

Original Article

Prognostic significance of EZH2 expression in patients with digestive cancers: a meta-analysis

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Abstract: Background: Enhancer of zeste 2 (EZH2), a key component of polycomb repressive complex 2 (PRC2), was of great importance in human cancer pathogenesis. Various studies examined the relationship between EZH2 overexpression with the clinical outcome in patients with digestive cancers, but yielded inconsistent results. Methods: Electronic databases updated to January 2015 were searched to find relevant studies. A meta-analysis was conducted with eligible studies which quantitatively evaluated the relationship between EZH2 overexpression and survival of patients with digestive cancers. Survival data were aggregated and quantitatively analyzed. Results: We performed a meta-analysis of 10 studies (n = 1,461 patients) that evaluated the correlation between EZH2 overexpression and survival in patients with digestive cancers. Combined hazard ratios suggested that EZH2 overexpression was associated with poor prognosis of overall survival (HR = 1.54, 95% CI: 1.27-1.81) in patients with esophageal cancer. In the stratified analysis, no significant risks were found among gastric cancer (HR = 0.66, 95% CI: 0.16-1.15) and colorectal cancer (HR = 0.91, 0.63-1.19), indicating EZH2 was not an indicator of poor prognosis in gastric cancer or colorectal cancer. Conclusions: EZH2 overexpression indicates a poor prognosis for patients with esophageal cancer, but not among gastric cancer or colorectal cancer.

Keywords: Enhancer of zeste homolog 2, esophageal cancer, colorectal cancer, gastric cancer, prognosis, meta-analysis

Introduction

Digestive system malignant tumors, with 3.4 million new diagnosed cases and 1.5 million deaths each year, are the most common cancers worldwide [1]. Digestive cancers are complex, multistep, multifactorial, and highly fatal diseases. Digestive cancers contain alimentary tract and digestive gland cancers. Among them, colorectal, gastric, and esophagus cancers were common cancers with high incidence and mortality in the world. Despite recent advances in treatment, the prognosis of patients with cancers in digestive system remains poor. Numerous studies have reported molecular predictors of prognosis of patients with digestive system cancers [2-4]. However, no this kind of specific molecular biomarker has been accepted commonly and used routinely until now. The clinically applicable biomarkers for prognosis analysis are urgently required.

Enhancer of zeste homolog 2 (EZH2) is a key component of the polycomb repressive com-

plex 2 (PCR2), which possesses histone methyltransferase activity and mediates gene silencing through posttranslational histone modifications [5]. EZH2 is frequently overexpressed in a wide variety of human malignancies such as breast cancer [6], prostate cancer [7] and lung cancer. In addition, it also promotes cancer development and progression through chromatin modification by epigenetic activation of oncogenic signaling cascades and silencing of tumor suppressor genes, and has been implicated in cell proliferation, differentiation, invasion, and metastasis [8]. Thus, it is acting with oncogenic properties.

Many studies have evaluated whether the overexpression of EZH2 may be a prognostic factor for survival in patients with digestive cancers. However, the results of the studies are inconclusive and no consensus has been reached. It is unknown whether differences in these investigations have been mostly due to their limited sample size or genuine heterogeneity. Thus, we conducted a meta-analysis of all available stud-

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Table 1. Main characteristics and results of the eligible studies

First author-year	Patients source	Histology	Stage	N pts	Method	Positive (%)	HR estimation	Survival results
Benard 2014	Netherlands	Colorectal cancer	I-IV	408	IHC	NA	HR and 95% CI 0.84 (0.60-1.18)	NS
Liu 2014	China	Colorectal cancer	I-IV	82	Realtime-PCR	NA	HR and 95% CI 2.51 (1.10-5.74)	Poor
Wang 2010	China	Colorectal cancer	I-IV	119	IHC	32.8	HR and 95% CI 3.21 (1.06-9.73)	Poor
Kodach LL 2010	Netherlands	Colorectal cancer	NA	72	IHC	46	Survival curves 1.42 (0.65-2.93)	NS
He 2012	China	Gastric cancer	I-IV	117	IHC	70	HR and 95% CI 1.88 (1.17-3.03)	Poor
Lee 2012	South Korea	Gastric cancer	I-IV	178	IHC	92.1	HR and 95% CI 0.11 (0.01-1.20)	NS
Matsukawa 2006	Japan	Gastric cancer	I-IV	83	IHC	56.6	Survival curves 1.72 (0.06-5.89)	NS
Ha 2008	South Korea	Esophageal cancer	I-IV	164	IHC	52.4	Survival curves 1.24 (0.83-1.68)	NS
Wang H 2013	China	Esophageal cancer	I-IV	102	IHC	65.7	Survival curves 2.47 (1.75-3.43)	Poor
Yamada 2011	Japan	Esophageal Cancer	I-IV	136	IHC	14	Survival curves 1.59 (1.25-2.03)	Poor

IHC, immunohistochemistry; NS, not significant; NA: not applicable; HR, hazard ratio; N pts, number of patients; PCR, polymerase chain reaction.

ies relating EZH2 with the clinical outcome in patients with digestive cancers including esophageal cancer, gastric cancer and colorectal cancer.

Materials and methods

Search strategy and study selection

The electronic databases PubMed, Embase and CNKI (China National Knowledge Infrastructure) were searched for studies to include in the present meta-analysis. An upper date limit of Jan 01, 2015 was applied; we used no lower date limit. Searches included the terms “esophageal or gastric or colorectal”, “cancer or carcinoma or tumour or neoplasm”, “EZH2”, “Enhancer of zeste homolog 2”, and “prognosis”. We also reviewed the Cochrane Library for relevant articles. The references reported in the identified studies were also used to complete the search.

Studies eligible for inclusion in this meta-analysis met the following criteria: (1) measure EZH2 expression in the primary colorectal cancer or gastric cancer or esophageal cancer with IHC (immunohistochemistry) or RealTime-PCR (polymerase chain reaction); (2) provide information on survival (i.e. overall survival [OS], studies investigating response rates only were excluded); (3) When the same author reported results obtained from the same patient population in more than one publication, only the most recent report, or the most complete one, was included in the analysis. Two reviewers (W.W. and F.W.) independently determined study eligibility.

Data extraction and quality assessment

The final articles included were assessed independently by two reviewers (W.W. and F.W.).

Data retrieved from the reports included author, publication year, patient source, histology, test method, positive, follow-up and survival data (Table 1). If data from any of the above categories were not reported in the primary study, items were treated as “not applicable”. We did no contact the author of the primary study to request the information. We did not use pre-specified quality-related inclusion or exclusion criteria and did not weigh each study by a quality score, because the quality score has not received general agreement for use in a meta-analysis, especially observational studies [9].

Statistical methods

For the quantitative aggregation of the survival results, we measured the impact of EZH2 overexpression on survival by HR between the two survival distributions. HRs and 95% confidence intervals (CIs) were used to combine as the effective value. If the HRs and their 95% CIs were given explicitly in the articles, we used crude ones. When these variables were not given explicitly, they were calculated from the available numerical data using methods reported by Parmar et al. [10].

Heterogeneity of the individual HRs was calculated with χ^2 tests according to Peto's method [11]. Heterogeneity test with inconsistency index (I^2) statistic and Q statistic was performed. If HRs were found to have fine homogeneity, a fixed effect model was used for secondary analysis; if not, a random-effect model was used. DerSimonian-Laird random effects analysis [12] was used to estimate the effect of EZH2 overexpression on survival. By convention, an observed HR > 1 implies worse survival for the group with EZH2 overexpression. The impact of EZH2 on survival was considered to

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Table 2. Meta-analysis: HR value in colorectal cancer, gastric cancer and esophageal cancer

	Nb	Patients	Combined HR (95% CI)	χ^2 heterogeneity test (P)
Overall	10	1461	1.15 (0.97-1.33)	0.000
Esophageal cancer	3	402	1.54 (1.27-1.81)	0.035
Gastric cancer	3	378	0.66 (0.16-1.15)	0.006
Colorectal cancer	4	681	0.91 (0.63-1.19)	0.272

Abbreviations: HR, hazard ratio; Nb, number of studies; CIs, confidence intervals.

be statistically significant if the 95% confidence interval (CI) did not overlap with 1. Horizontal lines represent 95% CIs. Each box represents the HR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (HR = 1.0).

Evidence of publication bias was sought using the methods of Egger et al. [13] and of Begg et al. [14]. Intercept significance was determined by the *t* test suggested by Egger ($P < 0.05$ was considered representative of statistically significant publication bias). All of the calculations were performed by STATA version 11.0 (Stata Corporation, College Station, TX).

Results

Study selection and characteristics

Ten studies [15-24] published between 2006 and 2014 were eligible for this meta-analysis. All reported the prognostic value of EZH2 status for survival in colorectal cancer or gastric cancer or esophageal cancer patients. The total number of patients included was 1461, ranging from 82 to 408 patients per study (median 146). The major characteristics of the 10 eligible publications are reported in **Table 1**. The studies were conducted in 4 countries (China, South Korea, Netherlands and Japan). Among the 10 studies, 8 studies (981 patients, 67.1%) were performed in Asian populations, and the remaining 2 studies (480 patients, 32.9%) followed Netherlands patients. All patients in the eligible studies were determined by pathological stage.

All of the studies reported the prognostic value of EZH2 status for survival in patients with lung cancer. Of the 10 studies, 5 directly reported HRs (multivariate analysis), while the other 5

studies provided survival curves. Five of the 10 studies identified EZH2 overexpression as an indicator of poor OS, and the other 5 studies showed no statistically significant impact of EZH2 overexpression on OS.

Meta-analysis

The results of the meta-analysis were shown in **Table 2** and **Figure 1**. Overall, the combined HR for all 10 eligible studies evaluating EZH2 overexpression on OS was (HR = 1.15, 95% CI: 0.97-1.33), suggesting that EZH2 overexpression was not associated with poor prognosis for combined effect of the three digestive cancer. No significant heterogeneity was observed among the studies ($Q = 5.89$, $I^2 = 74.7\%$, $P = 0.000$).

When grouped according to the various histological types of digestive cancer, the combined HRs of esophageal cancer was (HR = 1.54, 95% CI: 1.27-1.81), indicating EZH2 was an indicator of poor prognosis in esophageal cancer ($P = 0.035$ for heterogeneity test). However, the combined HRs of gastric cancer and colorectal cancer were (HR = 0.66, 95% CI: 0.16-1.15) and (HR = 0.91, 95% CI: 0.63-1.19), respectively, indicating EZH2 was not an indicator of poor prognosis in gastric cancer or colorectal cancer.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All 10 eligible studies investigating EZH2 overexpression on overall survival yielded a Begg's test score of $P = 0.348$ and an Egger's test score of $P = 0.461$, meanwhile according to the funnel plot (**Figure 2**), the absence of publication bias was found. Similar results were found for investigating EZH2 overexpression on overall survival of the three digestive cancers. These results suggested that there were no publication biases in the subgroup analyses.

Discussion

EZH2 is the catalytic subunit of PRC2, which catalyses the addition of methyl groups to lysine 27 on histone H3 (H3K27) in the promoters of target genes, leading to repression of gene transcription [25, 26]. The gene of EZH2, encoding a polycomb group protein, plays an important role in tumorigenesis and cancer

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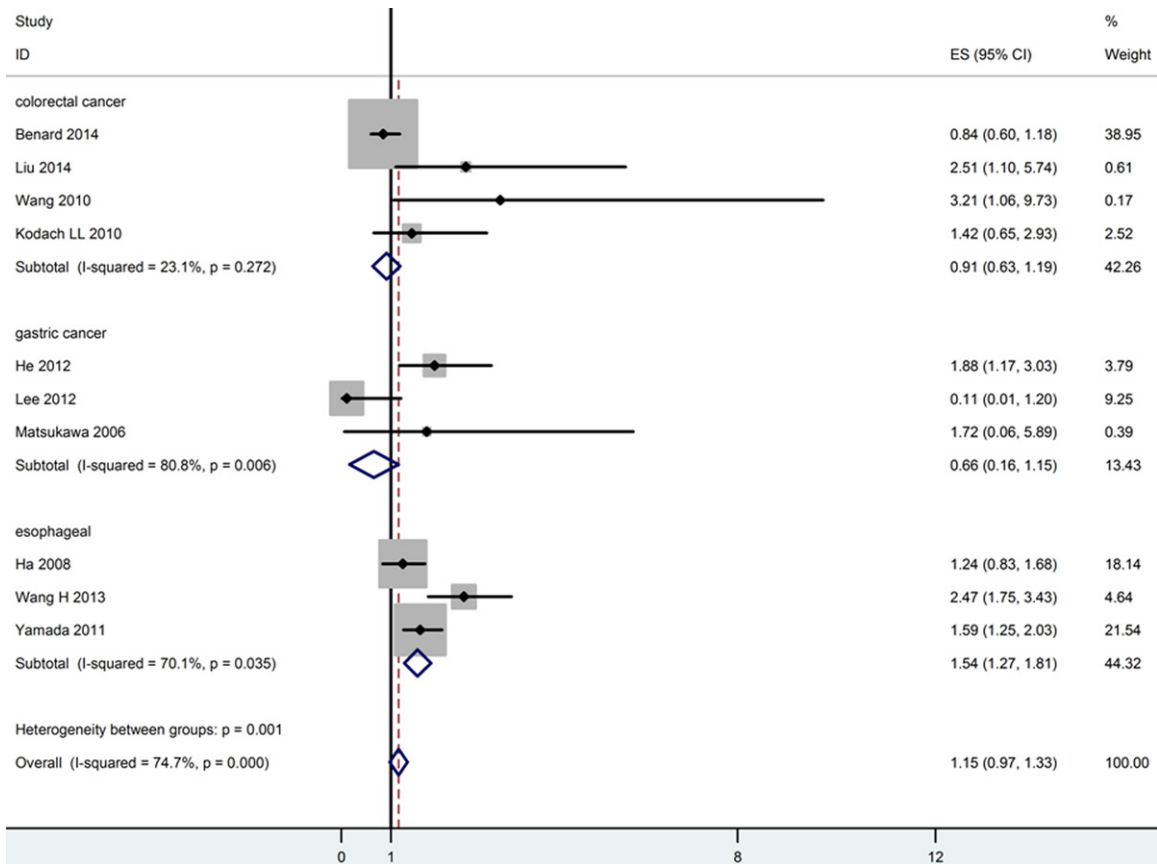


Figure 1. Meta-analysis (Forest plot) of the 10 evaluable studies assessing EZH2 in patients with digestive cancers stratified by different histological types for overall survival.

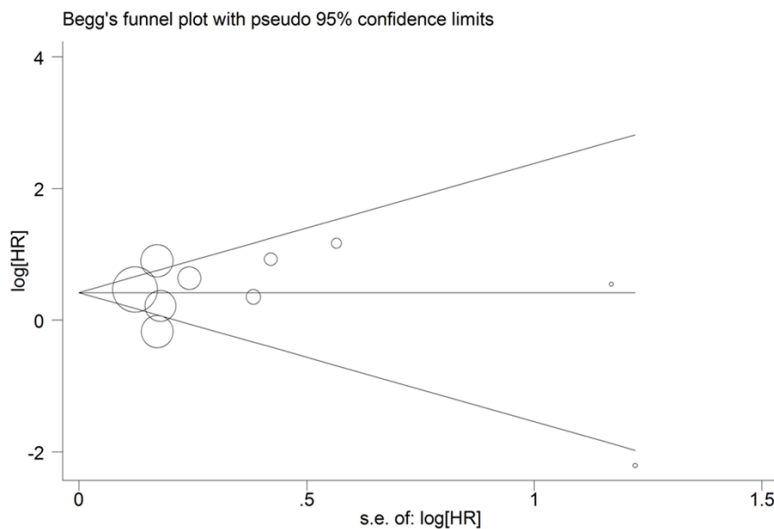


Figure 2. Funnel plot of the 10 evaluable studies assessing EZH2 in patients with digestive cancers for overall survival.

generations [26]. Disruption of EZH2 expression restricts cell proliferation and induces cell cycle arrest at the G2 phase, whereas the overexpression of EZH2 can shorten the G1 phase of the cell cycle and lead to cell accumulation in the S phase [28]. Furthermore, EZH2 protein, as a transcriptional repressor, may help the induction of transcriptional repression and participation in the controlling of gene expression patterns in the gastric epithelial cells, thereby resulting in the loss of tumor suppressor functions [29].

progression through epigenetic gene silencing and chromatin remodeling [27]. EZH2 is also capable of keeping the transcriptional repressive state of genes over successive cell

Our present meta-analysis is the first to evaluate the correlation between EZH2 overexpression and survival in patients with digestive cancers. This meta-analysis combined 10 pub-

lications including 1,461 patients with lung cancer to yield statistics, indicating different roles of EZH2 on overall survival in esophageal cancer, gastric cancer and colorectal cancer. Combined hazard ratios suggested that EZH2 overexpression was associated with poor prognosis of overall survival OS (HR = 1.54, 95% CI: 1.27-1.81) in patients with esophageal cancer. In the stratified analysis by histological types, significantly risks were not found among gastric cancer (HR = 0.66, 95% CI: 0.16-1.15) or colorectal cancer (HR = 0.91, 95% CI: 0.63-1.19), respectively, indicating EZH2 was not an indicator of poor prognosis in gastric cancer or colorectal cancer.

The heterogeneity issue was complicated in the systematic review and meta-analysis was. We found no significant heterogeneity among all studies included and subgroup analysis. Another potential source of bias is related to the method of HR and 95% CI extrapolation. If these statistics were not reported by the authors, we calculated them from the data available in the article. If this was not possible, we extrapolated them from the survival curves, necessarily making assumptions about the censoring process. Data for multivariate survival analysis reported in the article were included in the present systematic review with meta-analysis; if these data were not available, data calculated from survival curves by univariate analysis were included. These results should be confirmed by an adequately designed prospective study. Furthermore, the exact value of EZH2 overexpression status needs to be determined by appropriate multivariate analysis. Unfortunately, few prospectively designed prognostic studies concerning biomarkers have been reported; thus, our collection of many retrospective studies revealed more significance.

Publication bias [30] is a major concern for all forms of meta-analysis; positive results tend to be accepted by journals, while negative results are often rejected or not even submitted. The present analysis does not support publication bias; the obtained summary statistics likely approximate the actual average. However, it should be noted that our meta-analysis could not completely exclude biases. For example, the study was restricted to papers published in English and Chinese, which probably introduced bias.

To sum up, our meta-analysis is the first study to systematically estimate the association between EZH2 expression detected by IHC or Realtime-PCR and survival of patients with digestive cancers. As determined in our meta-analysis, we concluded that EZH2 overexpression was associated with poor overall survival in esophageal cancer, but not among gastric cancer or colorectal cancer. Thus, the detection of EZH2 expression may be of great value in determining the prognosis of esophageal cancer patients. However, given the limitations of our meta-analysis, further studies with more integral data and larger sample sizes are required to achieve a more widely applicable statistical analysis.

Disclosure of conflict of interest

None.

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