Review Article
Clinicopathological and prognostic relevance of EZH2 expression in renal cell carcinoma: a meta-analysis

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Abstract: Background: The prognostic value of zeste homolog 2 (EZH2) in renal cell carcinoma (RCC) has been reported in a large number of studies. However, the results from these studies are inconsistent and remain in conclusive. We conducted a systematic review and meta-analysis to explore the significance of EZH2 expression in patients with RCC. Methods: We searched PubMed, Embase, ISI Web of Knowledge and Cochrane Library to identify studies written in English. The methodological quality of the studies was also evaluated. Odds ratio (OR) and hazard ratio (HR) were calculated and summarized. Results: Eleven eligible studies including 2305 RCC patients were identified. We observed that EZH2 expression was significantly higher in the RCC tissue than the normal renal tissue (OR: 7.88, 95% CI 4.33-14.36, P < 0.00001). EZH2 expression was not associated with tumor type or sex (OR: 0.73, 95% CI 0.46-1.15, P = 0.18; OR: 1.20, 95% CI 0.94-1.52, P = 0.14). However, EZH2 expression was clearly associated with clinical staging (OR: 0.44, 95% CI 0.34-0.55, P < 0.00001), Fuhrman grading (OR: 0.55, 95% CI 0.42-0.72, P < 0.0001) and metastatic status (OR = 0.45, 95% CI 0.34-0.60, P < 0.00001). A statistically significant combined HR was detected for overall survival (OR: 2.85, 95% CI 2.05-3.98, P < 0.00001), progression-free survival (OR: 3.09, 95% CI 1.49-6.43, P = 0.002), and disease-free survival (OR: 2.69, 95% CI 1.74-4.17, P < 0.00001). The results of this meta-analysis suggest that EZH2 expression is associated with an increased risk of RCC and worsened survival of RCC patients. Aberrant EZH2 expression plays an important role in the carcinogenesis and prognosis of RCC.

Keywords: Zeste homolog 2, renal cell carcinoma, meta-analysis, clinicopathological, prognostic

Introduction

Renal cell carcinoma (RCC) accounts for an estimated 3% of all adult malignancies [1]. Despite the remarkably rapid advancement in the diagnosis and management of RCC, RCC incidence is still increasing in most countries. Advanced disease and distant metastases are still diagnosed in RCC patients [2, 3]. Thus, new targets and therapies are needed to improve patient outcomes. As a key component of PRC2 complex, EZH2 is involved in silencing various tumor suppressor genes. EZH2 over-expression is seen in tumorigenesis and correlates with a poor prognosis of several tumor types [4-7]. Many studies have shown that EZH2 is also aberrantly expressed in RCC. However, these results remain disputed due to the limited num-

ber of patients in individual studies. Actually, several studies claimed that increased EZH2 expression was associated with poor outcome of RCC patients, while others did not support the conclusion. In this study, we updated and analyzed published clinical investigations evaluating the expression of EZH2 in patients with RCC.

Methods

Search strategy and selection criteria

We searched PubMed, Embase, ISI Web of Knowledge and Cochrane Library to identify studies. The search ended on October 1, 2015, with no other date limit. We used the following search terms: "renal", "kidney", "tumor or can-
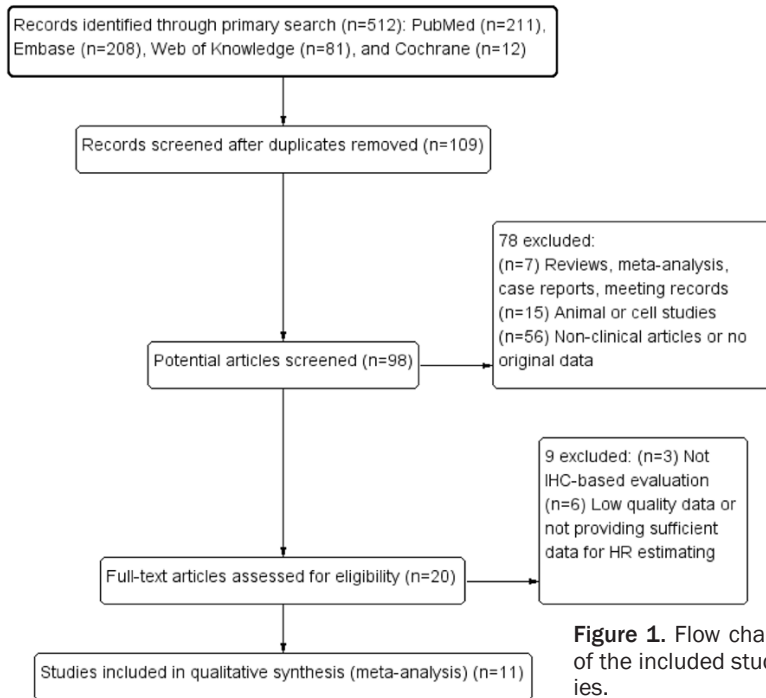
Figure 1. Flow chart of the included studies.

cer or carcinoma or neoplasm", "expression", "EZH2 or zeste homolog 2", and "prognosis or prognostic or outcome".

Inclusion criteria: (1) studies that included EZH2 expression in primary RCC tissues, (2) studies that revealed the relationship between EZH2 expression and RCC clinicopathological parameters and prognosis, and (3) studies that provided sufficient information to analyze a hazard ratio (HR) of overall survival (OS), progression free survival (PFS), disease free survival (DFS) and a 95% confidence interval (CI).

Data extraction and methodological assessment

Two reviewers independently extracted the following information from data from each study: (1) first author's surname, publication year, country, number of cases, immunohistochemical staining methods, antibody source, percentage rate of expression; (2) baseline data, including sample size, age, gender, follow-up period and treatment, EZH2 proportion, histological subtypes, pathological nuclear grade, and TNM stage, (3) statistical data such as HRs and their 95% CIs. We preferred to collect multivariate analysis data. If they were not available, data from univariate analyses of survival outcomes were extracted instead. The

quality of the selected articles was assessed according to the Newcastle-Ottawa Scale (NOS) [8]. Heterogeneity of investigation was evaluated to determine whether the data of the various studies could be analyzed for a meta-analysis. The data in this study was extracted from previous studies, ethic approval is waived.

Statistical analysis

A statistical analysis was conducted using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) and STATA 14.0 (Stata Corporation, TX). Heterogeneity was quantifiably assessed with Cochran's Q test [9] and an I^2 statistic [10, 11]. Some of the studies that provided a HR and 95% CI value were directly pooled. For studies in which these data were not provided directly, we obtained the value from the available data or by assessing Kaplan-Meier survival curves in an original study [12, 13]. When the I^2 statistic results were 0-50%, a fixed effect model was used to calculate parameters. If the I^2 statistic results were 50%-100%, a random-effects model was presented and considered to be more appropriate than a fixed-effects model. A *p* value < 0.05 was identified as statistically significant. Funnel plots and Begg's test were used to evaluate publication bias [14, 15].

Results

As shown in **Figure 1**, we initially included 11 studies in the final meta-analysis [16-26]. The total number of patients included in this meta-analysis was 2305. Their basic characteristics are summarized in **Table 1**. The patients came from 5 countries (Germany, Japan, Korea, Canada and China). IHC was the only method used to assess EZH2 expression in RCC tissues. There were differences in defining the cut-off values of positive EZH2 expression.

The pooled OR from four studies, which included 398 RCC and 263 normal renal tissues, is shown in **Figure 2A** (OR: 7.88, 95% CI: 4.33-

Table 1. Main characteristics of all the studies included in the meta-analysis

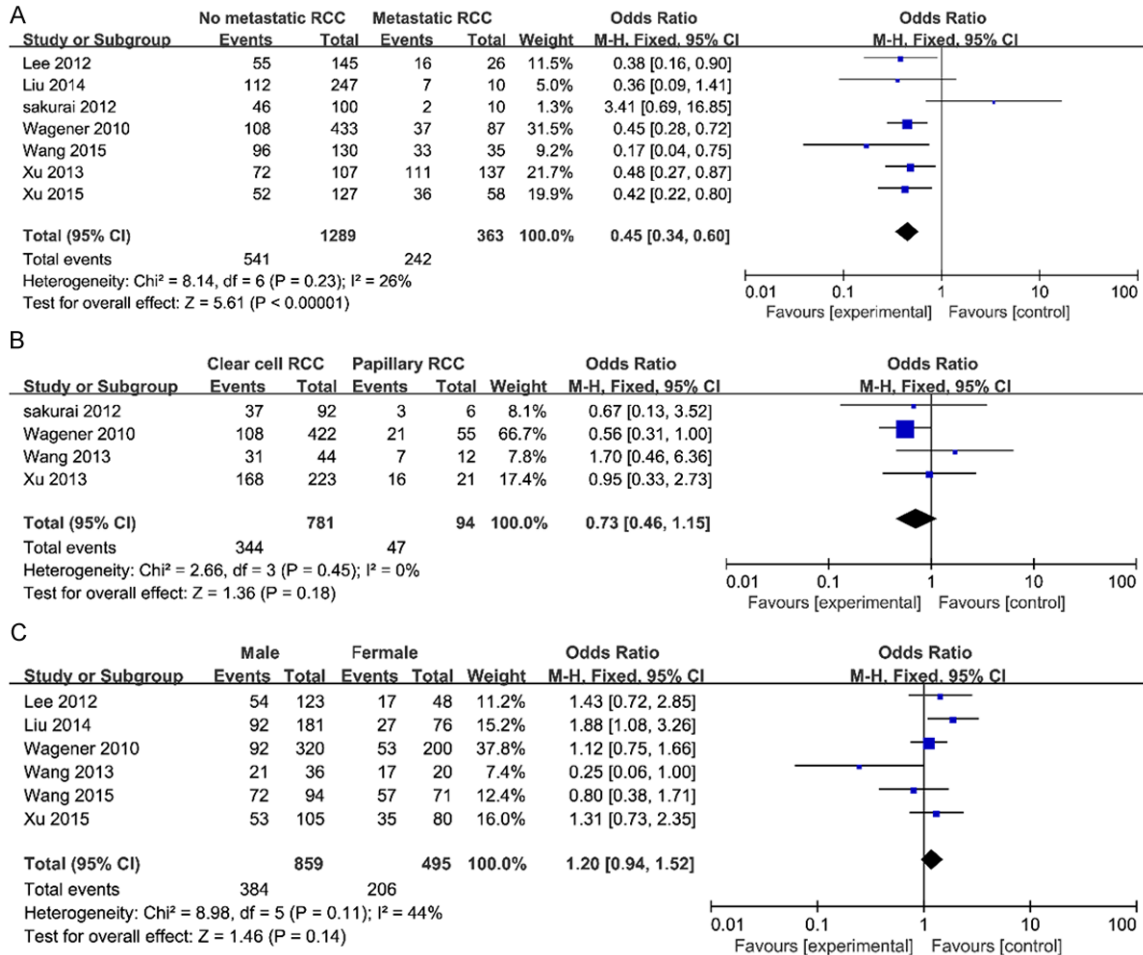| Study	Country	Patient	Histology	Methods	Antibody source	Criteria of EZH2 aberrant expression	Quality assessment (score)
Hinz_2009	Germany	119	Not known	IHC	R&D Systems	Semiquantitative scoring system	6
Li_2009	China	66	Clear cell (58) other (8)	IHC	Cell Signaling	Semiquantitative scoring system	7
Wagener_2010	Germany	520	Clear cell (422) other (98)	IHC	BD Transduction	Semiquantitative scoring system	7
Sakurai_2012	Japan	110	Clear cell (92) other (18)	IHC	BD Transduction	Nuclear staining positive cells > 50 %	8
Lee_2012	Korea	210	Clear cell (171) other(39)	IHC	LabVision and Zymed	Semiquantitative scoring system	6
Wang_2013	China	56	Clear cell (44) papillary (12)	IHC	Cell Signaling	Semiquantitative scoring system	7
Xu_2013	Canada	244	Clear cell (223) papillary (21)	IHC	BD Transduction	Semiquantitative scoring system	8
Liu_2013	China	373	Clear cell (342) other (31)	IHC	Cell Signaling	Semiquantitative scoring system	6
Liu_2014	China	257	Clear cell (241) other (16)	IHC	Cell Signaling	Semiquantitative scoring system	7
Xu_2015	China	185	Clear cell (185)	IHC	Cell Signaling	Semiquantitative scoring system	7
Wang_2015	China	165	Not known	IHC	Cell Signaling	Semiquantitative scoring system	6
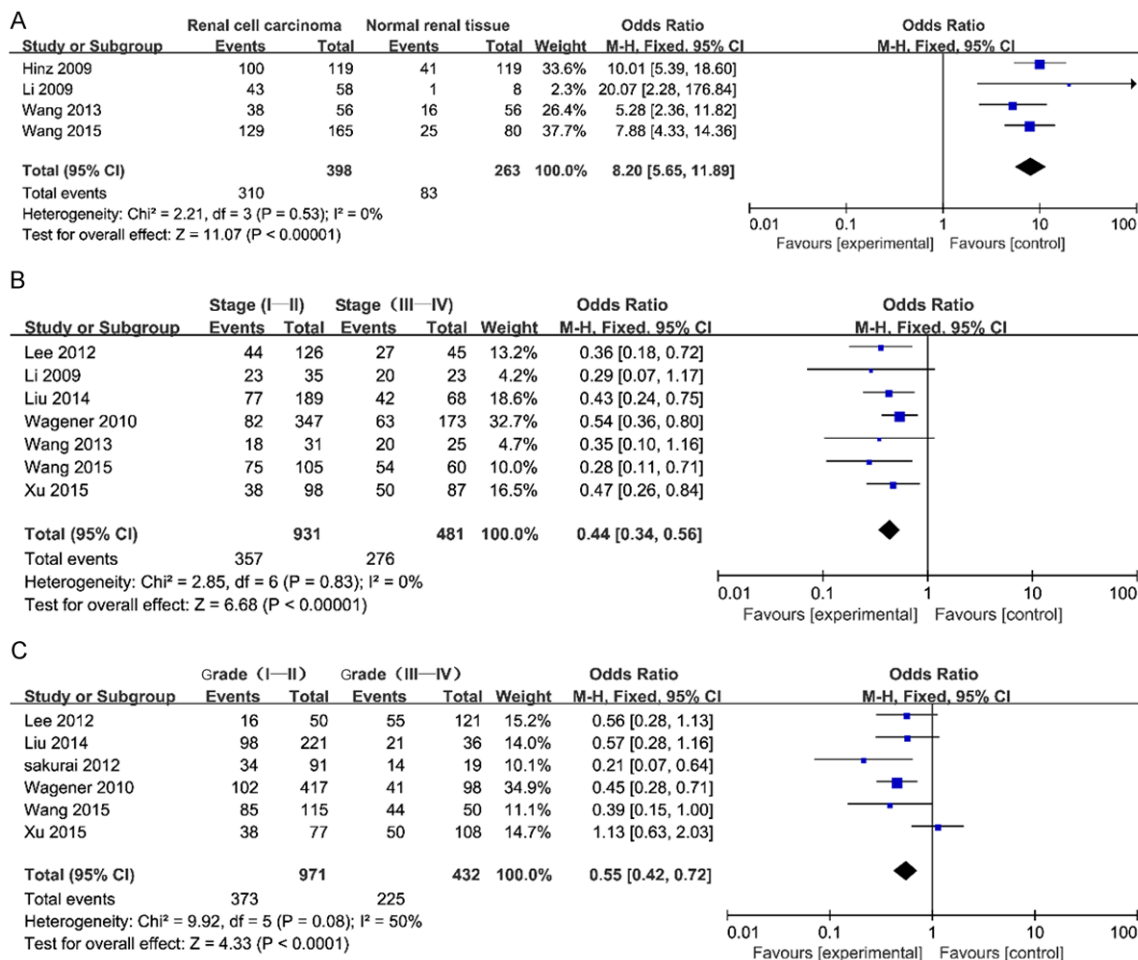
Figure 2. A. The pooled OR from five studies including 398 RCC and 263 normal renal tissues. B. A total of 1412 RCC patients were pooled from seven studies to assess whether EZH2 expression in RCC was associated with advanced stages of RCC. C. The pooled OR from five studies, which included 971 grade I and II, 432 grade III and IV. CI: confidence interval.

14.36, P < 0.00001). It indicates that EZH2 expression was markedly higher in RCC than in normal renal tissues and that EZH2 plays a key role in the pathogenesis of RCC. The pooled OR from seven studies, which included 868 early stage RCC (I and II) and 417 advanced RCC (III and IV) samples, is shown in **Figure 2B** (OR: 0.44, 95% CI: 0.34-0.55, P < 0.00001). The pooled OR indicates that the EZH2 protein expression was higher in the advanced RCC (III and IV) group than in the early RCC (I and II) group. EZH2 may play an important role in the clinical stage of RCC. The pooled OR from six studies, which included 971 grade I and II and 432 grade III and IV, is shown in **Figure 2C** (OR: 0.55, 95% CI 0.42-0.72, P < 0.0001). It indicates that EZH2 expression was significantly higher in the RCC patients with high Fuhrman grades than in those with low Fuhrman grades.

As shown in **Figure 3A**, aberrant EZH2 expression was markedly higher in metastatic RCC than in nonmetastatic RCC (OR: 0.45, 95% CI 0.34-0.60, P < 0.00001). As shown in **Figure 3C**, aberrant EZH2 expression was not associated with the tumor type in the RCC patients (OR: 0.73, 95% CI 0.46-1.15, P = 0.18). As shown in **Figure 3B**, aberrant EZH2 expression was also not associated with the gender of the RCC patients (OR = 1.20, 95% CI 0.94-1.52, P = 0.14).

EZH2 expression was significantly correlated with OS (OR: 2.85, 95% CI 2.05-3.98, P < 0.00001; **Figure 4A**), RFS (OR: 3.09, 95% CI 1.49-6.43, P = 0.002; **Figure 4B**), and DFS (OR: 2.69, 95% CI 1.74-4.17, P < 0.00001; **Figure 4C**). These results suggest that the up-regulation of EZH2 expression might lead to a poorer prognosis in RCC patients.

A

Study or Subgroup	No metastatic RCC Events	Total	Metastatic RCC Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2012	55	145	16	26	11.5%	0.38 [0.16, 0.90]
Liu 2014	112	247	7	10	5.0%	0.36 [0.09, 1.41]
sakurai 2012	46	100	2	10	1.3%	3.41 [0.69, 16.85]
Wagener 2010	108	433	37	87	31.5%	0.45 [0.28, 0.72]
Wang 2015	96	130	33	35	9.2%	0.17 [0.04, 0.75]
Xu 2013	72	107	111	137	21.7%	0.48 [0.27, 0.87]
Xu 2015	52	127	36	58	19.9%	0.42 [0.22, 0.80]
Total (95% CI)		1289		363	100.0%	0.45 [0.34, 0.60]
Total events	541		242			

Heterogeneity: Chi² = 8.14, df = 6 (P = 0.23); I² = 26%
Test for overall effect: Z = 5.61 (P < 0.00001)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [experimental] Favours [control]

B

Study or Subgroup	Clear cell RCC Events	Total	Papillary RCC Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
sakurai 2012	37	92	3	6	8.1%	0.67 [0.13, 3.52]
Wagener 2010	108	422	21	55	66.7%	0.56 [0.31, 1.00]
Wang 2013	31	44	7	12	7.8%	1.70 [0.46, 6.36]
Xu 2013	168	223	16	21	17.4%	0.95 [0.33, 2.73]
Total (95% CI)		781		94	100.0%	0.73 [0.46, 1.15]
Total events	344		47			

Heterogeneity: Chi² = 2.66, df = 3 (P = 0.45); I² = 0%
Test for overall effect: Z = 1.36 (P = 0.18)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [experimental] Favours [control]

C

Study or Subgroup	Male Events	Total	Fermale Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2012	54	123	17	48	11.2%	1.43 [0.72, 2.85]
Liu 2014	92	181	27	76	15.2%	1.88 [1.08, 3.26]
Wagener 2010	92	320	53	200	37.8%	1.12 [0.75, 1.66]
Wang 2013	21	36	17	20	7.4%	0.25 [0.06, 1.00]
Wang 2015	72	94	57	71	12.4%	0.80 [0.38, 1.71]
Xu 2015	53	105	35	80	16.0%	1.31 [0.73, 2.35]
Total (95% CI)		859		495	100.0%	1.20 [0.94, 1.52]
Total events	384		206			

Heterogeneity: Chi² = 8.98, df = 5 (P = 0.11); I² = 44%
Test for overall effect: Z = 1.46 (P = 0.14)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [experimental] Favours [control]

Figure 3. A. The pooled OR from seven studies, which included 1289 non-metastatic RCC and 363 metastatic RCC. B. The pooled OR from five studies, which included 781 ccRCC and 94 pRCC. C. A total of 1354 RCC patients with either gender pooled in six studies. CI: confidence interval.
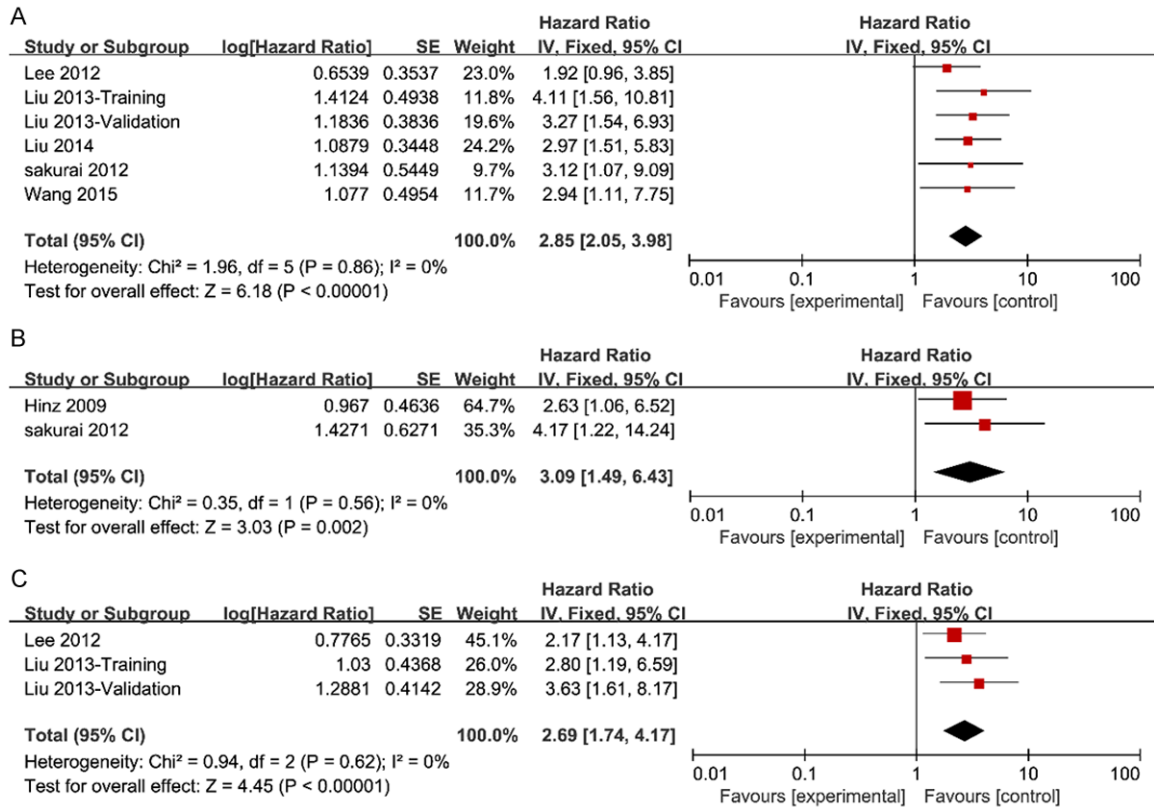
We used a sensitivity analysis to test whether the inclusion criteria of the individual studies affected the final results. All the results of the sensitivity analysis are shown in **Figure 5A-I**. Our data showed that no study had an obvious impact on the overall results, which indicated that our results of clinicopathological parameters and prognosis analyses were relatively stable and credible.

Discussion

Mounting evidence has shown that both genetic and epigenetic modifications play crucial roles in RCC carcinogenesis. EZH2 is the catalytically active constituent of the polycomb repressive complex 2 (PRC2) and participate in repressing gene expression through methylation of histone H3 on lysine 27 (H3K27), while EZH2 expression is associated with the meth-

ylation class of RCC tumors [27, 28]. Increased EZH2 expression has been shown to promote cell proliferation and inhibit apoptosis in RCC cell lines [29]. Upregulation of EZH2 expression has been correlated with bone metastasis in RCC [30]. Liang et al. found that MiR-138 is a tumor-suppressor miRNA that induces renal carcinoma cell senescence by downregulating EZH2 expression [31]. Hirata et al. demonstrated that long non-coding RNA MALAT1 promotes aggressive RCC through interactions with EZH2 [32]. These studies described the precise expression and prognostic impact of EZH2 in RCC; but the roles of EZH2 expression in RCC and clinical significance have not been thoroughly investigated. Our pooled data showed that (a) RCC tissue had a higher expression than normal renal tissue and that EZH2 plays a key role in the pathogenesis of RCC; (b) EZH2

A

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Fixed, 95% CI
Lee 2012	0.6539	0.3537	23.0%	1.92 [0.96, 3.85]
Liu 2013-Training	1.4124	0.4938	11.8%	4.11 [1.56, 10.81]
Liu 2013-Validation	1.1836	0.3836	19.6%	3.27 [1.54, 6.93]
Liu 2014	1.0879	0.3448	24.2%	2.97 [1.51, 5.83]
sakurai 2012	1.1394	0.5449	9.7%	3.12 [1.07, 9.09]
Wang 2015	1.077	0.4954	11.7%	2.94 [1.11, 7.75]
Total (95% CI)			**100.0%**	**2.85 [2.05, 3.98]**

Heterogeneity: Chi² = 1.96, df = 5 (P = 0.86); I² = 0%
Test for overall effect: Z = 6.18 (P < 0.00001)

B

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Fixed, 95% CI
Hinz 2009	0.967	0.4636	64.7%	2.63 [1.06, 6.52]
sakurai 2012	1.4271	0.6271	35.3%	4.17 [1.22, 14.24]
Total (95% CI)			**100.0%**	**3.09 [1.49, 6.43]**

Heterogeneity: Chi² = 0.35, df = 1 (P = 0.56); I² = 0%
Test for overall effect: Z = 3.03 (P = 0.002)

C

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Fixed, 95% CI
Lee 2012	0.7765	0.3319	45.1%	2.17 [1.13, 4.17]
Liu 2013-Training	1.03	0.4368	26.0%	2.80 [1.19, 6.59]
Liu 2013-Validation	1.2881	0.4142	28.9%	3.63 [1.61, 8.17]
Total (95% CI)			**100.0%**	**2.69 [1.74, 4.17]**

Heterogeneity: Chi² = 0.94, df = 2 (P = 0.62); I² = 0%
Test for overall effect: Z = 4.45 (P < 0.00001)

Figure 4. A. The six studies included that investigated the relationship between overall survival (OS) and EZH2 expression. (Liu 2013-Training = training sets, Liu 2013-Validation = validation sets). B. The two studies included investigated the relationship between progression-free survival (PFS) and EZH2 expression. C. The two studies included investigated the relationship between disease-free survival (DFS) and EZH2 expression. CI: confidence interval, RCC: renal cell carcinoma, SE: standard error.

expression was associated with the clinical staging, Fuhrman grade and metastatic status in RCC patients; (c) EZH2 expression was not strongly associated with gender or tumor type in the RCC patients; (d) RCC patients with high expression of EZH2 had a lower survival rate than those with a low expression; (e) EZH2 negatively regulated Y-box-binding protein 1 (YB-1), and positively regulated E-cadherin in RCC cells [24, 26]. Epithelial-mesenchymal transition (EMT) is a characteristic of cancer cell intravasation and metastasis. Loss of E-cadherin expression is a hallmark of EMT, and YB-1 regulates EMT-related factors by translational control; therefore, EZH2 modulates EMT signaling and promotes cancer cell migration and invasion in RCC cells [33, 34]; (f) The increased expression observed in solid tumor types has been linked to many factors including loss of EZH2-targeting miRNAs (e.g., mir-101, mir-26A) [19, 35]; and (g) in addition, this study have shown EZH2 promotes tumor progression by increasing vascular endothelial

growth factor (VEGF) expression in ccRCC [25]. The past studies demonstrated that VEGF-targeted therapies are standard treatment for metastatic renal cell carcinoma (mRCC) [36]. Objective is to study the relationship between EZH2 expression level and prognosis of renal cell carcinoma and to provide more objective evidence for the treatment of renal cell carcinoma.

Several limitations of this study need to be acknowledged. In the studies included, the antibodies used in detecting EZH2 expression were not the same. The definition of cut off value was also different. Besides, other clinical factors such as race, age, and different chemotherapies in each study might lead to bias. Non-English studies, unpublished studies, and studies that did not provide plenty data in HRs calculated did not contribute to assessing of the predictive value of EZH2 for survival. These approaches may have produced errors because of possible inaccurate reading. Finally, although
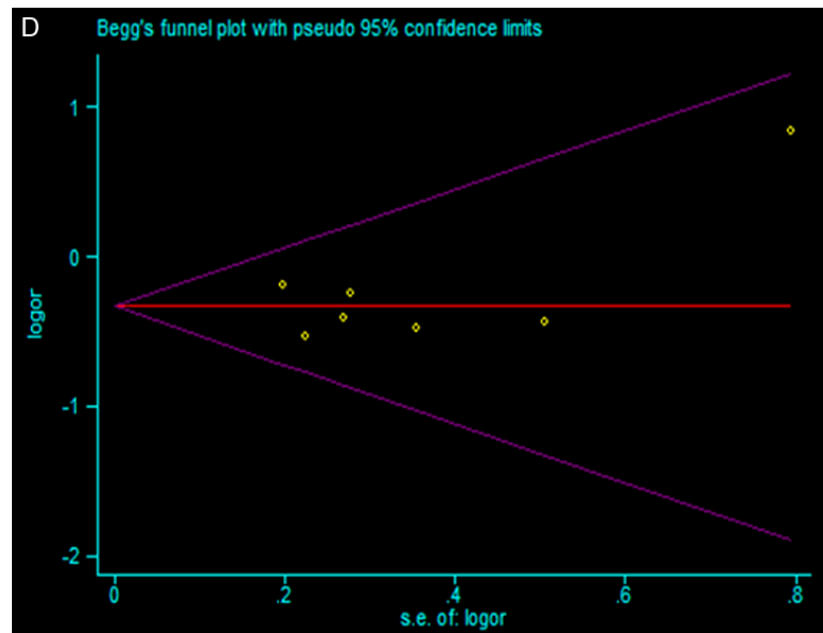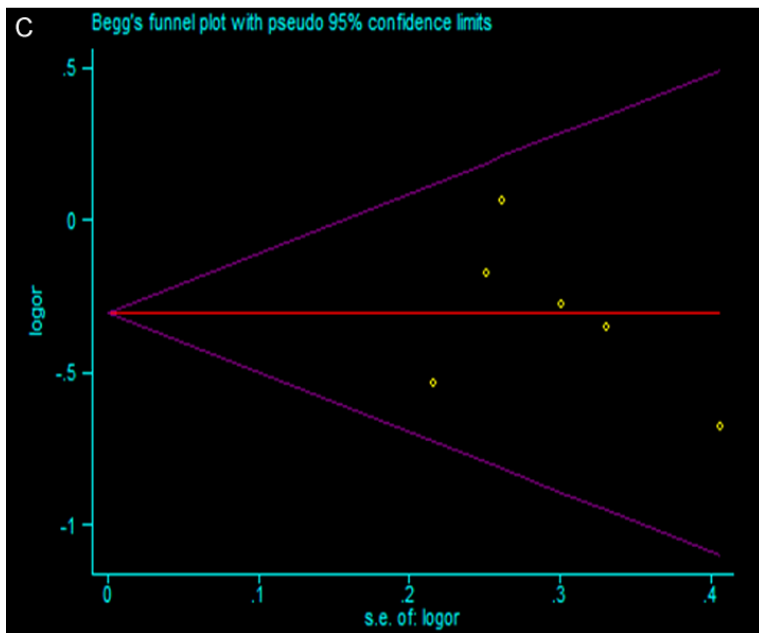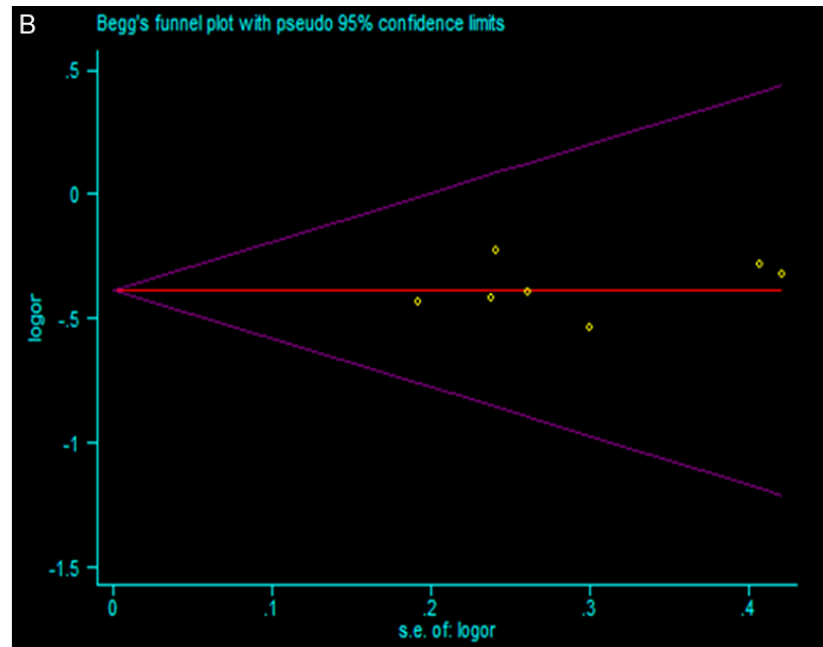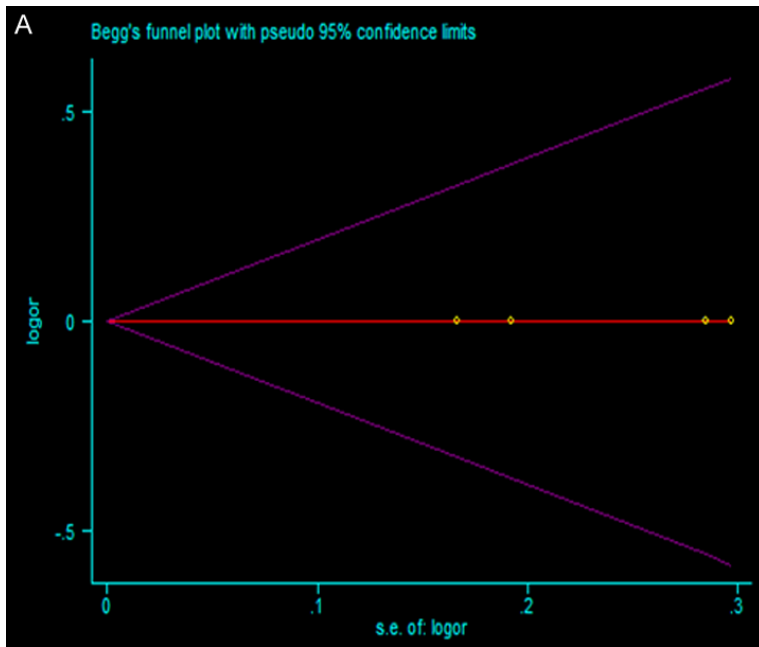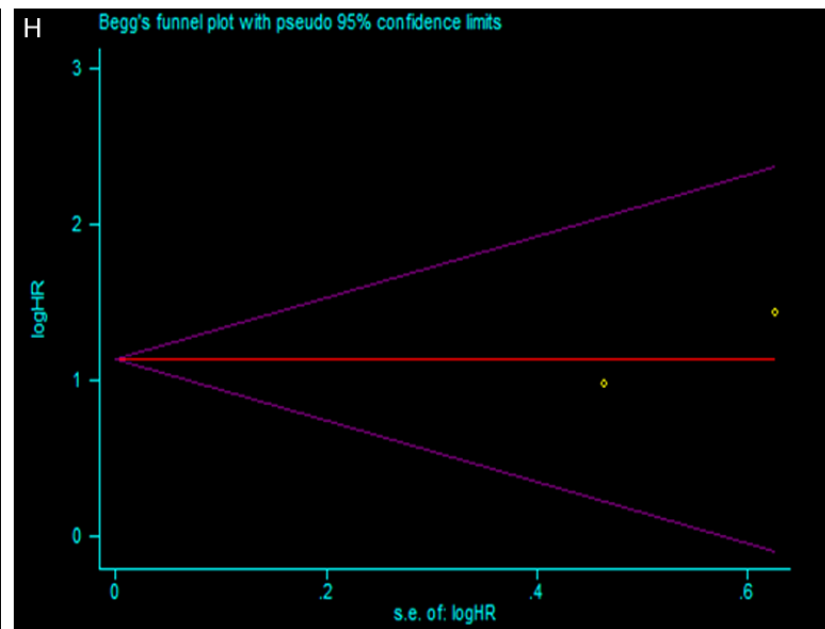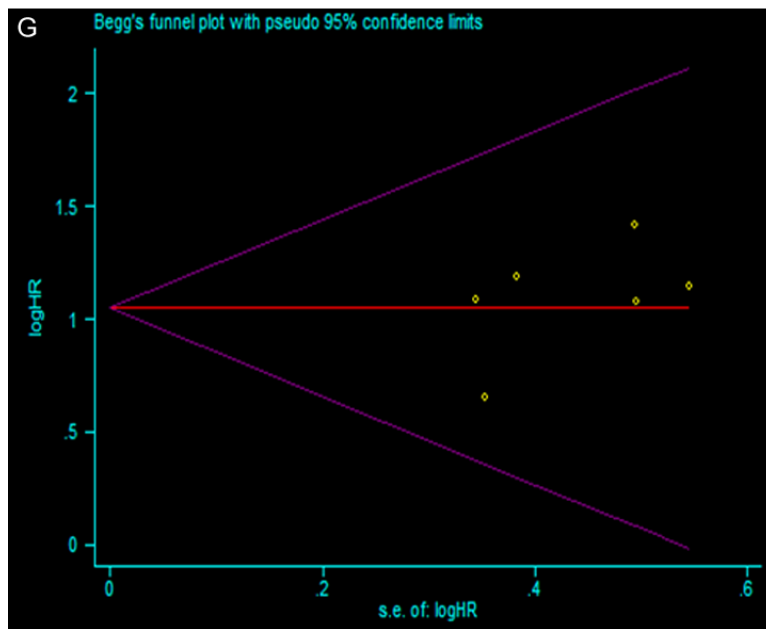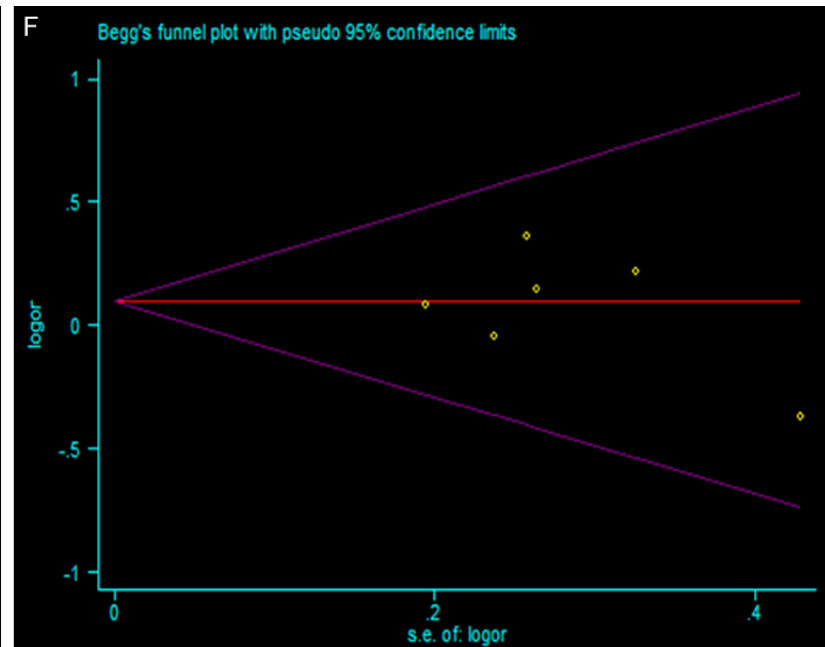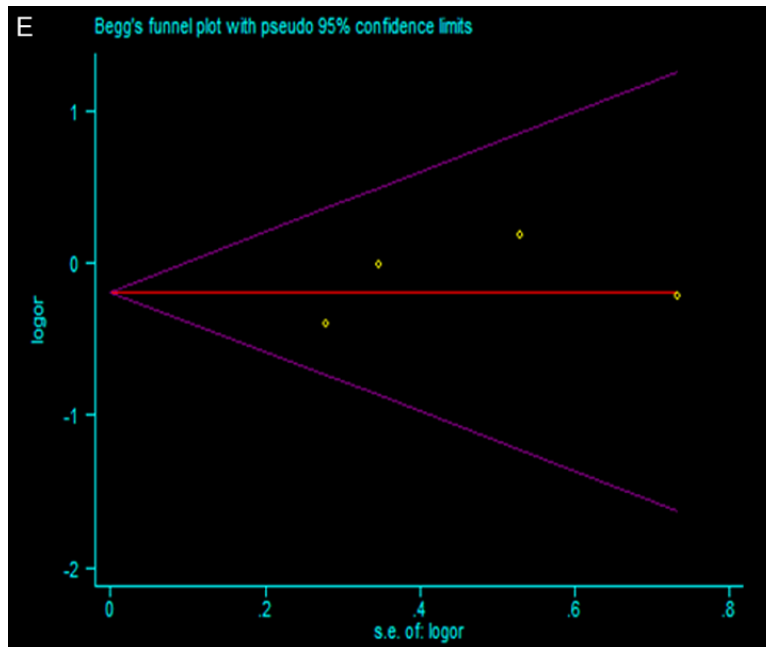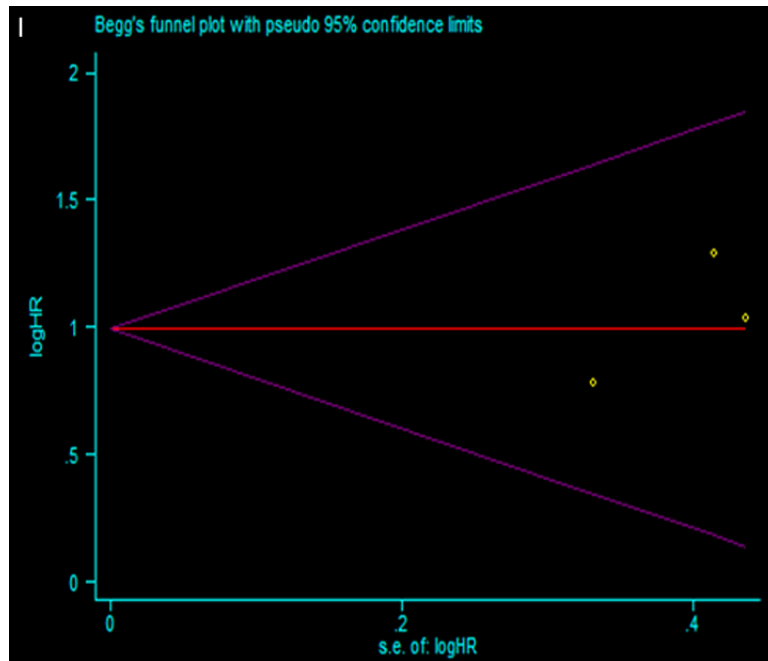
Figure 5. A. The funnel plots were largely symmetric, suggesting that there were no publication biases in the meta-analysis of EZH2 expression and clinicopathological features. Begg's funnel plot from four studies compared RCC and normal renal tissue. B. Begg's funnel plot from seven studies was used to determine EZH2 expression in RCC of different clinical stages. C. Begg's funnel plot from six studies compared EZH2 expression between high Fuhrman grades (III and IV) and low Fuhrman grades (I and II). D. Begg's funnel plot from four studies determined the relationship between EZH2 expression and metastatic status in RCC. E. Begg's funnel plot from six studies was used to determine the EZH2 expression and tumor type in the RCC patients. F. Begg's funnel plot from six studies was used to determine the relationship between EZH2 expression and sex in RCC. G. Begg's Funnel plots for OS. H. Begg's Funnel plots for PFS. I. Begg's Funnel plots for DFS.

we included 11 studies comprised of 2305 cases for this meta-analysis, some studies were categorized for subgroup analysis and several survival subgroup analysis lacks corresponding data. Therefore, more well-designed and large-scale trials are expected to confirm these findings.

To our knowledge, this meta-analysis is the first study to systematically evaluate the association between EZH2 expression and clinicopathological features and prognostic factors in RCC. Clear cell RCC is a major type of RCC and is more aggressive than othertypes of RCC. However, only one study determined that there was no significant difference in EZH2 expression between clear cell and other types of RCC (P = 0.18). Though larger well-designed studies with more ethnic groups, as well as larger population studies, are required, our meta-analysis demonstrated that EZH2 has a detrimental effect on the clinicopathological features and metastatic status in RCC. Therefore, it could serve as an independent prognostic factor of OS, RFS, and DFS. EZH2 may be a novel candidate for RCC genotyping and an indicator for predicting the prognosis of RCC patients.

Acknowledgements

Disclosure of conflict of interest

None.

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