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Review Article

Prognostic significance of Bmi-1 expression in patients with esophageal cancer: a meta-analysis

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Abstract: Objective: The prognostic role of B-cell-specific moloney leukemia virus insertion site 1 (Bmi-1) in esophageal cancer remains controversial. Therefore, a meta-analysis was performed to assess the association between Bmi-1 expression and prognostic effect in esophageal cancer patients. Methods: Eligible studies were identified by searching the online databases PubMed, Web of Science and China National Knowledge Infrastructure (CNKI) up to 20 October 2015. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to explore the relationships of Bmi-1 with patient survival. Results: A total of 11 eligible studies dealing with esophageal cancer were included in the analysis: 10 were dealing with overall survival, and 4 were with disease-free survival. High Bmi-1 was associated with poor overall survival (HR = 1.48, 95% CI 1.05-2.09, *P* = 0.026), and poor disease-free survival (HR = 1.42, 95% CI 1.06-1.90, *P* = 0.019). In subgroup analysis, high expression of Bmi-1 also predicted poor overall survival in Asian patients (HR = 1.90, 95% CI 1.48-2.44, *P* = 0.000), and esophageal squamous cell carcinoma patients (HR = 1.82, 95% CI 1.43-2.32, *P* = 0.000). Conclusions: This meta-analysis suggests that high Bmi-1 expression is associated with poor prognosis in esophageal cancer patients.

Keywords: Bmi-1, esophageal cancer, prognosis, meta-analysis

Introduction

Esophageal cancer (EC) is the eighth most frequent cancer and the sixth leading cause of cancer death worldwide [1]. Although the diagnosis and treatment of EC has improved, the 5-year survival rate for advanced stage disease is less than 20% [2]. Therefore, it is important for us to find a new molecular marker that could predict and improve the prognosis and reduce the mortality of patients with EC.

B-cell-specific moloney murine leukemia virus integration site 1 (Bmi-1) was first isolated as a proto-oncogene that cooperated with c-myc in generating lymphomas in a transgenic murine model [3]. It is a transcriptional repressor that belongs to the polycomb group (PcG) family of proteins involved in axial patterning, cell cycle regulation, hematopoiesis, apoptosis and senescence [4, 5]. Bmi-1 is correlated with poor prognosis in many malignant tumors, including lung cancer, gastric cancer, epithelial ovarian cancer and EC [6-9]. Recently, many studies have explored the prognostic role and clinicopathological outcomes in patients with EC, but the results remains controversial. Several studies showed that the high expression of Bmi-1 is positively associated with poor prognosis in EC [9, 10]. However, some studies described that Bmi-1 could not predict the prognosis of EC [11, 12]. Since the association of Bmi-1 overexpression with the prognosis of EC was not clear, a meta-analysis is necessary to comprehensively evaluate the prognostic significance of Bmi-1 expression in patients with EC.

Materials and methods

Literature search and selection criteria

We searched PubMed, Web of Science and China National Knowledge Infrastructure (CNKI) up to 20 October 2015 to identify relevant studies. We retrieved articles with combination of the following key words: "B-cell-specific moloney

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

Study	Year	Study location	Ethnicity	Study design	Pathological type	Method	Cutoff	Follow-up (month)	Number (patients)	Quality score#	Outcome	HR estimate
He	2009	China	Asian	R	ESCC	RT-PCR	Median	Mean 60	70	7	OS	SC
Liu	2010	China	Asian	R	ESCC	IHC	IRS≥4	Mean 25	171	8	OS	Reported
Yamada	2011	Japan	Asian	R	ESCC	IHC	Median	71.5 (2-164)	136	8	DFS	SC
Choy	2012	USA	Caucasian	R	ESCC	IHC	≥10%	39 (0.03-142)	34	8	OS	SC
Choy	2012	USA	Caucasian	R	EAC	IHC	≥10%	39 (0.03-142)	110	8	OS	SC
Ha	2012	Korea	Asian	R	ESCC	IHC	IRS≥6	Over 60	164	8	OS, DFS	Reported
Lv	2012	China	Asian	R	ESCC	RT-PCR	Median	42 (8-84)	70	7	OS	SC
Yoshikawa	2012	Japan	Asian	R	ESCC	IHC	≥5%	30 (3-120)	78	8	OS, DFS	Reported
Zhang	2014	China	Asian	R	ESCC	IHC	≥5%	Mean 60	80	8	OS	SC
Honing	2014	Holland	Caucasian	R	EAC	IHC	IRS≥6	40.5 ± 36.7	94	8	OS, DFS	Reported
Hwang	2014	China	Asian	R	ESCC	IHC	One-third	13 (0.3-57.4)	41	7	OS	SC

Study design is described as prospective (P) or retrospective (R). ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; RT-PCR: real-time PCR; IHC: immunohistochemistry; IRS: immunoreactivity score; OS, overall survival; DFS, disease free survival; SC, survival curve; #Study quality was judged based on the Newcastle-Ottawa scale (range, 1-9). Median: ≥50% of tumor cells positive; ≥10%: ≥10% of tumor cells positive; ≥5%: ≥5% of tumor cells positive; one-third: one-third of tumor cells positive.

leukemia virus insertion site 1", "Bmi-1", "Bmi1", "esophageal squamous cell carcinoma", "esophageal cancer", "esophageal carcinoma", "esophageal adenocarcinoma", and "esophageal cancer". The citation lists associated with the studies were used to identify additional eligible studies. The reviews and bibliographies were also manually inspected to find related articles.

Inclusion and exclusion criteria

Inclusion criteria for this meta-analysis were as follows: (1) Bmi-1 expression evaluated in the human EC tissues; (2) EC should be confirmed by histopathology; (3) evaluation of the relationships between Bmi-1 expression and overall survival (OS) or disease-free survival (DFS); (4) sufficient information provided to estimate the hazard ratios (HRs) with their 95% confidence intervals (CIs). The exclusion criteria were as follow: (1) letter, case report, review, and conference abstract without original data; (2) articles had no sufficient data to calculate the HR; and (3) overlapping article or those with duplicate data.

Data extraction and quality assessment

All data were evaluated and extracted independently by two authors (HLX and DYY). The following data were recorded: first author's name, year of publication, ethnicity, method, pathological type, total number of patients, cut-off value, the time of follow-up, outcome and HR estimate.

Study quality was assessed independently by two investigators (LZL and ZYY) according to the Newcastle-Ottawa Scale (NOS) quality assessment scale [13]. Three aspects were considered in the NOS criteria: (1) subject selection, 0~4; (2) comparability of subject, 0~2; (3) clinical outcome: 0~3. The range of NOS scores is from 0 to 9; and a score ≥7 means a good quality. Disagreements were resolved by discussion among all authors.

Statistical analysis

HRs with 95% CIs were calculated to estimate the association of Bmi-1 expression with OS and DFS. The HR with 95% CI in each eligible study was directly extracted from report. If only Kaplan-Meier curves were available, data were extracted indirectly from survival curves to extrapolate HRs with their 95% CIs, using previously described methods [14, 15]. The χ^2-based Q statistical test and the I^2 statistic were used to evaluate the heterogeneity among studies [16]. If the heterogeneity was significant between studies (I^2>50% or P<0.10), the random-effects model was used; otherwise, the fixed-effects model was used [17]. Funnel plots and Egger's linear regression test were applied to investigate publication bias [18]. The statistical analyses were performed using STATA version 12.0 software (Stata Corporation, Collage Station, Texas, USA). All P values were two-sided and a P<0.05 was considered statistically significant.

Results

Study characteristics

After careful read and selection, a total of 11 studies [9-12, 19-24] were retrieved according

Table 2. Main meta-analysis results of Bmi-1 expression in patients with esophageal cancer

Analysis	Number of studies	Number of patients	HR (95% CI)	*P* value	Heterogeneity	
					I^2 (%)	*P* value
OS						
Overall	10	912	1.48 (1.05-2.09)[b]	0.026*	61.2	0.006
Ethnicity						
Asian	7	674	1.90 (1.48-2.44)[a]	0.000*	32.5	0.180
Caucasian	3	238	0.90 (0.66-1.23)[a]	0.519	0.0	0.600
Pathological type						
ESCC	8	708	1.82 (1.43-2.32)[a]	0.000*	33.5	0.161
EAC	2	204	0.89 (0.65-1.24)[a]	0.505	0.0	0.319
HR estimate						
Survival curves	6	405	1.40 (0.81-2.43)[b]	0.229	64.4	0.015
Reported	4	507	1.58 (0.97-2.58)[b]	0.066	64.8	0.036
Method						
RT-PCR	2	140	1.66 (0.98-2.81)[a]	0.061	0.0	0.503
IHC	8	772	1.46 (0.96-2.22)[a]	0.077	68.7	0.002
DFS	4	472	1.42 (1.06-1.90)[a]	0.019*	34.7	0.204

ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; HR: hazard ratio; CI: confidence intervals. OS, overall survival; DFS, disease free survival. *Indicates that the difference was statistically significant. [a]Fixed-effects model. [b]Random-effects model.

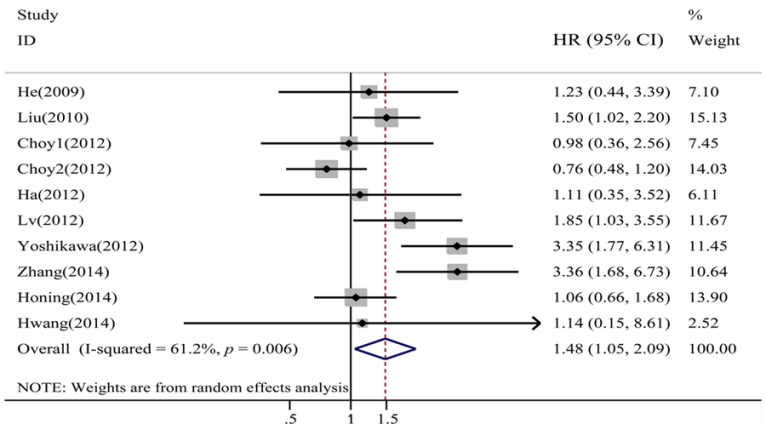

Figure 1. Forest plots for the relationship between Bmi-1 expression and overall survival.

to the inclusion and exclusion criteria. The main characteristics of the included studies are listed in **Table 1**. The total number of patients included was 1048, ranging from 34 to 171 per study. Among these 11 studies, 8 studies evaluated Asian patients, and 3 studies evaluated Caucasian patients. Nine studies investigated esophageal squamous cell carcinoma (ESCC) patients, and two studies investigated esophageal adenocarcinoma (EAC) patients. HRs with 95% CIs were reported directly in five studies, and extrapolated from Kaplan-Meier curves in six studies. The OS was observed in ten studies, and the DFS was presented in four studies. There were two major approaches for the evaluation of Bmi-1 in EC: quantitative real-time polymerase chain reaction and immunohistochemistry. The NOS scores of all included studies were ≥7.

Bmi-1 expression and OS in patients with EC

The main results of this meta-analysis are listed in **Table 2**. Meta-analysis of OS was conducted in 10 studies. Our analysis suggested that high expression of Bmi-1 was significantly associated with poor OS (HR = 1.48, 95% CI 1.05-2.09, *P* = 0.026) with heterogeneity (I^2 = 61.2%, *P* = 0.006) (**Figure 1**). Subgroup analysis was performed by ethnicity, pathological type and HR estimate, and the main results are shown in **Table 2**. In the ethnicity subgroup, high Bmi-1 expression predicted poor OS in Asians (HR = 1.90, 95% CI 1.48-2.44, *P* = 0.000), but not in Caucasians (HR = 0.90, 95% CI 0.66-1.23, *P* = 0.519) (**Figure 2**). When grouped according to pathological type, a

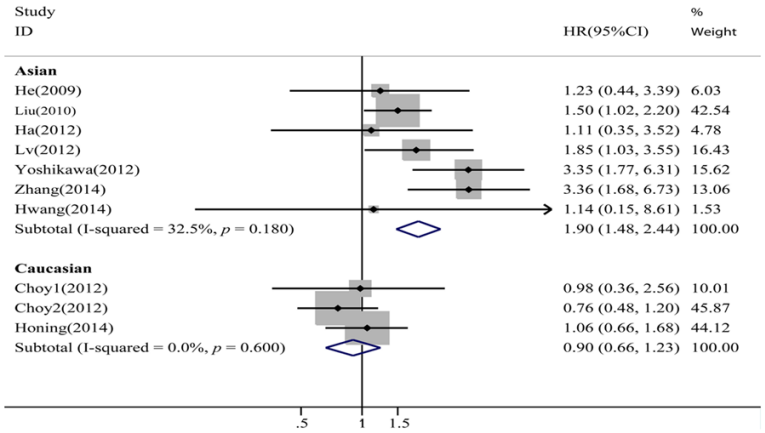

Figure 2. Forest plots for the relationship between Bmi-1 expression and overall survival stratified by ethnicity.

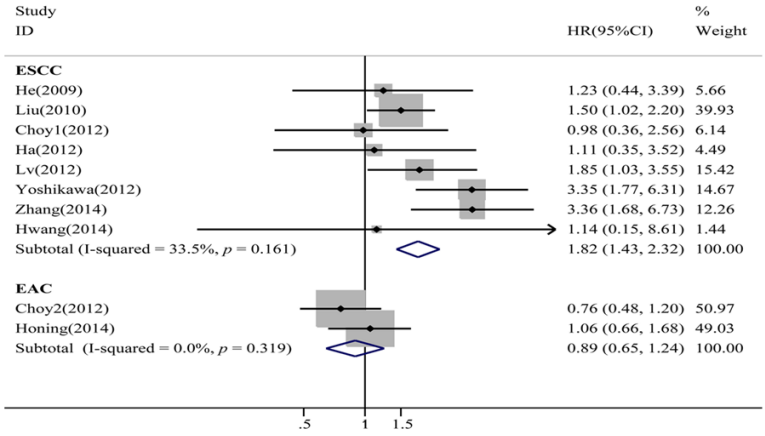

Figure 3. Forest plots for the relationship between Bmi-1 expression and overall survival stratified by pathological type.

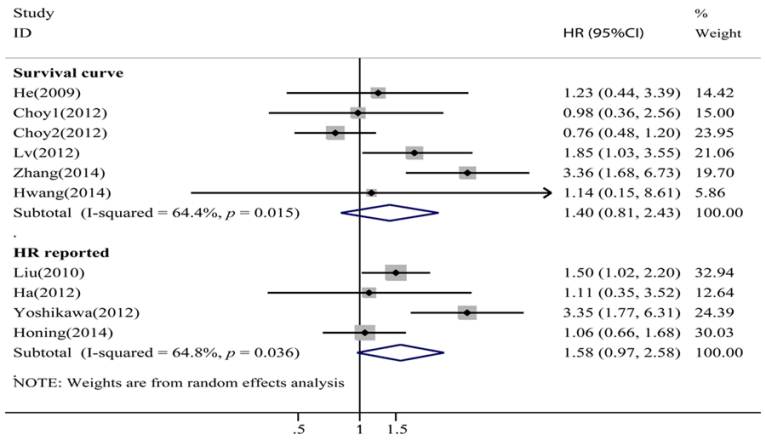

Figure 4. Forest plots for the relationship between Bmi-1 expression and overall survival stratified by HR estimate.

significant relationship between Bmi-1 expression and OS was observed in esophageal squamous cell carcinoma (ESCC) patients (HR = 1.82, 95% CI 1.43-2.32, $P = 0.000$), but not in esophageal adenocarcinoma (EAC) patients (HR = 0.89, 95% CI 0.65-1.24, $P = 0.505$) (**Figure 3**). In addition, no significant relevance was observed in subgroups of HR reported directly in articles (HR = 1.58, 95% CI 0.97-2.58, $P = 0.066$), and HR estimated indirectly by survival curves (HR = 1.40, 95% CI 0.81-2.43, $P = 0.229$) (**Figure 4**).

Bmi-1 expression and DFS in patients with EC

Four studies including 472 patients were eligible for the final analysis. Our analysis suggested that high Bmi-1 expression was significant associated with worse DFS (HR = 1.42, 95% CI 1.06-1.90, $P = 0.019$) without heterogeneity ($I^2 = 34.7\%$, $P = 0.204$) (**Figure 5**).

Sensitivity analyses and publication bias

In order to evaluate the influence of single studies on the pooled HRs, we performed a sensitivity analysis by estimating the average HR in the absence of each study. The results indicated that no individual studies significantly influenced the pooled HRs (**Figure 6**).

As shown in **Figure 7**, funnel plots demonstrated no evidence of obvious asymmetry in any of the included studies for OS or DFS, and Egger's test also showed no obvious publication bias in the studies for either of the two outcomes (OS, $P = 0.726$; DFS, $P = 0.863$).

Discussion

The up-regulation of Bmi-1 expression was observed in various types of human cancers,

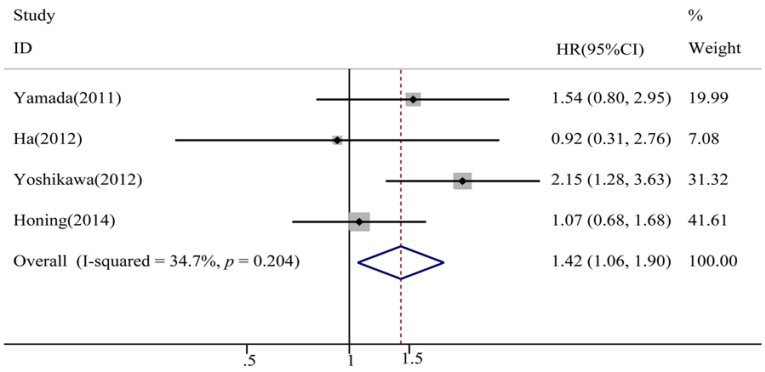

Figure 5. Forest plots for the relationship between Bmi-1 expression and disease-free survival.

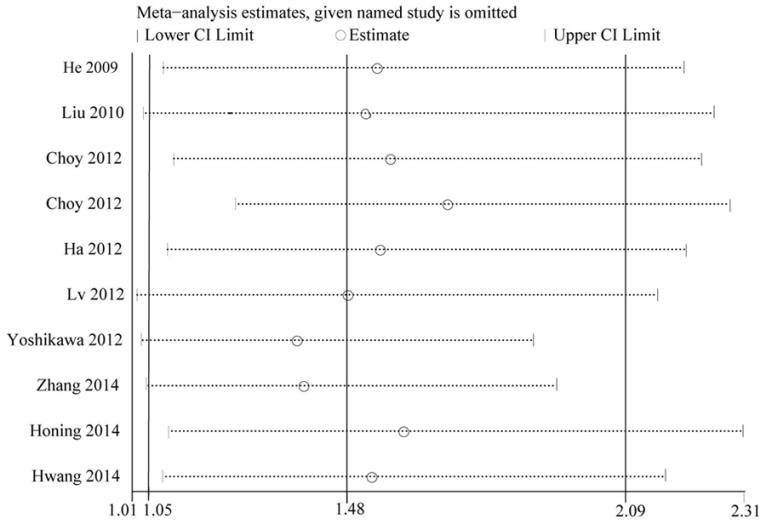

Figure 6. Sensitivity analysis for the pooled HRs in OS. The analysis was conducted by estimating the average HR in the absence of each study. HRs = hazard ratios; OS = overall survival.

including lung cancer [25], ovarian cancer [26] and nasopharyngeal carcinoma [27], which indicates that Bmi-1 might be considered as one of potential biomarkers for cancer prognosis. The human Bmi-1 gene is located at zone 13 of the 10th short arm of the chromosome, i.e., 10p13, which contains 10 exons and 10 introns. The NK4a/ARF site is the downstream control site of the Bmi-1 gene. Bmi-1 controls the self-renewal, proliferation and cell cycle of cancer stem cells by regulating $p16^{Ink4a}$/Rb and/or $p14^{ARF}$/MDM2/p53 tumor suppressor pathways [5, 28]. In addition, elevated Bmi-1 expression can induce stem-like properties and epithelial-mesenchymal transition (EMT), an initiating event of tumor metastasis acts by repressing the tumor suppressor PTEN or cooperating with Twist1 [29, 30]. Many studies have reported that Bmi-1 was involved in the development of EC; however, its prognostic significance for EC patients remains controversial. Therefore, we conducted this meta-analysis to determine whether Bmi-1 could be a prognosis factor in EC.

The present systematic review included 11 studies with 1048 patients to evaluate the prognostic effect of Bmi-1 overexpression in EC, representing the most comprehensive summary of available evidence on this topic so far. Bmi-1 overexpression was found to be associated with both worse OS (HR = 1.48, 95% CI 1.05-2.09, $P = 0.026$) and worse DFS (HR = 1.42, 95% CI 1.06-1.90, $P = 0.019$) in EC. Specifically, Bmi-1 overexpressed patients have a 48% higher risk of death and a 42% higher risk of disease recurrence compared with those without Bmi-1 overexpression. It may suggest that detected Bmi-1 expression could be a prognostic factor in esophageal cancer.

There are some possible limitations in the present meta-analysis. Firstly, the cut-off value of Bmi-1 expression was not consistent among included studies, and our conclusion may be less powerful. The cut-off value may be different with the actual value and would influence the effectiveness of Bmi-1 as a prognostic marker in esophageal cancer. In future, a large multicenter study using the same detection method and cut-off of Bmi-1 expression may be helpful to obtain more accurate results. Secondly, only two studies focused on EAC patients, which made it difficult to draw a firm conclusion on the prognostic value of Bmi-1 for EAC patients. Thirdly, HRs extrapolated from survival curves might be less reliable than reported directly in articles.

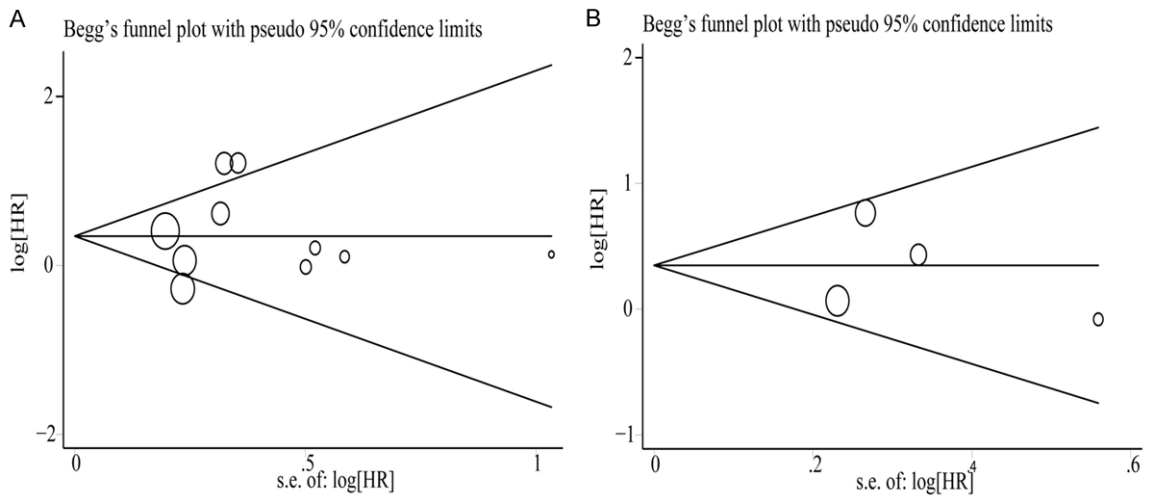

Figure 7. Funnel plots of the meta-analysis assessing (A) Bmi-1 expression and overall survival (B) Bmi-1 expression and disease-free survival.

In summary, the present meta-analysis showed elevated Bmi-1 expression levels to be closely associated with poor prognosis in EC patients. More multi-center clinical investigations with larger sample sizes should be conducted to confirm these findings.

Disclosure of conflict of interest

None.

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References

[1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[2] Wang J, Ge J, Zhang XH, Liu JY, Yang CM and Zhao SL. Endoscopic submucosal dissection versus endoscopic mucosal resection for the treatment of early esophageal carcinoma: a meta-analysis. Asian Pac J Cancer Prev 2014; 15: 1803-6.

[3] van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H and Berns A. Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. Cell 1991; 65: 737-52.

[4] van der Lugt NM, Domen J, Linders K, van Roon M, Robanus-Maandag E, te Riele H, van der Valk M, Deschamps J, Sofroniew M, van Lohuizen M, et al. Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. Genes Dev 1994; 8: 757-69.

[5] Jacobs JJ, Kieboom K, Marino S, DePinho RA and van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature 1999; 397: 164-8.

[6] Zhang X, Sun J, Wang H, Lou Y, Zhang Y, Sha H, Feng J and Han B. IGF-1R and Bmi-1 expressions in lung adenocarcinoma and their clinicopathologic and prognostic significance. Tumour Biol 2014; 35: 739-45.

[7] Lu H, Sun HZ, Li H and Cong M. The clinicopathological significance of Bmi-1 expression in pathogenesis and progression of gastric carcinomas. Asian Pac J Cancer Prev 2012; 13: 3437-41.

[8] Abd El hafez A, El-Hadaad HA. Immunohistochemical expression and prognostic relevance of Bmi-1, a stem cell factor, in epithelial ovarian cancer. Ann Diagn Pathol 2014; 18: 58-62.

[9] He XT, Cao XF, Ji L, Zhu B, Lv J, Wang DD, Lu PH and Cui HG. Association between Bmi1 and clinicopathological status of esophageal squamous cell carcinoma. World J Gastroenterol 2009; 15: 2389-94.

[10] Yoshikawa R, Tsujimura T, Tao L, Kamikonya N and Fujiwara Y. The oncoprotein and stem cell renewal factor BMI1 associates with poor clinical outcome in oesophageal cancer patients undergoing preoperative chemoradiotherapy. BMC Cancer 2012; 12: 461.

[11] Choy B, Bandla S, Xia Y, Tan D, Pennathur A, Luketich JD, Godfrey TE, Peters JH, Sun J and Zhou Z. Clinicopathologic characteristics of

high expression of Bmi-1 in esophageal adenocarcinoma and squamous cell carcinoma. BMC Gastroenterol 2012; 12: 146.
[12] Hwang CC, Nieh S, Lai CH, Tsai CS, Chang LC, Hua CC, Chi WY, Chien HP, Wang CW, Chan SC, Hsieh TY and Chen JR. A retrospective review of the prognostic value of ALDH-1, Bmi-1 and Nanog stem cell markers in esophageal squamous cell carcinoma. PLoS One 2014; 9: e105676.
[13] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-5.
[14] Parmar MK, Torri V and Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998; 17: 2815-34.
[15] Tierney JF, Stewart LA, Ghersi D, Burdett S and Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8: 16.
[16] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-60.
[17] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-88.
[18] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-34.
[19] Liu WL, Guo XZ, Zhang LJ, Wang JY, Zhang G, Guan S, Chen YM, Kong QL, Xu LH, Li MZ, Song LB and Zeng MS. Prognostic relevance of Bmi-1 expression and autoantibodies in esophageal squamous cell carcinoma. BMC Cancer 2010; 10: 467.
[20] Ha SY, Kim SH. Co-expression of Bmi1 and EZH2 as an independent poor prognostic factor in esophageal squamous cell carcinoma. Pathol Res Pract 2012; 208: 462-9.
[21] Lv J, Cao XF, Ji L, Zhu B, Wang DD, Tao L and Li SQ. Association of beta-catenin, Wnt1, Smad4, Hoxa9, and Bmi-1 with the prognosis of esophageal squamous cell carcinoma. Med Oncol 2012; 29: 151-60.
[22] Zhang Y, Zhang YL, Chen HM, Pu HW, Ma WJ, Li XM, Ma H and Chen X. Expression of Bmi-1 and PAI-1 in esophageal squamous cell carcinoma. World J Gastroenterol 2014; 20: 5533-9.
[23] Honing J, Pavlov KV, Meijer C, Smit JK, Boersma-van Ek W, Karrenbeld A, Burgerhof JG, Kruyt FA and Plukker JT. Loss of CD44 and SOX2 expression is correlated with a poor prognosis in esophageal adenocarcinoma patients. Ann Surg Oncol 2014; 21 Suppl 4: S657-64.
[24] Yamada A, Fujii S, Daiko H, Nishimura M, Chiba T and Ochiai A. Aberrant expression of EZH2 is associated with a poor outcome and P53 alteration in squamous cell carcinoma of the esophagus. Int J Oncol 2011; 38: 345-53.
[25] Vonlanthen S, Heighway J, Altermatt HJ, Gugger M, Kappeler A, Borner MM, van Lohuizen M and Betticher DC. The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with INK4A-ARF locus expression. Br J Cancer 2001; 84: 1372-6.
[26] Zhang F, Sui L and Xin T. Correlations of BMI-1 expression and telomerase activity in ovarian cancer tissues. Exp Oncol 2008; 30: 70-4.
[27] Song LB, Zeng MS, Liao WT, Zhang L, Mo HY, Liu WL, Shao JY, Wu QL, Li MZ, Xia YF, Fu LW, Huang WL, Dimri GP, Band V and Zeng YX. Bmi-1 is a novel molecular marker of nasopharyngeal carcinoma progression and immortalizes primary human nasopharyngeal epithelial cells. Cancer Res 2006; 66: 6225-32.
[28] Park IK, Morrison SJ and Clarke MF. Bmi1, stem cells, and senescence regulation. J Clin Invest 2004; 113: 175-9.
[29] Song LB, Li J, Liao WT, Feng Y, Yu CP, Hu LJ, Kong QL, Xu LH, Zhang X, Liu WL, Li MZ, Zhang L, Kang TB, Fu LW, Huang WL, Xia YF, Tsao SW, Li M, Band V, Band H, Shi QH, Zeng YX and Zeng MS. The polycomb group protein Bmi-1 represses the tumor suppressor PTEN and induces epithelial-mesenchymal transition in human nasopharyngeal epithelial cells. J Clin Invest 2009; 119: 3626-36.
[30] Wu KJ. Direct activation of Bmi1 by Twist1: implications in cancer stemness, epithelial-mesenchymal transition, and clinical significance. Chang Gung Med J 2011; 34: 229-38.