | 82/77 | Median= 61 | 1995-2009 | Median= 41.6 (1.2-168) | OS, DFS | The proportion of tumor cells with complete membrane staining of any intensity (<50%) | MVA | 8 |
| Yu *et al*. (2011) (21) | China | Gastric cancer | 337 | 248/89 | Mean= 59 | 2001-2003 | Mean= 37 (21-73) | OS | Negative: No staining Low: <25% Immunoreactive tumor cells | MVA | 8 |
| Zlobec *et al*. (2008) (22) | Switzerland | Rectal cancer | 482 | 251/231 | Mean= 68.7 (36-96) | 1987-1996 | Median= 51 (0-150) | CSS | <70% Immunoreactive tumor cells | UVA | 7 |
| Guo *et al*. (2006) (18) | USA | Colorectal cancer | 345 | 190/155 | Mean= 67.99 (31-94) | NA | Median= 44.85 | OS, DFS | Membrane staining intensity (<1, negative) | UVA (OS) MVA (DFS) | 8 |
| Jubb *et al*. (2005) (16) | Germany | Colorectal cancer | 330 | 150/180 | Mean= 69 (28-88) | 1990-1995 | Median= 50.4 (5-136.8) | OS, RFS | <10% Or ≥10% of stained weakly | UVA | 7 |

CSS: Cancer-specific survival; DFS: disease-free survival; DSS: disease-specific survival; EPHB2: ephrin receptor B2; IHC: immunohistochemistry; MFS: metastasis-free survival; MVA: multivariate analysis; NA: not available; NOS: Newcastle-Ottawa Scale; OS: overall survival; RFS: recurrence-free survival; UVA: univariate analysis.

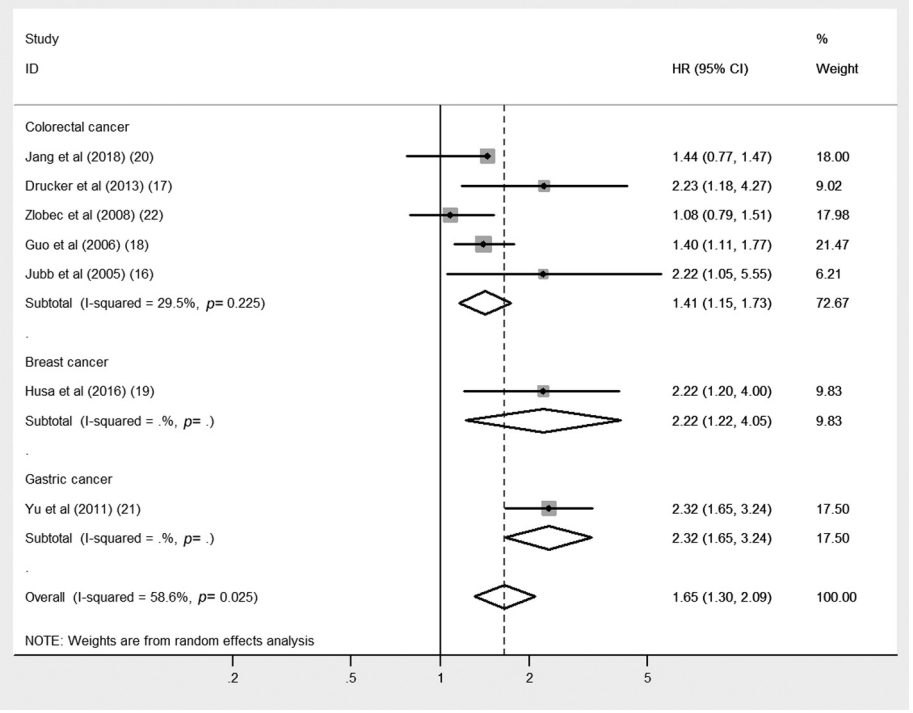


Figure 3. *Forest plot of studies evaluating ephrin receptor B2 expression and overall survival stratified by cancer type. CI: Confidence intervaI; HR: hazard ratio.*

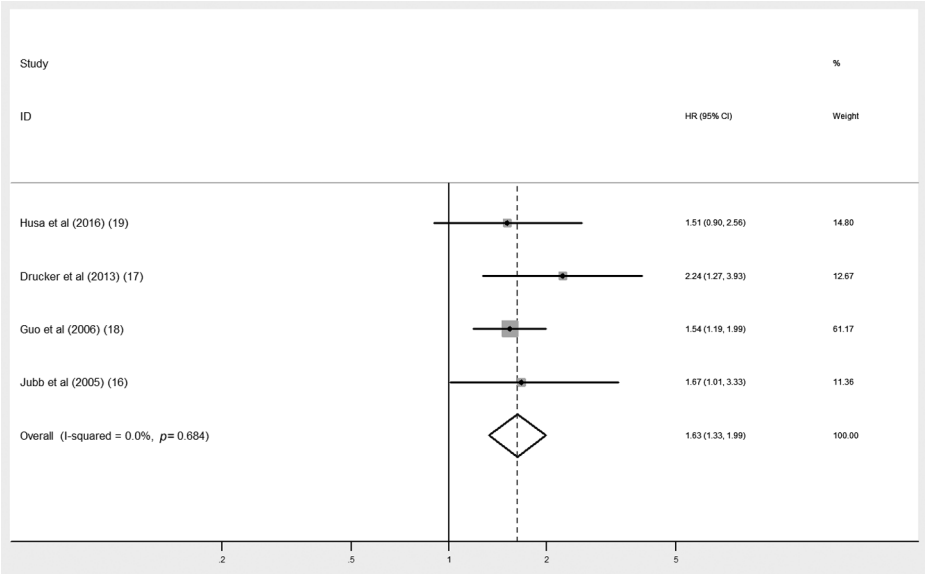


Figure 4. *Forest plot for the relationship between ephrin receptor B2 expression and disease-free survival. CI: Confidence intervaI; HR: hazard ratio.*

associated with poor DFS. With respect to subgroup analysis, low expression of EPHB2 was found to be related to the progression of the disease in CRC (HR=1.65, 95% CI=1.32-2.05, $p<0.001$) (Figure 5).

*Relationship between low expression of EPHB2 and clinicopathological variables.* Low expression of EPHB2 was significantly correlated with higher tumor grade and stage [odds ratio (OR)=3.04, 95% CI=1.70-5.42, $p<0.001$;

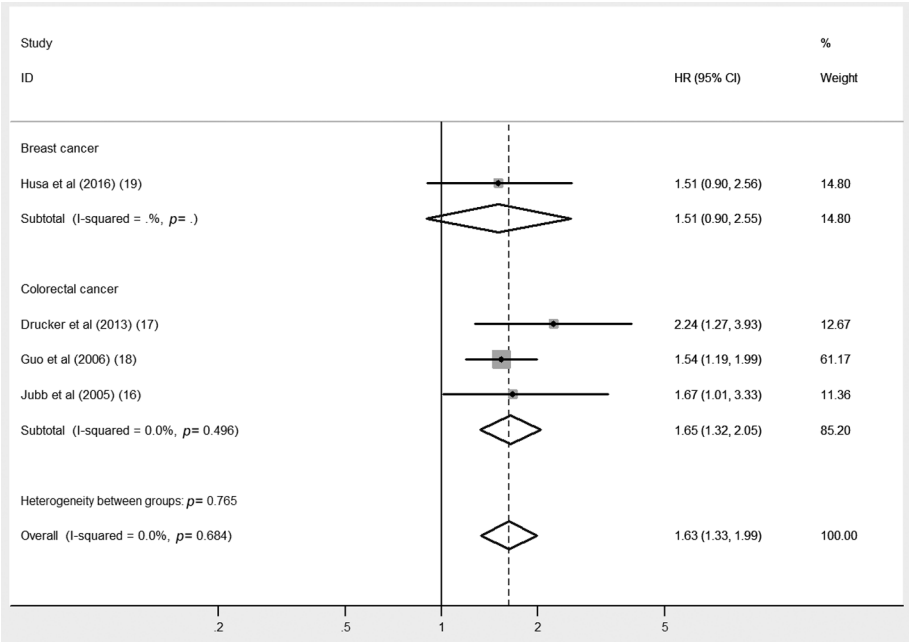


Figure 5. *Forest plot of studies evaluating ephrin receptor B2 expression and disease-free survival stratified by cancer type. CI: Confidence intervaI; HR: hazard ratio.*

Table II. *The relationship between low expression of ephrin receptor B2 and clinicopathological variables in patients with cancer.*

| Variable | Number of studies | Number of patients | Pooled OR (95% CI) | *p*-Value | Heterogeneity | | |
|---|---|---|---|---|---|---|---|
| | | | | | $I^2$ (%) | *p*-Value | Model |
| Tumor grade (high *vs*. low) | 5 | 1624 | 3.04 (1.70-5.42) | <0.001 | 67.9 | 0.014 | Random |
| Tumor stage (high *vs*. low) | 4 | 1279 | 1.82 (1.11-2.99) | 0.018 | 64.3 | 0.038 | Random |
| Lymph node metastasis (present *vs*. absent) | 3 | 1120 | 2.13 (1.64-2.77) | <0.001 | 46.5 | 0.155 | Fixed |
| Overall stage (high *vs*. low) | 3 | 1249 | 2.14 (1.71-2.69) | <0.001 | 0.0 | 0.616 | Fixed |

CI: Confidence interval; OR: odds ratio.

and OR=1.82, 95% CI=1.11-2.99, $p$=0.018], lymph node metastasis (OR=2.13, 95% CI=1.64-2.77, $p$<0.001), and higher overall stage (OR=2.14, 95% CI=1.71-2.69, $p$<0.001) (Table II, Figure 6).

*Publication bias*. The funnel plots for OS and DFS were visually asymmetrical but Egger's test did not statistically prove publication bias (for OS, $p$=0.211; for DFS, $p$=0.424) (Figure 7A and B).

The filled funnel plot for OS revealed that the pooled HR was slightly lower but still statistically significant (HR=1.40, 95% CI=1.09-1.79, $p$=0.009) compared with the initial pooled results (Figure 7C), and the results for DFS showed that association was unchanged (HR=1.63, 95% CI=1.33-1.99, $p$<0.001) (Figure 7D).

*Sensitivity analysis*. Sensitivity analysis was conducted to investigate the influence of individual studies and the consistency of pooled results. The results for OS and DFS were still significant (for OS, HR=1.55, 95% CI=1.35-1.77; for DFS, HR=1.63, 95% CI=1.33-1.99), therefore our initial pooled results were consistent and reliable even if the effects of individual studies were excluded (Figure 8).

## Discussion

EPH plays important roles in various physiological functions including cell adhesion and migration, development of the nervous system, and angiogenesis (5-8). In addition to these *in vivo* functions, EPH is believed to induce carcinogenesis (8). Some studies have shown EPH overexpression in various

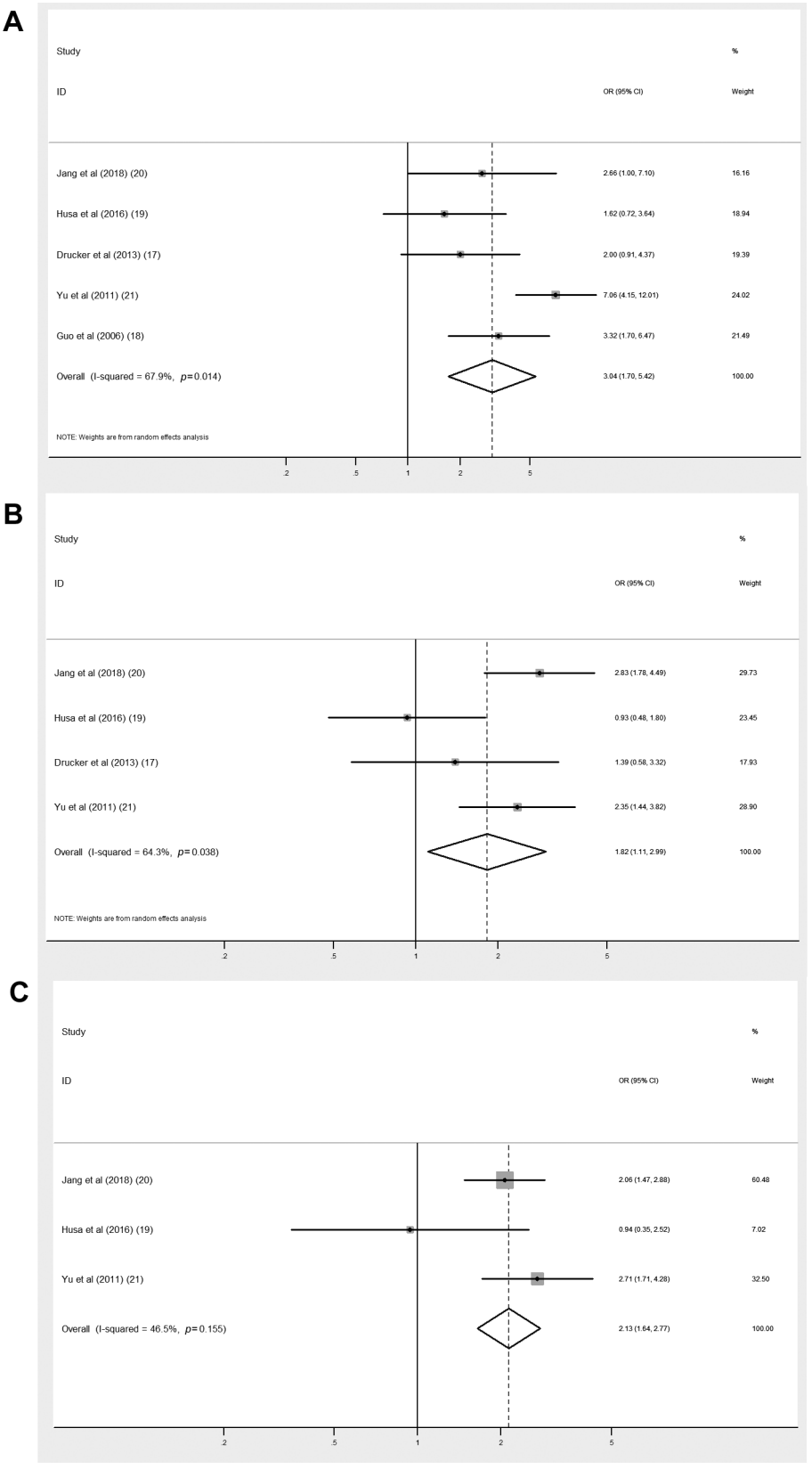


Figure 6. *Continued*

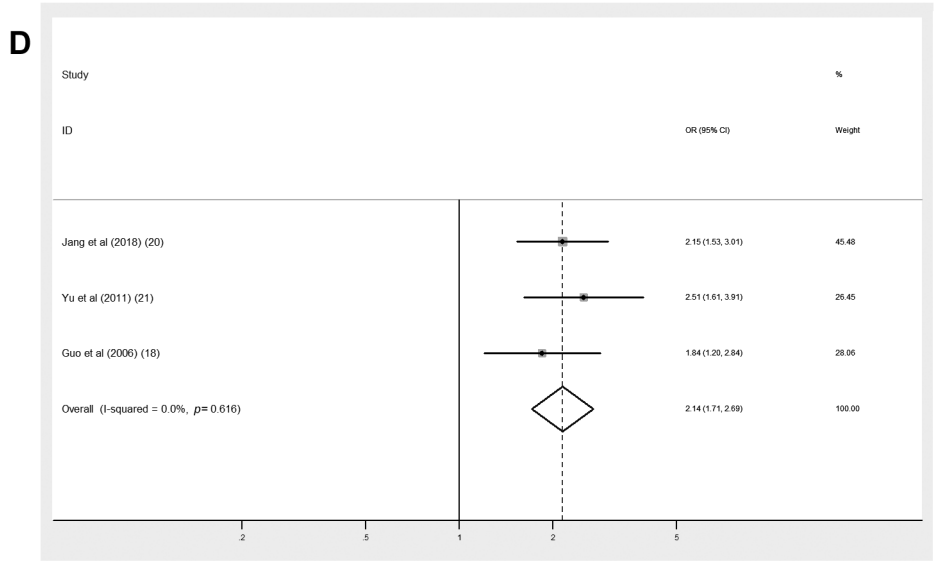


Figure 6. *Forest plot for the relationship between ephrin receptor B2 expression and clinicopathological variables. (A) tumor grade, (B) tumor stage, (C) lymph node metastasis, and (D) overall stage. CI: Confidence intervaI; OR: odds ratio.*

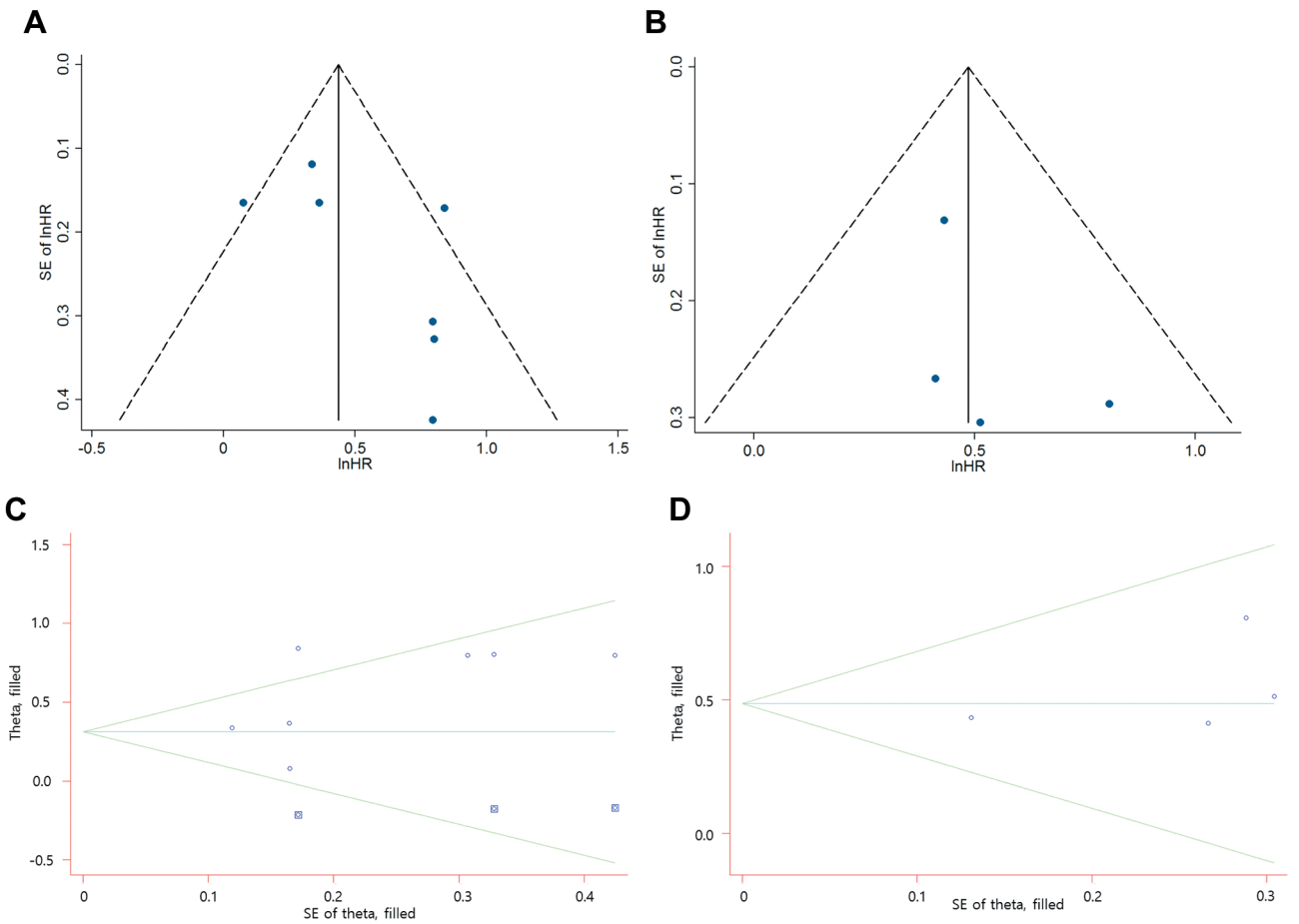


Figure 7. *Funnel plot (A, B) and trim and fill method (C, D) for the relationship between ephrin receptor B2 expression and overall survival (A, C) and disease-free survival (B, D) with 95% confidence intervaIs.CI: Confidence intervaI; LnHR: logn hazard ratio; SE: standard error.*

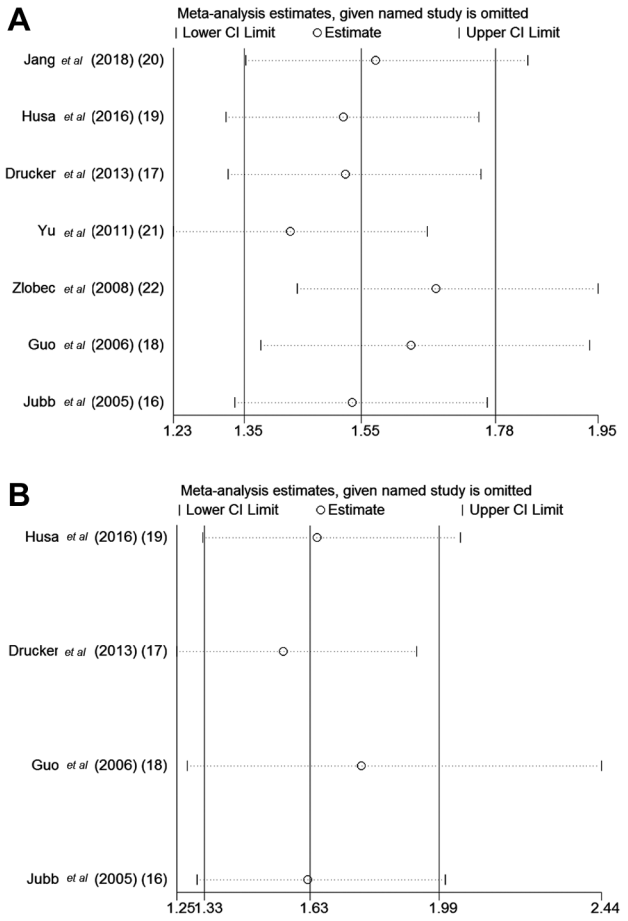


Figure 8. *Sensitivity analysis for the relationship between ephrin receptor B2 expression and overall survival (A) and with disease-free survival (B). CI: Confidence intervaI.*

tumor types, such as lung, breast, thyroid, gastrointestinal, pancreatic, prostatic, and ovarian cancer (9-15).

EPHB2 is considered a target for the WNT signaling pathway, which is frequently hyperactivated early in the colorectal adenoma to adenocarcinoma sequence (3, 16). Recently, EPHB2 expression was demonstrated in a number of human cancer types, and reported to be related to the prognosis of colorectal, gastric and breast cancer (8, 16-22).

In this study, we investigated the relationship between EPHB2 expression and the prognosis of cancer in an integrated manner. We demonstrated that low expression of EPHB2 was significantly correlated with unfavorable clinical outcome of OS and DFS, and was associated with higher tumor grade and stage, lymph node metastasis, and higher overall stage. Moreover, we demonstrated that our findings remained significant, even though the effects of individual study results were excluded. Our results suggest that EPHB2 expression can predict the prognosis of patients with cancer. To the best of our knowledge, this is the first meta-analysis to assess the prognostic role of EPHB2 expression in cancer.

There are several limitations to the present study. Firstly, heterogeneity could not be prevented in the analysis for OS. Secondly, the various cut-off values used with studies of EPHB2 expression may have affected our results. We hope that more studies on the prognosis of EPHB2 expression will be conducted in the future. However, in the present study, we showed that EPHB2 expression may be a valuable prognostic marker in patients with cancer.

## Conflicts of Interest

The Authors declare no potential conflicts of interest exist.

## Authors' Contributions

H.M. Koh, C.L. Hyun, B.G. Jang and H.J. Lee designed this study; H.M. Koh, C.L. Hyun and B.G. Jang searched the databases and inspected all candidate articles; H.M. Koh and H.J. Lee extracted and analyzed the data; H.M. Koh and B.G. Jang assessed the quality of the included studies; H.M. Koh wrote the article, and all Authors reviewed the article.

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