Effect of enhancer of zeste homolog 2 mutations on the prognosis of patients with myelodysplastic syndrome

A meta-analysis

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Abstract

Background: Gene mutations with important prognostic roles have been identified in patients with myelodysplastic syndrome (MDS). Overall, it is not yet fully clear whether enhancer of zeste homolog 2 (EZH2) is affected and contributes to the disease in MDS patients. Thus, we performed a meta-analysis to investigate the effects of EZH2 mutations on the prognosis of patients with MDS.

Methods: We searched English-language databases (PubMed, Embase, and Cochrane Library) for studies published on the effects of EZH2 mutations in MDS patients. The study had to include at least 1 of the following indices as therapeutic evaluation data: overall survival (OS), transformation time to leukemia, and International Prognostic Scoring System risk. Revman, version 5.2 software was used for all statistical processing. We calculated the risk ratio and the 95% confidence interval (CI) of continuous variables, and determined the hazard ratio and 95% CI of time-to-event data.

Results: We included 5 studies with a total enrolment of 994 patients. There was a significant adverse effect on OS in the EZH2-mutation group compared to the unmutated group (hazard ratio = 2.47, 95% CI: 1.37–4.47, P < .00001), while the heterogeneity was relatively high (I² = 68%). There was no significant correlation between EZH2 mutations and IPSS risk (low/int-1 vs int-2/high) (odds ratio: 0.69, 95% CI: 0.14–3.39, P = .65), with significant heterogeneity (I² = 78%). The analysis did not show significant publication bias in the studies.

Conclusion: This meta-analysis indicated an adverse effect of EZH2 mutations with regard to OS in patients with MDS. However, larger cohort trials are still needed to better understand the prognostic impacts of EZH2 mutations on MDS patients.

Abbreviations: 95% CI = 95% confidence interval, EZH2 = enhancer of zeste homolog 2, HR = hazard ratio, IPSS = international prognostic scoring system, MDS = myelodysplastic syndrome, NOS = Newcastle-Ottawa-scale, OS = overall survival.

Keywords: enhancer of zeste homolog 2, mutation, myelodysplastic syndrome, prognosis

1. Introduction

Myelodysplastic syndrome (MDS) is a type of myeloid neoplasm characterized by ineffective hematopoiesis, morphological dysplasia, and peripheral blood cytopenia. MDS carries a high risk of progression to acute myeloid leukemia (AML).[1] In recent years, epigenetic abnormalities and gene mutations in MDS patients have been gradually revealed with the development of next-generation sequencing. Most patients with MDS present with at least 1 abnormal gene mutation.[2,3] The risk stratification for MDS patients is categorized according to clinical characteristics of peripheral blood and bone marrow, and also the karyotype. The crucial role of gene mutations in MDS has become increasingly important in clinical practice. Several gene mutations have been shown to be associated with the prognosis of MDS.[4,5] It is therefore of great importance to achieve a better understanding of the role of gene mutations in MDS, so that we can better predict the prognosis of patients with this disorder.

Enhancer of zeste homolog 2 (EZH2) is a catalytic subunit of PRC2 (Polycomb Repressive Complex 2), the core components of the Polycomb group of proteins, which are epigenetic regulators of development and which could affect the proliferation and differentiation of cells via epigenetic silencing of important growth regulatory genes.[6,7]

Some studies have shown that EZH2 mutations are associated with the prognoses of solid tumors[8–10] as well as MDS.[11] However, there is still a lack of systematic studies on EZH2 mutations and their clinical relevance. Therefore, we here summarize relevant studies, with a focus on the effects of EZH2 mutations on the overall survival (OS) and other clinical
characteristics of patients with MDS, to provide new insight into the diagnosis, treatment, and prognosis of the disorder.

2. Methods

2.1. Retrieval strategy

We searched English-language databases—namely, PubMed, Embase, and Cochrane Library—for articles published on the effects of EZH2 mutations on MDS patients, using the search strategy “EZH2 AND MDS OR myelodysplastic syndrome.” After reading the titles and abstracts, the documents were screened and the full texts were read. The appropriate documents were selected according to the inclusion and exclusion criteria. We also searched for relevant articles from available references to avoid omissions. For raw data not provided in the literature, we contacted the author for access.

2.2. Literature inclusion criteria and exclusion criteria

The inclusion criteria were as follows:

(1) the study used second-generation sequencing to detect prognostic gene mutations and focused on EZH2 mutations;
(2) a confirmed diagnosis of MDS, according to the World Health Organization classification;
(3) the article was published in English;
(4) the study included at least 1 of the following indices as therapeutic evaluation data: OS, transformation time to leukemia, and international prognostic scoring system (IPSS) risk; and
(5) the data in the article could be used to calculate the hazard ratio (HR) with 95% confidence intervals (CIs).

The exclusion criteria were the following:

(1) the article was an expert review, case summary, case article, or meeting record;
(2) studies with insufficient data for calculating the incidence and/or HRs with 95% CIs;
(3) the results of the study did not include any effect of EZH2 mutations on the OS, transformation time to leukemia, or IPSS risk.

If more than 1 published article was from the same study, the results of the most recently published article were considered; if the recent article did not provide definite results, the results of the previous articles were used.

2.3. Literature effect index

The clinical effects of different regimens were evaluated by the effect indicators of OS and IPSS risk.

2.4. Data extraction

According to the retrieval strategy and retrieval database, 2 researchers independently searched and excluded the articles that did not meet the inclusion criteria. The data extracted from the literature included author, publication time, regions, ages, sex, classifications, stratifications, average follow-up time, and other indicators. The results of multivariate analyses were preferred.

2.5. Quality assessment and control

All titles and abstracts of retrieved articles were independently reviewed by 2 investigators (W. X. X. and H.X.Y) for the inclusion/exclusion criteria. Any divergent opinions were resolved through discussion. The Newcastle–Ottawa scale (NOS) quality assessment[12] was used to evaluate the quality of each individual study. The NOS comprises 9 items. A score of 9 indicates that all standards are met. In general, studies with a score of 7 or higher are considered high quality.

2.6. Statistical analysis

Revman, version 5.2 software was used for all statistical processing. The heterogeneities between subgroups were evaluated by the standard Chi-squared test and P-statistic. A value of \( P < 0.05 \) suggested that there was no heterogeneity using the fixed effect model. A value of \( I^2 > 50\% \) indicated the existence of heterogeneity using a random effect model, and identified the source of heterogeneity as much as possible. Based on the research included in the analyses, we calculated risk ratio and 95% CI of continuous variables, and found the HR and 95% CI of time-to-event data. If the HR could not be directly obtained from the article, we used the Engauge Digitizer V, version 4.1 calculation method[13] Funnel plots were used to estimate publication bias. A value of \( P < .05 \) was considered statistically significant.

2.7. Ethics statement

All data sources and statistical analyses were based on previously published studies; thus, no ethical approval and patient consent was required.

3. Results of meta-analysis

3.1. The basic status of the included literature

A total of 109 articles were retrieved, and 93 articles were excluded by reading titles, abstracts, and the type of study. According to the inclusion and exclusion criteria, 11 articles were excluded because they did not provide enough information. Finally, 5 articles met the inclusion criteria (Fig. 1).

3.2. Characteristics of the studies

There were 5 studies[14–18] included in our analysis. In a total of 994 patients, there were 82 patients with EZH2 mutations (8.2%) and 912 patients without mutations. The specific characteristics of the studies are listed in Table 1. The NOS was used to evaluate the quality of each included study, and the NOS scores are listed in Table 2.

3.3. Effect index

3.3.1. OS. The meta-analysis included 4 studies with EZH2 mutations. The results showed that patients with EZH2 mutations could have an adverse prognosis regarding the OS (HR = 2.47, 95% CI: 1.37–4.47, \( P < .00001 \)), while the heterogeneity was relatively high (\( I^2 = 68\% \)) (Fig. 2).

3.3.2. Correlation with the IPSS risk. Several studies also mentioned that the EZH2 mutation might be associated with IPSS risks among patients with MDS. However, the conclusions
Table 1
Characteristics of included studies.

| Author, year | Region | Median age (range) | Sex (male/female) | WHO subtype, n | Karyotype subtype, n | IPSS, n | Median Follow-up, (range) | N. |
|--------------|--------|--------------------|-------------------|----------------|----------------------|--------|--------------------------|----|
| Bejar 2011[14] | USA    | 70                 | 306/133           | 279            | 105/55               | 310     | 55/67                    | 110 | 105/101 | 32 | 4.44y | 439 |
| Nikoloski 2010[15] | USA   | NR                 | NR/16[14]         | NR             | NR                   | NR      | NR/16                    | 7   | 85/57  | 16 | NR      | 104 |
| Wang 2013[16] | China  | 51 (16-81)         | 106/47            | 86             | 28/39                | 89/39   | 17                       | 20  | 192/60 | 30 | NR      | 304 |
| Wu 2016[17]  | China  | 57 (11-89)         | 162/142           | 181            | 52/71                | 43/13   | 28                       | –   | –      | –  | NR      | 84  |

Int-1 = intermediate-1 group, Int-2 = intermediate-2 group, NA = not applicable, NR = not reported, RCMD = refractory cytopenia with multilineage dysplasia, RCUD = refractory cytopenia with unilineage dysplasia, RARS = refractory anemia with ringed sideroblasts, RAEB-1 = refractory anemia with excess blasts-1, RAEB-2 = refractory anemia with excess blasts-2.

Table 2
Quality assessment of individual study. (NOS, Newcastle–Ottawa quality assessment score).

| Author, year | Representativeness of the exposed cohort | Selection of the non-exposed cohort | Ascertainment of exposure | Outcome of interest was not present at start | Comparability | Assessment of outcome | Follow-up long enough | Adequacy of follow up | Score |
|--------------|----------------------------------------|------------------------------------|--------------------------|-------------------------------------------|---------------|----------------------|-----------------------|-----------------------|-------|
| Bejar 2011[15] | 1                                      | 1                                  | 1                        | 1                                         | 1             | 1                    | 1                     | 1                     | 8     |
| Nikoloski 2010[14] | 1                                      | 1                                  | 1                        | 1                                         | 1             | 1                    | 1                     | 0                     | 7     |
| Wang 2013[16]  | 1                                      | 1                                  | 1                        | 2                                         | 1             | 1                    | 1                     | 0                     | 8     |
| Wu 2016[17]   | 1                                      | 1                                  | 1                        | 1                                         | 1             | 1                    | 1                     | 0                     | 7     |
| Cedena 2017[18] | 1                                      | 1                                  | 1                        | 1                                         | 1             | 1                    | 1                     | 0                     | 7     |
were inconsistent after we summarized the data. There was no significant correlation between EZH2 mutation and the IPSS risk (low/int-1 vs int-2/high) (odds ratio: 0.69, 95% CI: 0.14–3.39, P = .65), with significant heterogeneity (I² = 78%) (Fig. 3).

3.3.3. Publication bias. Publication bias was assessed using funnel plots (Fig. 4), which did not show significant publication bias in the included studies.

4. Discussion

Genetic alterations in patients with MDS have been important because of their significant prognostic effects. Identifying prognosis-related gene mutations and developing precision therapies based on risk stratification and potential targets will, therefore, be of great importance in the overall diagnosis and treatment of MDS.

EZH2, located at Cr.7q36.1, has been recognized as an associated gene on chromosome 7 in MDS patients. EZH2 contributes to the process of cell division and has important roles in embryonic development. It also plays an important role in controlling hematopoietic stem cell self-renewal, as well as promoting cell proliferation and cell cycle progression. EZH2 is the functional catalytic subunit of the PRC2 (Polycomb-repressive complex-2) complex. The EZH2 protein serves as a histone methyltransferase that catalyzes H3 methylation on lysine 27 when assembled in the PRC2 complex, leading to silencing of downstream tumor suppressor genes.

Several studies have reported that EZH2 plays a crucial role in leukemogenesis. EZH2 mutations enhance the activity of methyltransferase and the level of H3K27me3, which affects the expression of tumor suppressor genes to cause malignancy. EZH2 mutations have also been associated with decreased survival of patients with other myeloid malignancies, including MDS/MPN, myelofibrosis, and chronic myelomonocytic leukemia.

However, the effect of EZH2 mutations on biological functions is still controversial. In MDS patients, the mutation frequency of EZH2 is relatively low (~6%–7%) including missense, nonsense, and frame shift mutations. Although our study reported that patients with EZH2 mutations had poorer prognoses with regard to OS when compared with the unmutated group, there was still some controversy. A study by Ernst et al analyzed a larger cohort of patients, including those with MDS and MDS/myeloproliferative neoplasm. Of these MDS patients, 6% had EZH2 mutations, and no prognostic significance was seen between the patients with and without EZH2 mutations.

Even the study conducted by Wu et al suggested that mutations in EZH2 had no significant impact on the OS of patients, when compared with control patients. Only 1 study mentioned the effect of EZH2 mutations on the risk of transformation to AML. The results from Wang et al showed that patients with EZH2 mutations more easily progressed to AML, when compared with control patients (P = .039). Another study conducted by Cedena et al suggested that EZH2 mutations alone did not affect the response to azacitidine in patients with MDS (P = .075). However, according to multivariate analysis, there was no difference in the response to azacitidine treatment. Thus, provided there was a TP53 or EZH2 mutation, the patient showed an adverse effect and decreased OS.

Three studies referred to correlations between EZH2 mutations and IPSS risks in MDS patients. Wu, et al reported that EZH2 mutations were more common in high-risk subtypes [refractory anemia with excess-1/-2/] than in refractory cytopenia.
with unilineage dysplasia/refractory anemia with ringed sideroblasts/refractory cytopenia with multilineage dysplasia cases. In addition, patients with EZH2 mutations had higher IPSS scores than those without mutations. By contrast, Bejar et al reported that most patients with EZH2 mutations had low or -1 risk according to the IPSS. However, the presence of EZH2 mutations was strongly associated with a decreased OS. Overall, the results showed that lower risk patients with MDS, who had EZH2 mutations, may require more aggressive treatment than would be predicted by the IPSS.

There are also relevant studies mentioning the contribution of EZH2 to other hematological malignancies such as lymphoma or essential thrombocytopenia. The EZH2 mutation may also be found in patients with germinal center B-cell like diffuse large B-cell lymphoma, which is also associated with proliferation and growth of malignant cells. In addition, the OS of essential thrombocythemia was influenced by the presence of EZH2 mutations.

However, limitations of this meta-analysis should be considered. Because the frequency of EZH2 mutations was low, some of the studies contained small numbers of patients. Consequently, the results require confirmation using a larger patient cohort.

Further studies focused on the function of EZH2 mutations should be conducted by systemic functional analysis based on cell biology and animal experiments. The contribution of EZH2 mutations to epigenetic dysregulation in MDS remains to be fully understood, and numerous questions and difficulties remain to be addressed. In view of promising EZH2-targeted therapies, the solution to this challenge should be the highest priority.

5. Conclusion

Our study summarized the published literature and revealed an adverse prognostic effect of EZH2 mutations in patients with MDS. Regarding the effect of IPSS risk, no significant correlation was found. However, mutations of EZH2 may be a promising prognostic factor and therapeutic target for MDS patients, although further clinical trials are needed to better understand the prognostic impact of EZH2 mutations on this disorder.

Author contributions

Xinyue Huang wrote the manuscript and was responsible for the data analysis. Xiaoxue Wang: responsible for the revisions.

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