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ORIGINAL ARTICLE

# Association between H3K4 methylation and cancer prognosis: A meta-analysis

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## Abstract

**Background:** Histone H3 lysine 4 methylation (H3K4 methylation), including mono-methylation (H3K4me1), di-methylation (H3K4me2), or tri-methylation (H3K4me3), is one of the epigenetic modifications to histone proteins, which are related to the transcriptional activation of genes. H3K4 methylation has both tumor inhibiting and promoting effects, and the prognostic value of H3K4 methylation in cancer remains controversial. Therefore, we performed a systematic review and meta-analysis to examine the association between H3K4 methylation and cancer prognosis.

**Methods:** A comprehensive search of PubMed, Web of Science, ScienceDirect, Embase, and Ovid databases was conducted to identify studies investigating the association between H3K4 methylation and prognosis of patients with malignant tumors. The data and characteristics of each study were extracted, and the hazard ratio (HR) at a 95% confidence interval (CI) was calculated to estimate the effect.

**Results:** A total of 1474 patients in 10 studies were enrolled in this meta-analysis. The pooled HR of 1.52 (95% CI 1.02–2.26) indicated that patients with a lower level of H3K4me2 expression were expected to have shorter overall survival, while the pooled HR of 0.45 (95% CI 0.27–0.74) indicated that patients with a lower level of H3K4me3 expression were expected to have longer overall survival.

**Conclusion:** This meta-analysis indicates that increased H3K4me3 expression and decreased H3K4me2 expression might be predictive factors of poor prognosis in cancer. Further large cohort studies are needed to confirm these findings.

## Introduction

Histone modification plays an important role in epigenetic regulation. The N-terminal residues of the four core histones (H2A, H2B, H3, and H4) are targets for posttranslational modifications, which include acetylation, methylation, and phosphorylation.[1] Histone modification contributes to basic cellular functions, such as cell cycle, growth, and apoptosis, by influencing gene transcription, DNA replication, DNA recombination, DNA repair, and other molecular processes. Abnormal histone modification is responsible for tumor development and progression.[2]

Histone methylation may either activate or inhibit the expression of downstream genes, depending on the site and degree of methylation.[3] Histone H3 lysine 4 (H3K4) methylation refers to the modification occurring at the fourth lysine residue from the N-terminus of histone H3. H3K4 methylation is associated with transcriptional activation and elongation. The fourth lysine residues of histone H3 can be mono-methylated (H3K4me1), di-methylated (H3K4me2), and tri-methylated (H3K4me3), which greatly enhances the diversity of the regulatory function of histone methylation.[4]

Some studies have demonstrated that low expression of H3K4me2 and high expression of H3K4me3 are associated with poor prognoses of malignancies.[5,6] For example, Liu *et al.* found that the median survival time of patients with low H3K4me2 and high H3K4me2 expression were 11.3 and 18.6 months, respectively.[6] However, Ellinger *et al.*

showed that in univariate analysis, patients with low level H3K4me1-3 expression suffered from shorter cancer-specific survival. Nevertheless, after multivariate analysis, no significant correlation was found between H3K4me1-3 expression and survival.[7]

The connection between these epigenetic changes and cancer prognosis has emerged as a leading trend in clinical practice and translational research. Few studies have examined the association between H3K4 methylation and cancer survival and those that have provided conflicting results.[5–8] Furthermore, it is unclear whether sources of heterogeneity among studies, such as the subtypes of H3K4 methylation or the variation used to classify H3K4 methylation, may have contributed to these discrepancies.

Given the growing number of studies in the literature and the increasing interest in the role of H3K4 methylation in cancer survival, we systematically reviewed the literature examining the association between H3K4 methylation and survival in cancer to conduct a comprehensive meta-analysis to quantify the magnitude of risk.

# Methods

## Search strategy

In this study, we investigated the association between H3K4 methylation levels and prognosis of malignant tumors. We systematically searched PubMed, Web of Science, ScienceDirect, Embase, and Ovid databases for all relevant articles published up to March 2018. The search terms included: ("Histone H3 lysine 4 methylation OR H3K4 methylation OR Histone H3 Lys4 methylation OR histone H3K4 methylation") AND ("Immunohistochemistry") AND ("Neoplasm OR carcinoma OR sarcoma OR cancer") AND ("prognosis OR outcome OR survival").

## Study selection

The inclusion criteria were as follows: (i) studies that described the clinical characteristics and pathological patterns of patients with malignant tumors; (ii) studies that provided information on immunohistochemical evaluation of H3K4 methylation levels; (iii) studies with detailed demographic data (e.g. age, gender, and sample size); (iv) studies that defined overall survival (OS) as a clinical endpoint; and (v) studies that reported hazard ratio (HR) with 95% confidence interval (CI) for OS.

## Data extraction and quality assessment

The two authors independently extracted the data, including the first author's name, year of publication, country, sample size, types of disease, the total number of cases with different H3K4 methylation levels, and HR values with 95% CI for OS. The quality of each included study was assessed using the Newcastle–Ottawa Scale (NOS). Studies that received a score of six or higher were considered high quality.

## Statistical analysis

Meta-analysis was performed using Stata 12.0 (Stata Corp., College Station, TX, USA). The heterogeneity across individual studies was evaluated by Cochran's Q-test and $I^2$ index. If $I^2 \leq 50\%$, a fixed effect model was estimated, otherwise, a random effect model was used. Potential publication bias was assessed using Begg's and Egger's line regression tests. Sensitivity analysis was conducted by eliminating one study at a time in order to analyze the stability of the pooled results.

# Results

## Systematic search

A total of 1181 full-text articles were identified through the database search (Fig 1). After removing duplicate publications, 741 articles remained for systematic title and abstract analysis. After screening the titles and abstracts of each article, 723 articles that did not meet the inclusion criteria, such as articles that did not report patient prognosis and articles unrelated to H3K4 methylation, were excluded. Eighteen articles passed preliminary screening, thus the full text was reviewed. Articles that failed to report the HR with 95% CI for OS were also excluded. Eventually, seven articles were included.

## Study characteristics and quality assessment

The seven included articles, three of which contained two study cohorts, focused on H3K4me2 and H3K4me3 methylation states and were published between 2007 and 2017, with a total of 10 studies and 1474 cases (Table 1). Of the 10 included studies, six studies from five published articles involved 659 cancer patients associated with H3K4me2. The histopathological types of cancers included non-small cell lung, pancreatic, colorectal, and esophageal squamous cell cancers. Another four studies from two articles involved 815 cancer patients associated with H3K4me3, including liver and cervical cancers. The quality assessment showed that all of the studies included in this meta-analysis were of high quality (NOS ≥ 6). The basic characteristics of the included studies are summarized in Table 1.

  

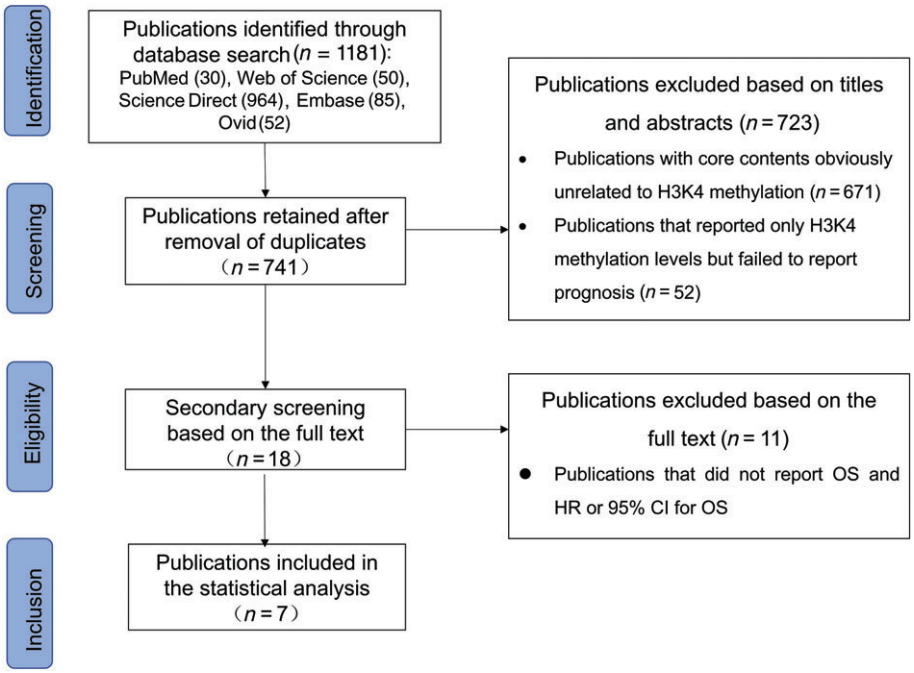


**Figure 1** Flow diagram of study inclusion. CI, confidence interval; HR, hazard ratio; OS, overall survival.

**Table 1** Basic characteristics of the included studies

| Item | Publication year | Country | Disease type | Number of cases | Low H3K4 expression (n) | High H3K4 expression (n) | OS HR (95% CI) | NOS |
|---|---|---|---|---|---|---|---|---|
| H3K4me2 | | | | | | | | |
| Barlési *et al.*[9] | 2007 | France | Non-small cell lung cancer | 62 | 36 | 26 | 1.990 (1.000–3.990) | 9 |
| Benard *et al.*[10] | 2014 | Netherlands | Colon cancer | 112 | 58 | 54 | 0.735 (0.429–1.266) | 8 |
| Manuyakorn *et al.*[11] (RTOG cohort) | 2010 | Thailand | Pancreatic cancer | 194 | 120 | 74 | 1.480 (1.050–2.080) | 8 |
| Manuyakorn *et al.*[11] (UCLA cohort) | 2010 | Thailand | Pancreatic cancer | 140 | 91 | 49 | 2.390 (1.530–3.730) | 8 |
| Tamagawa *et al.*[12] | 2012 | Japan | Colorectal cancer | 54 | 28 | 26 | 2.959 (1.277–6.849) | 8 |
| Tzao *et al.*[13] | 2008 | China | Esophageal squamous cell carcinoma | 97 | 58 | 39 | 0.950 (0.530–1.700) | 8 |
| H3K4me3 | | | | | | | | |
| He *et al.*[14] (Testing cohort) | 2011 | China | Liver cancer | 168 | 91 | 77 | 0.278 (0.178–0.434) | 8 |
| He *et al.*[14] (Validation cohort) | 2011 | China | Liver cancer | 147 | 71 | 76 | 0.371 (0.194–0.712) | 8 |
| Beyer *et al.*[15] (Nucleus) | 2017 | Germany | Cervical cancer | 250 | 29 | 221 | 0.822 (0.295–2.294) | 8 |
| Beyer *et al.*[15] (Cytoplasm) | 2017 | Germany | Cervical cancer | 250 | 158 | 92 | 0.665 (0.377–1.172) | 8 |

CI, confidence interval; HR, hazard ratio; NOS, Newcastle–Ottawa Scale; OS, overall survival.

## Publication bias

Both Begg's and Egger's tests were used to evaluate the publication bias of the studies included in the meta-analysis. For the studies investigating H3K4me2 and H3K4me3 expression levels and the prognosis of malignant tumors, the results of Begg's testing showed $P$ values of 0.707 and 0.734, respectively. Meanwhile, the results of Egger's testing showed $P$ values of 0.876 and 0.311 for studies of H3K4me2 and H3K4me3, respectively. Neither Begg's nor Egger's publication bias tests provided a statistically

 

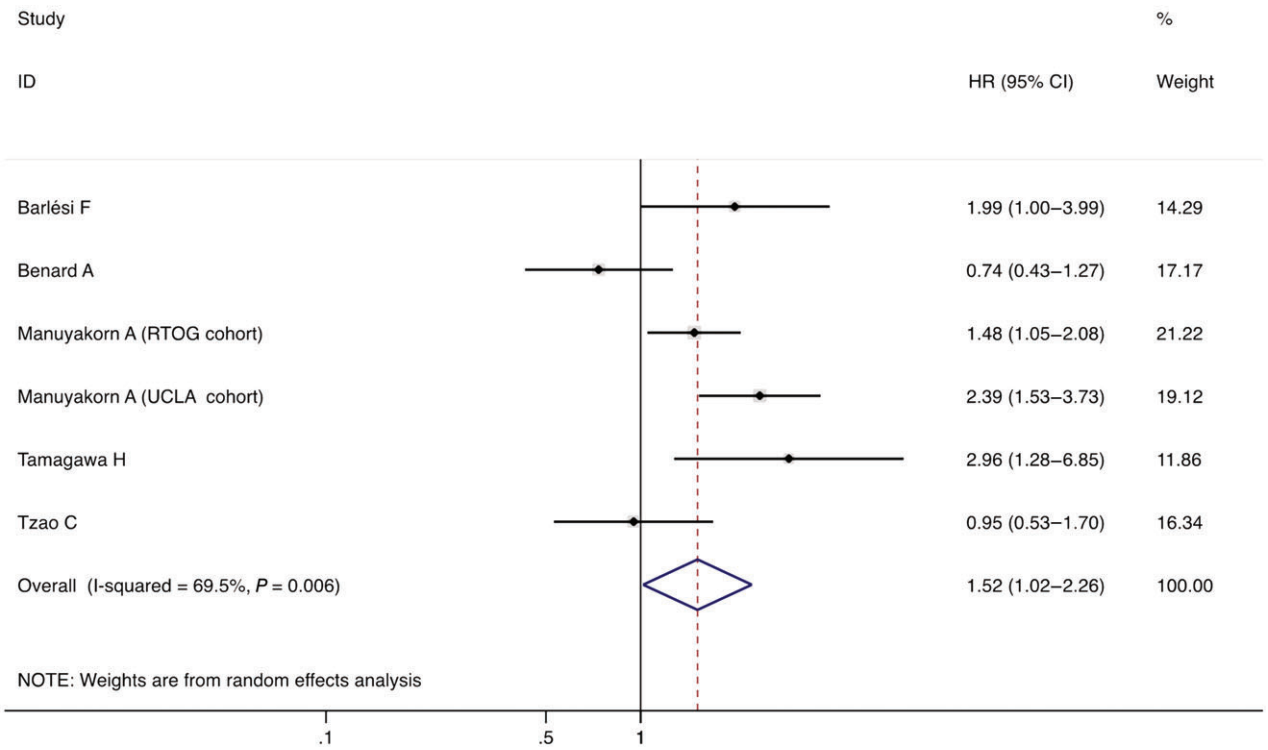


**Figure 2** Forest plot depicting the association between H3K4me2 and the overall survival (OS) of the patients. CI, confidence interval; HR, hazard ratio.

significant finding ($P > 0.05$), indicating that no obvious publication bias existed in the 10 studies included in this meta-analysis.

### Association between H3K4me2 and H3K4me3 and overall survival

For the association between H3K4me2 expression and the prognosis of malignant tumors, a total of five studies ($n = 659$) were included. The random effect model was used to pool results across the studies in presence of moderate heterogeneity ($I^2 = 69.5\%$; $P = 0.006$). As shown in Figure 2, patients with low level H3K4me2 expression demonstrated relatively poorer OS (HR 1.52, 95% CI 1.02–2.26). Four studies ($n = 815$) were included for the association between H3K4me3 expression and the prognosis of malignant tumors. The random effect model was applied to pool results across the studies in the presence of moderate heterogeneity ($I^2 = 60.4\%$; $P = 0.056$). As shown in Figure 3, patients with low level H3K4me3 expression experienced relatively better OS (HR 0.45, 95% CI 0.27–0.74). When each individual study was removed the HR did not significantly change, indicating that the results were relatively stable (Fig S1).

## Discussion

Histone methylation is an essential part of histone modification, which involves a variety of biological processes, including chromatin reorganization, translational regulation, and DNA damage response.[16] Abnormal histone methylation is closely associated with tumor development and progression. H3K4 methylation is an evolutionarily conserved histone modification that regulates cell growth, migration, invasion, and angiogenesis, which further contributes to tumorigenesis.[17] Studies have shown that distinct methylation states of H3K4 can lead to different prognoses in cancer patients. However, a limited number of studies have been conducted and yielded inconclusive results with regard to the relationship between H3K4 methylation and the prognosis of patients with malignant tumors. Therefore, this systematic review and meta-analysis was conducted to assess whether the level of H3K4 methylation could predict the prognosis of cancer patients.

The meta-analysis included a total of 1474 cases with malignant tumors, including non-small cell lung, esophageal squamous cell, colorectal, pancreatic, liver, and cervical cancers. Our results indicated that decreased H3K4me2 expression is significantly correlated with poorer OS (HR 1.52, 95% CI 1.02–2.26), while reduced H3K4me3 expression is significantly associated with better OS

  

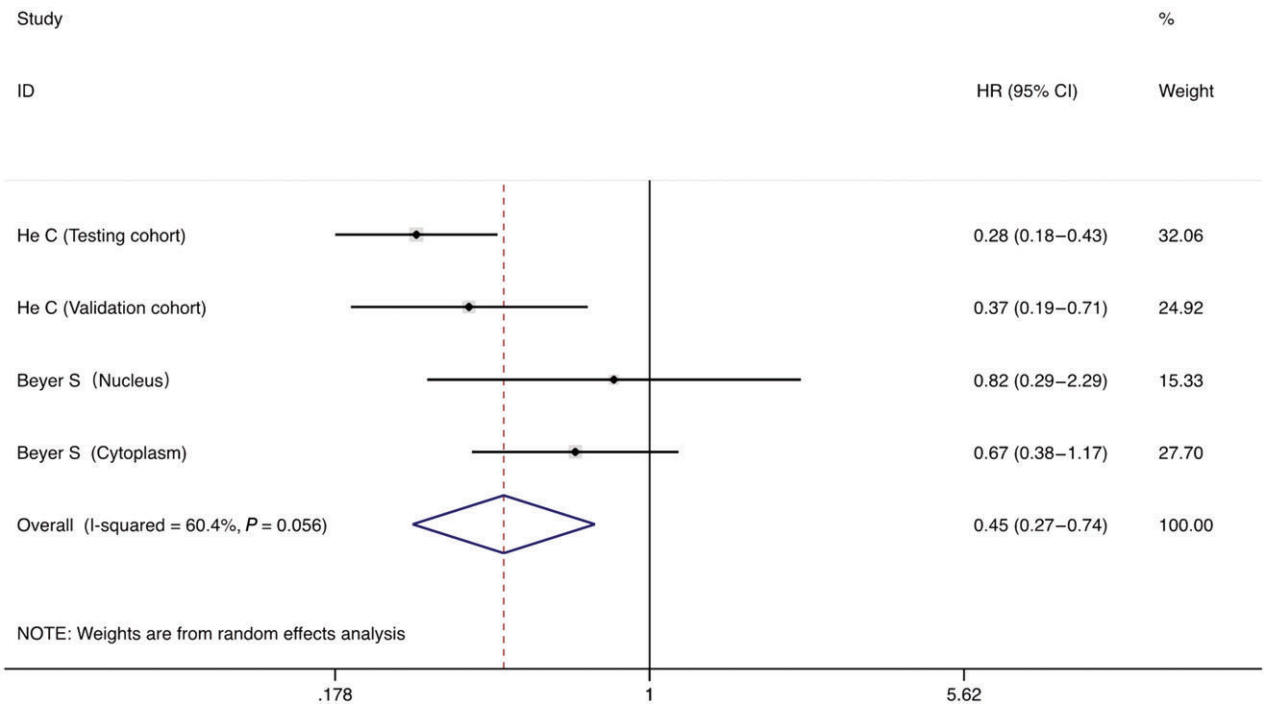


**Figure 3** Forest plot depicting the association between H3K4me3 and the overall survival (OS) of the patients. CI, confidence interval; HR, hazard ratio.

(HR 0.45, 95% CI 0.27–0.74). These results suggest that distinct methylation states of H3K4 may exert different effects on the biological behaviors of tumors. As transcriptional activators, H3K4me2 and H3K4me3 could promote the expression of downstream genes. Meanwhile, the positive or negative regulatory effects of H3K4me2 and H3K4me3 on carcinogenesis are highly dependent on their interaction with biological and environmental factors.[17–19] Because the effects of H3K4 methylation may be rather complex, it is important to investigate the influence of different H3K4 methylation patterns on the prognosis of patients with malignant tumors.

This meta-analysis has several limitations. Most of the included studies were relatively small and involved different types of cancer. However, all of the studies included in this meta-analysis were of high quality, assessed by NOS, and reflected the exact association of H3K4 methylation with patient prognosis. Additionally, because most malignant tissues exhibited similar biological behaviors, it is reasonable to assume that H3K4me2 and H3K4me3 expression levels could be used as prognostic factors to help identify a subgroup of patients with poor prognosis at high risk and to further provide clues for specified molecular typing. In particular, low H3K4me2 or high H3K4me3 expression should be recognized as prognostic factors of poorer survival in multiple cancer types.

The results of our meta-analysis suggest that decreased levels of H3K4me2 and H3K4me3 expression are associated with adverse and protective effects on the survival of diverse cancer patients, respectively. Although a moderate amount of inter-study heterogeneity means that no firm conclusions can be drawn, a sensitivity test revealed that the results were relatively stable. Further studies need to be conducted with a particular focus on the mechanism of carcinogenesis to elucidate the roles of histone methylation in different types of cancer.

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## Disclosure

No authors report any conflict of interest.

 

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** Sensitivity analysis of the association between (a) H3K4me2 and (b) H3K4me3 expression and overall survival.

  