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Original Article

EZH2 overexpression as a biomarker of poor prognosis in prostate cancer

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Abstract: A number of studies have investigated the prognostic role of enhancer of zeste homolog 2 (EZH2) expression for patients with prostate cancer (PCa), however, the results were controversial. The current study was aimed to comprehensively explore the association between EZH2 and clinical outcomes of PCa by using meta-analysis. The electronic databases of Pubmed, Embase and Web of Science were searched. Combined hazard ratio (HR) and 95% confidence interval (CI) were computed using Stata 12.0 software. Seven studies with 1120 patients were included. The results demonstrated that high EZH2 expression was associated with poor recurrence-free survival (RFS) in PCa (HR=1.87, 95% CI=1.57-2.23, P<0.001). Subgroup analysis showed that EZH2 overexpression had enhanced prognostic significance for patients in Western countries (HR=2.22, 95% CI=1.61-3.05, P<0.001). In addition, elevated EZH2 expression also predicted poor RFS in patients receiving radical prostatectomy (RP) (HR=1.85, 95% CI: 1.55-2.21, P<0.001) and when immunohistochemistry staining (IHC) was used to detect EZH2 (HR=1.87, 95% CI: 1.55-2.24, P<0.001). In conclusion, EZH2 overexpression was distinctly correlated with poor patient RFS in PCa. EZH2 could serve as a prognostic biomarker in PCa patients.

Keywords: EZH2, prognosis, meta-analysis, prostate cancer

Introduction

Prostate cancer (PCa) is a prevalent cancer form in males in Western countries [1]. In the USA, PCa is the most frequently diagnosed cancer and the second leading cause of cancer related deaths in men, only next to lung cancer [2]. PCa epidemiology differs globally among various geographical regions and ethnic populations, ranging from highest incidence rate in African-American people in the USA to lowest rate in some Asian regions such as China, Thailand and India [3, 4]. Although the age-adjusted death rates of PCa have declined over the past two decades [5], PCa is still a major threat for elderly men and poses a heavy financial burden worldwide [1]. It has been established that age, race and a family history of the disease are causative risk factors for PCa [6]. Recent efforts suggest that epigenetic abnormalities are common in human cancer and facilitate tumor occurrence and progression [7] and elucidating of epigenetic changes could provide implications for cancer prevention and treatment.

Enhancer of zeste homolog 2 (EZH2), a catalytic core protein of the Polycomb Repressor Complex 2 (PRC2), has intrinsic histone methyltransferase (HMTase) activity and has been involved in gene silencing of target genes implicated in fundamental cellular processes [8]. Accumulated evidence also showed that EZH2 also played a pivotal role in progression and metastasis of several cancers including breast cancer [9, 10], bladder cancer [11], liver cancer [12] and prostate cancer [13]. Furthermore, EZH2 could promote tumor angiogenesis through VEGF stimulation in a paracrine circuit manner [14]. EZH2 is biologically functioned as a transcriptional repressor that silences more than 200 tumor suppressor genes [15]. Its oncogenic properties made EZH2 a promising risk indicator and target for cancer therapy [16]. Vast work has been done to investigate the prognostic value of EZH2 expression in PCa

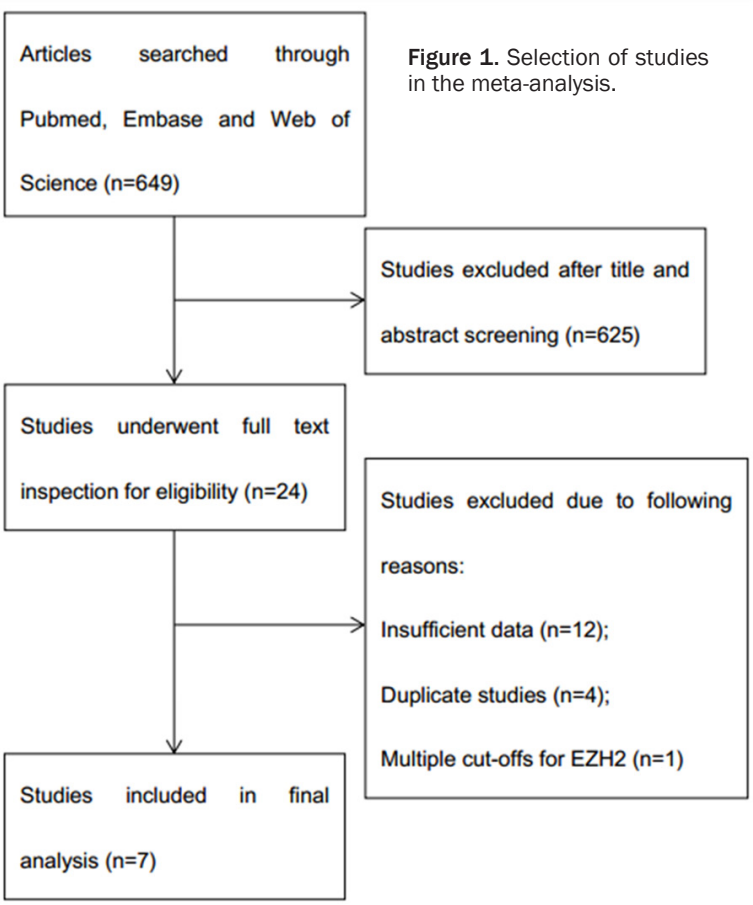

Figure 1. Selection of studies in the meta-analysis.

patients, however, the results are still contradictory and inconclusive according to previous studies [13, 17-22]. Meta-analysis is an analytical approach which combines conflicting data and pools the results to provide relatively objective conclusions through aggregated sample size. We therefore carried out a meta-analysis to systematically and comprehensively examine the impact of elevated expression on the prognosis of PCa patients.

Material and methods

Literature search

Pubmed, Embase and Web of Science databases were searched for relevant studies. The following keywords and MeSH terms were used in combination: "EZH2", "Zeste homolog 2", "Enhancer of zeste homologue 2", "prostatic neoplasms", "prostate cancer" and "prostate carcinoma". The publication language was restricted to English and the last search was on May 2016. The above references of articles and relevant reviews were also screened for for additional studies.

Inclusion and exclusion criteria

Eligible studies should meet the following inclusion criteria: (i) pathological confirmation of PCa diagnosis; (ii) EZH2 expression were measured by any approach; (iii) studies investigated the association between EZH2 and survival of PCa patients; (iv) the hazard ratio (HR) and 95% confidence interval (CI) were reported in text or can be calculated according to Tierney's method [23]; (v) if duplicate studies from the same research group ware found, the one with largest sample was selected; (vi) published in English with full text availability. Accordingly, the exclusion criteria were: (i) nonhuman studies; (ii) reviews, meeting abstracts and repeated studies; (iii) published in other languages than English.

Data extraction

Two investigators (XB, Gu and XS, Gao) extracted the following items from eligible studies independently: first author's name, publication year, study location, number of patients, clinical stage of disease, treatment, follow-up duration, detection method of EZH2, HR and 95% CI and survival information. Any disagreement was settled by discussion between the two investigators.

Statistical analysis

Hazard ratios (HRs) and 95% confidence intervals (CIs) were combined to measure the prognostic value of EZH2 expression for PCa. An HR>1 indicated patients with high EZH2 expression had poor survival outcomes whereas an HR<1 showed the opposite trend. Heterogeneity among studies was measured by the Q test and I^2 test. *P* value of Q test (P_h)<0.1 or I^2>50% indicated significant heterogeneity, then random-effects model was used to pool the data, otherwise a fixed-effects model was applied. Publication bias was tested by using Begg's test. Stata (version12.0, Stata Corporation, TX, USA) were used to conduct all statistical analy-

Table 1. Basic characteristics of included studies

Author	Ref.	Year	Study location	Patients (n)	Clinical stage	Treatment	Follow-up (m) median/range	Detection method	EZH2 + n (%)	HR estimation	Survival analysis
Varambally	[13]	2002	USA	64	Localized PCa	RP	80	IHC	10 (15.6)	HR and 95% CI	RFS
Bachmann	[17]	2006	Norway	104	Localized PCa	RP	104 (20-179)	IHC	9 (8.7)	HR and 95% CI	RFS
Tolonen	[18]	2011	Finland	207	Localized PCa	RP	56.5 (8-104)	IHC	107 (51.7)	HR and 95% CI	RFS
Li	[19]	2013	China	129	Localized PCa	RP	31 (6-60)	IHC	58 (45)	HR and 95% CI	RFS
Hoogland	[20]	2014	The Netherlands	426	Localized PCa	RP	113.3 (0-203.8)	IHC	10 (2.3)	HR and 95% CI	RFS
Jacobs	[21]	2014	USA	54	Localized PCa	Radiotherapy	32.6 (2.8-84.6)	IHC	9 (18.8)	HR and 95% CI	RFS
Vieira	[22]	2014	Portugal	136	Localized PCa	RP	105 (3-145)	RT-PCR	102 (75)	HR and 95% CI	RFS

PCa: Prostate cancer; RP: Radical prostatectomy; IHC: Immunohistochemistry staining; RT-PCR: Reverse Transcription-Polymerase Chain Reaction; RFS: Recurrence-free survival.

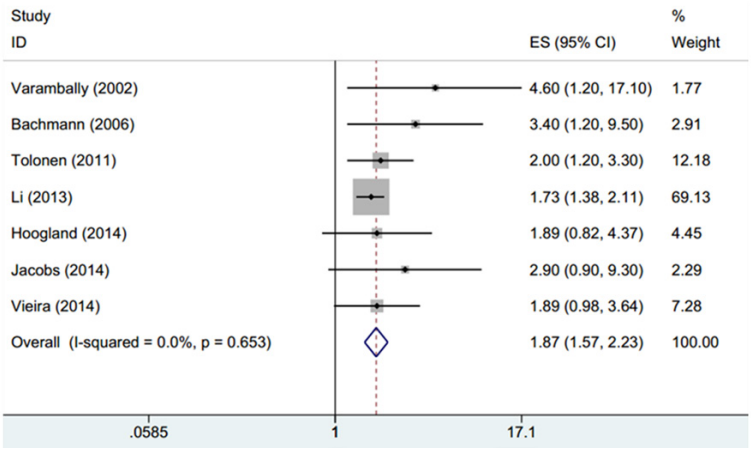

Figure 2. Forest plots showing prognostic value of EZH2 expression on RFS in PCa.

ses. P<0.05 was considered as statistically significant.

Results

Study selection

The study screening and selection process was displayed in **Figure 1**. A total of 649 relevant studies were identified initially and 625 studies were excluded after title and abstract reading. Twenty-four full text articles were further evaluated and 17 studies were discarded because they lacked necessary information, were duplicate studies or used multiple EZH2 cut-off values. At last, seven studies [13, 17-22] published from 2002 to 2014 were included for meta-analysis.

Characteristics of included studies

The main characteristics of included studies were shown in **Table 1**. Two studies [13, 21] were conducted in the USA, and the other five studies were performed in Norway [17], Finland [18], China [19], the Netherlands [20] and Portugal [22], respectively. The total sample size was 1120, ranging from 54 to 426. Six studies [13, 17-21] used immunohistochemistry staining (IHC) to detect EZH2 expression and one study [22] used RT-PCR. All studies investigated the association between EZH2 and recurrence-free survival (RFS) of PCa patients.

Meta-analysis

The pooled results were calculated based on values of HR and 95% CI from each individual study (**Figure 2**). Overall, the combined HR was 1.87 with 95%CI: 1.57-2.23, P<0.001 and there was no heterogeneity among studies (I^2=0, P_h=0.653). The results indicated that elevated EZH2 expression generally predicted shorter RFS. To further investigate the prognostic value of EZH2, subgroup analysis stratified by study location, treatment and detection method was conducted. As shown in **Table 2**, high EZH2 expression showed enhanced prognostic significance for PCa patients in Western countries (HR=2.22, 95% CI: 1.61-3.05, P< 0.001), additionally, there was good homogeneity (I^2=0, P_h=0.769). Moreover, results from subgroup analysis also suggested that EZH2 overexpression was associated with poor RFS in patients receiving radical prostatectomy (RP) (HR=1.85, 95% CI: 1.55-2.21, P<0.001; I^2=0, P_h=0.606) and when IHC was used to detect EZH2 (HR=1.87, 95% CI: 1.55-2.24, P<0.001; I^2=0, P_h=0.525). There was no heterogeneity between studies for meta-analysis, therefore, the fixed-effects model was adopted.

Publication bias

Potential publication bias in this meta-analysis was measured by using Begg's funnel plots test. The Begg's *p* value was 0.072 and the funnel plots were symmetric (**Figure 3**), showing no significant publication bias. Thus, our results were statistically credible.

Discussion

To our knowledge, the present meta-analysis independently investigated the prognostic value of EZH2 overexpression for PCa patients for the first time. Our combined data from 1120 subjects suggested that high EZH2 expression was collectively correlated with poor RFS in PCa, in addition, elevated EZH2 had more significant prognostic function for patients in Western countries. The consistent prognostic role was also remained for patients undergoing surgery and when using IHC to detect EZH2. There was also no heterogeneity or significant publication bias in our meta-analysis, guaranteeing the reliability of our results.

Table 2. Main results of meta-analysis

Variables	Studies (n)	Patients (n)	Effects model	Combined HR (95% CI)	*P*-value	Heterogeneity	
						I^2 (%)	P_h
Overall	7	1120	FEM	1.87 (1.57-2.23)	<0.001	0	0.653
Study location							
Western countries	6	991	FEM	2.22 (1.61-3.05)	<0.001	0	0.769
Asian countries	1	129	-	1.73 (1.4-2.14)	<0.001	-	-
Treatment							
RP	6	1066	FEM	1.85 (1.55-2.21)	<0.001	0	0.606
Radiotherapy	1	54	-	2.9 (0.9-9.32)	0.074	-	-
Detection method							
IHC	6	984	FEM	1.87 (1.55-2.24)	<0.001	0	0.525
RT-PCR	1	136	-	1.89 (0.98-3.64)	0.056	-	-

FEM: Fixed-effects model; P_h: *p*-value for heterogeneity test.

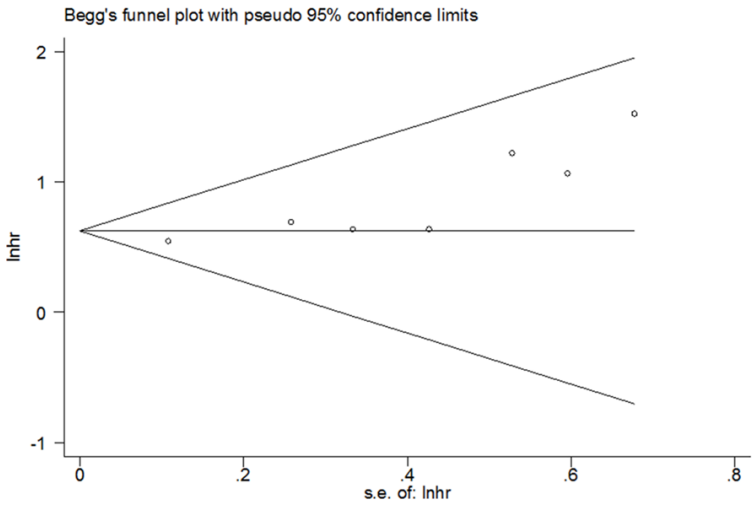

Figure 3. Publication bias examined by Begg's funnel plot.

EZH2 is a histone methyltransferase and EZH2 dysregulation leads to epigenetic aberrations, which could further salience tumor suppressor genes and induce cell proliferation and invasion [24]. EZH2 was first found to be associated with aggressive and metastatic disease status of PCa in 2002 [13]. Since then, more and more studies investigated the promotive role of EZH2 in PCa development. Bryant *et al.* showed that endogenous EZH2 promoted proliferation and invasiveness of both androgen-responsive and androgen-refractory PCa cells [25]. What's more, EZH2 also can function as an epigenetic silencer in PCa etiology and act with Polycomb Repressive Complexes (PRC1 and PRC2) in a cooperative manner [26]. Besides, in a castration-resistant prostate cancer (CRPC) model, EZH2 was also shown to be a PRC2-independent coactivator transcription factors, including androgen receptor (AR), which was a well-established factor for PCa progression and hormone therapy resistance [27]. Therefore, EZH2 had biological rationale to be explored as a prognostic biomarker for patients with PCa.

We have also noted that several recently published meta-analyses investigated the prognostic value of EZH2 in various cancer types, including non-small cell lung cancer (NSCLC) [28], breast cancer [29], digestive cancers [30], and all cancer forms [31, 32]. In the previous studies, investigators found that EZH2 overexpression was associated with poor overall survival (OS) in NSCLC [28] and breast cancer [29]. The previous evidence was in line with the results of our current study. Moreover, our study including subgroup analysis identified EZH2 as a more promising biomarker for patients in Western countries and patients receiving RP, which specified the appropriate population for EZH2 monitoring.

Several limitations still need to be addressed. First, only full-text published papers were included. As we all know, papers with positive results are prone to be published and are easier to be found, compared with negative results papers. Therefore, selection bias may be introduced. Second, in subgroup analysis, in the

groups of Asian countries, radiotherapy and RT-PCR, there was only one study for each group and the results could be potentially influenced by the single report. Therefore, more studies are needed.

In conclusion, the present meta-analysis suggested that high EZH2 expression was associated with poor RFS in patients with PCa, especially for patients in Western countries and patients receiving surgery. To strengthen our findings, large sample size studies are required to explore the relation between EZH2 and prognosis for PCa patients.

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Disclosure of conflict of interest

None.

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