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# Correlation between EZH2 and CEP55 and lung adenocarcinoma prognosis

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ABSTRACT

*Objective:* Recently, accumulated evidence indicates that the enhancer of zeste homologue 2 (EZH2) is highly expressed in a wide range of cancer types, including NSCLC. The downstream genes regulated by EZH2 were screened using bioinformatics analysis. This study aimed to analyse the correlation between the downstream genes of EZH2 and the prognosis of lung adenocarcinoma.

*Methods:* Expression and methylation data of lung adenocarcinoma were downloaded from The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/) database, and data were categorized into EZH2 overexpression and EZH2 downregulation groups according to EZH2 expression. The genes that showed opposite trends of methylation and expression changes were screened, and the association of gene expression was calculated. Based on the String database, a protein association analysis was conducted to identify genes related to EZH2, which are referred to as EZH2 regulation candidate genes. According to gene expression (GSE27262) and methylation (GSE66836) chip data in the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) database, the genes with differential expression and methylation in lung adenocarcinoma tissues were analysed, and the trends of EZH2 regulation candidate gene expression and methylation were verified to identify the EZH2 regulation candidate genes. Subsequently, MethHC (http://methhc.mbc.nctu.edu.tw/php/index.php) and UALCAN (http://ualcan.path.uab.edu/index.html) were employed to verify changes in the expression and methylation of EZH2 downstream regulation candidate genes and to analyse the correlation between these genes and the prognosis of lung adenocarcinoma.

*Results:* Expression and methylation data of lung adenocarcinoma were downloaded from TCGA database and categorized into EZH2 overexpression and EZH2 downregulation groups according to EZH2 expression. A total of 337 genes that showed opposite trends of methylation and expression changes were obtained. The protein association analysis using the String (https://string-db.org/) database showed that 61 genes interact with EZH2 and 61 genes represent EZH2 downstream regulation candidate genes. Moreover, 222 genes obtained from GSE27262 and GSE66836 chip data were negatively correlated with methylation and expression changes, and centrosomal protein 55 (CEP55) was identified as the EZH2 downstream regulation candidate gene. CEP55 was upregulated in lung adenocarcinoma tissues and showed low methylation. According to gene expression data from TCGA database, CEP55 and EZH2 exhibit higher levels in lung adenocarcinoma tissue than in adjacent normal tissue. Finally, the survival analysis revealed that EZH2 is not associated with the prognosis of lung adenocarcinoma, while CEP55 is related to lung adenocarcinoma prognosis.

*Conclusion:* Taken together, these results indicate that changes in EZH2 expression lead to changes in CEP55 expression in lung adenocarcinoma, and these changes are associated with its prognosis.

## 1. Introduction

Lung cancer, one of the most common cancers worldwide, remains the leading cause of death from cancer. There were 226,160 new cases of lung cancer and 160,340 cases of lung cancer deaths in 2012 [39]. With a lower early diagnosis rate of lung cancer, lung cancer is mostly diagnosed in the high metastasis stage of the tumour and is difficult to cure, with a poor prognosis [32]. The survival rate of lung cancer within 5 years is approximately 15% [21]. Moreover, lung adenocarcinoma is a main subtype of lung cancer with a poor chemotherapeutic effect, and a large number of patients show resistance to cisplatin [33]. Due to the lower early diagnosis rate, limited

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chemotherapeutic effect, and poor prognosis of lung adenocarcinoma, biomarkers with high sensitivity and specificity would be helpful in identifying its pathological changes, and new biomarkers would predict prognosis and provide a specific treatment strategy for lung adenocarcinoma to obtain better clinical outcomes.

Epigenetic inheritance is regarded as the genetic modification of chromatin that affects gene expression and related processes of other DNAs but does not directly change the DNA coding sequence [18]. Epigenetic inheritance includes DNA methylation, histone modification, and noncoding RNA expression, affecting some functions, such as individual biological metabolism, DNA repair capacity, and immunity [18,25]. Epigenetic abnormality often occurs in human cancers and is involved in the occurrence and progression of cancer, possibly representing a biomarker for cancer diagnosis and prognosis [9]. At present, the epigenetic event that has been most investigated is DNA methylation, the chemical covalent modification for cytosine. Changes in DNA methylation in lung cancer present as the hypermethylation of tumour suppressor genes [17,22]. Hypomethylation affects genome stability and induces high expression of oncogenes [10,12]. DNA methylation is a novel potential biomarker for lung cancer diagnosis and prognosis with high sensitivity and specificity [2].

In recent years, an increasing number of studies have paid more attention to the methylation of genes in lung cancer. It cannot be ignored that the factor that results in gene methylation serves as a modulatory factor for the potential carcinogenesis of lung cancer. It is of great significance for cancer progression, diagnosis, and prognosis to understand the regulatory roles of epigenetic modifying factors in methylation changes. As a method of genome modification, methylation is dependent on specific epigenetic modifying factors, including the regulation of methyltransferase [50]. Enhancer of zeste homologue 2 (EZH2) is a common methyltransferase. EZH2 overexpression is related to a poor prognosis of lung cancer. EZH2 is highly expressed in multiple cancers, such as cervical [28], mammary [14], prostatic [31], renal [45], gastric [15], and lung [51]. In lung cancer tissues, upregulated EZH2 is associated with histological differentiation, pathological tumour-lymph node metastasis staging and smoking history [5]. As an epigenetic modifying factor, EZH2 promotes the occurrence and progression of cancer by regulating downstream molecules via chromatin modifications, including epigenetic inheritance, stimulating a carcinogenic signal or silencing tumour suppressor genes [44].

Aberrant expression of EZH2 is correlated with the progression of lung adenocarcinoma. However, studies on EZH2-regulating downstream molecules in lung adenocarcinoma are lacking. Therefore, in the present study, bioinformatics was adopted to screen downstream genes regulated by EZH2, and the expression chip data of lung adenocarcinoma and methylation chip data in the GEO database were examined to verify the expression of downstream genes and methylation differences to analyse the correlations between EZH2 and its downstream genes with the prognosis of lung adenocarcinoma, providing new insight into the epigenetic modification/regulatory mechanism of lung adenocarcinoma and direction for its early diagnosis and prognosis.

## 2. Materials and methods

### 2.1. TCGA lung adenocarcinoma methylation and RNA-Seq data

The expression and methylation data of lung adenocarcinoma and normal tissue were downloaded in open source data generated from The Cancer Genome Atlas (TCGA) genome data analysis centre (https://cancergenome.nih.gov/). The Genomic Data Commons Application Programming Interface (GDC API) in TCGA was adopted to retrieve and download data to generate the methylation and expression profiles of lung cancer genes [48]. The beta value in the methylation profile represents the methylation degree calculated by Illumina Infinium Human Methylation 450 K arrays, which represents the intensity ratio of gene methylation to nonmethylation [47]. After normalisation using the RNA-Seq by expectation maximization (RSEM) in the expression profile, the expression value was used to refer to the mRNA level [26]. The 453 cases of methylation and RNA-Seq data were obtained from TCGA database and were used in the subsequent analyses.

### 2.2. Detection of the methylation and expression of lung cancer driven by EZH2 in TCGA

To screen the downstream genes that result in changes in methylation and expression caused by differences in EZH2 expression and methylation in lung cancer, the samples were first assigned to either the EZH2 overexpression or EZH2 downregulation group according to the EZH2 expression level of the gene expression profile. To compare the differences in gene expression between the EZH2 overexpression and EZH2 downregulation groups, limma [41] of R package was adopted and the log2 fold change (log2FC) of the gene was calculated. After correction, the $P$ value was represented as *adj.P.Val*, and if $|\log FC| > 1$ and the gene of *adj.P.Val* was identified as the differential gene, the volcano map of the differentially expressed gene was drawn. The *t*-test was performed to distinguish the differential methylation site between the EZH2 overexpression and EZH2 downregulation groups, and the site with a $p$ value of $< 0.05$ was regarded as the differential methylation site. The methylation level of the gene affected the gene expression level: the lower the methylation level, the higher expression level and vice versa [3,49]. Moreover, jvenn (http://jvenn.toulouse.inra.fr/app/example.html) was adopted to compare genes with up- or downregulated expression and genes with up- or downregulated methylation to screen genes with the opposite trend of methylation and expression, which were considered EZH2 downstream regulation candidate genes.

### 2.3. Functional analysis of EZH2 downstream regulation candidate genes using GO and KEGG analyses

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/home.jsp) provides a set of complete annotation tools to help us understand the biological significance of a large number of genes and classifies gene function and analyses the functional annotation clustering and functional annotation table for a list of given genes [19]. The DAVID was adopted for the functional analysis of EZH2 downstream regulation candidate genes using GO and KEGG and the obtained GO and KEGG functional enrichment results were visualized using ImageGP (http://www.ehbio.com/ImageGP/index.php/Home/Index/index.html).

### 2.4. Association analysis of EZH2 and genes driven by EZH2

The profile of EZH2 and its downstream regulation candidate genes was extracted according to TCGA gene expression profiles in lung adenocarcinoma. R language was adopted to calculate the Pearson correlation coefficient of downstream regulation candidate genes with EZH2 expression and the correlation of expression among genes. The String (https://string-db.org/) database provides protein-protein interaction information, including direct (physical) and indirect (functional) associations [42]. The protein-protein interaction (PPI) network constructed with EZH2 downstream regulation candidate genes was obtained from the String database in Cytoscape 3.6.0 software [38]. The default scoring threshold of interaction in the String database was 0.4 and the subnetwork constructed with those genes interacting with EZH2 was further extracted. Next, the EZH2 driving genes and the genes that interact with EZH2 were constructed into a network.

### 2.5. Expression and methylation of genes in lung adenocarcinoma using the Gene Expression Omnibus (GEO)

Expression (GSE27262) and methylation (GSE66836) chip data of

genes in lung adenocarcinoma were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) and used to analyse differences between the expression and methylation of genes in lung adenocarcinoma. GSE27262 was used to examine gene expression in tumour and adjacent normal tissues from 25 patients with lung adenocarcinoma. The gene annotation platform used was the GPL570-[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The affy package of R language [13] was adopted for background correction and standardized pretreatment for the gene expression profile. The limma package [41] was employed to screen the differential genes, and the screening threshold of differential genes was set as $adj.P.Val < 0.05$ and $|\log2FC| > 1$. A heat map of differentially expressed genes was drawn. GSE66836 was used for methylation sequencing data of the whole genome from 164 lung adenocarcinoma tissues and 19 normal tissues detected by the Illumina Infinium 450 K array. The wateRmelon package of R language [35] was adopted for standardized pretreatment for 450 K methylation data. The minif package [1] was used for the differential methylation analysis of methylation data to compare the differentially methylated genes between lung adenocarcinoma and normal tissues. The expression and methylation differences of genes in lung adenocarcinoma in the GEO database were compared. jvenn was employed to screen genes that showed the opposite trend of methylation and expression changes in lung adenocarcinoma.

### 2.6. Chip data analysis using the GEO

According to the EZH2 downstream regulation candidate genes obtained from TCGA database and lung adenocarcinoma genes obtained from the GEO database that showed the opposite trend of methylation and expression changes, the differences between these two types of genes were compared to screen EZH2 downstream regulation candidate genes with differential expression and methylation in lung adenocarcinoma. Centrosomal protein 55 (CEP55) was revealed as an EZH2 downstream regulation gene. Next, CEP55 gene expression data were extracted from the expression profile in TCGA database and GSE27262 chip data in the GEO database. Changes in CEP55 expression in the EZH2 overexpression and EZH2 downregulation groups in TCGA expression profile and the differential expression of CEP55 in lung adenocarcinoma in GSE27262 chip data were analysed.

### 2.7. Changes in EZH2 and CEP55 expression in lung adenocarcinoma in TCGA database

The expression and methylation differences in the EZH2 overexpression and EZH2 downregulation groups were analysed to identify EZH2 regulatory genes. The expression and methylation of EZH2 and its regulatory genes in lung adenocarcinoma and adjacent normal tissues were further verified.

UALCAN (http://ualcan.path.uab.edu/index.html) was used to analyse TCGA data. UALCAN was used to examine gene expression in tumour and normal samples and changes in factors associated with various tumours, such as tumour stage, tumour classification, race, weight, and other clinicopathological features [7]. The expression differences of CEP55 and EZH2 were identified in UALCAN. The MethHC (http://methhc.mbc.nctu.edu.tw/php/index.php) database provides DNA methylation data in TCGA database [20]. Methylation changes in CEP55 and EZH2 in lung adenocarcinoma and adjacent normal tissues were compared using the MethHC database.

### 2.8. Correlation analysis of EZH2 and CEP55 expression

CEP55 expression and methylation differences were driven by EZH2 expression. The differential expression and methylation of CEP55 and EZH2 in lung adenocarcinoma tissues were verified in the GEO and TCGA databases. For further analysis of the regulatory potential of EZH2 on CEP55, the association between CEP55 and EZH2 was analysed. EZH2 and CEP55 expression profiles were extracted from TCGA lung adenocarcinoma expression profiles and GSE27262 chip data. GraphPad Prism 6 was employed to analyse the Pearson correlation coefficient of EZH2 and CEP55 expression, and correlation graphs of EZH2 and CEP55 were drawn.

### 2.9. Effects of EZH2 and CEP55 expression on the prognosis of lung adenocarcinoma

Changes in EZH2 expression may regulate CEP55 in lung adenocarcinoma, and both EZH2 and CEP55 are differentially expressed in lung adenocarcinoma. To investigate the clinical effects of gene expression, specifically CEP55 expression on patients with lung adenocarcinoma, the effects of EZH2 and CEP55 expression changes on the viability of patients with lung adenocarcinoma were investigated. Because UALCAN data originate from TCGA level 3 RNA-Seq and the clinical data of cancer (except for the analysis of gene expression changes in tumours), the effect of gene expression on patient survival conditions was analysed. UALCAN was used to analyse the correlation between EZH2 and CEP55 expression and the prognosis of lung adenocarcinoma.

### 2.10. Statistical analyses

All quantitative data were analysed by t-tests or one-way ANOVA with an open source software package in the R programming language. Statistical significance was considered at $p < 0.05$.

## 3. Results

### 3.1. A total of 337 genes were identified as EZH2 downstream regulation candidate genes in TCGA

RNA-Seq and 450 K methylation data of 453 patients with lung adenocarcinoma were downloaded from TCGA database to obtain the expression and methylation profiles. Samples were grouped into the EZH2 overexpression or EZH2 downregulation groups according to the EZH2 expression level, as shown in Fig. 1A. The limma package was employed to screen the differentially expressed genes between the EZH2 overexpression and EZH2 downregulation groups, with a threshold of $|\log FC| > 1$ and $adj.P.Val < 0.05$. The volcano map of the differentially expressed genes is shown in Fig. 1B. In total, 480 genes were clearly upregulated and 275 were clearly downregulated. Information regarding the differentially expressed genes is shown in Supplemental Table 1. The *t*-test ($p$ value $< 0.05$) was used to identify the differential methylation site between the EZH2 overexpression and EZH2 downregulation groups. The same differential mode of differential methylation site in the gene promoter region suggests that differential methylation exists in the gene promoter region. A total of 18,165 differentially expressed genes were obtained in the EZH2 overexpression and EZH2 downregulation groups (Supplemental Table 2). Compared with the EZH2 downregulation group, the EZH2 overexpression group had 7552 genes with downregulated methylation and 2945 genes with upregulated methylation and downregulated EZH2 methylation. Genes that showed the opposite trend of methylation and expression changes were further screened. As shown in Fig. 1C, the EZH2 overexpression group had 95 genes with overexpressed methylation but reduced expression, 242 genes with overexpressed expression but reduced methylation and decreased EZH2 methylation but elevated EZH2 expression. These 337 genes were thus identified as EZH2 downstream regulation candidate genes.

### 3.2. The functions of EZH2 downstream candidate genes are closely related to the progression of lung adenocarcinoma

A total of 337 EZH2 downstream candidate genes screened from

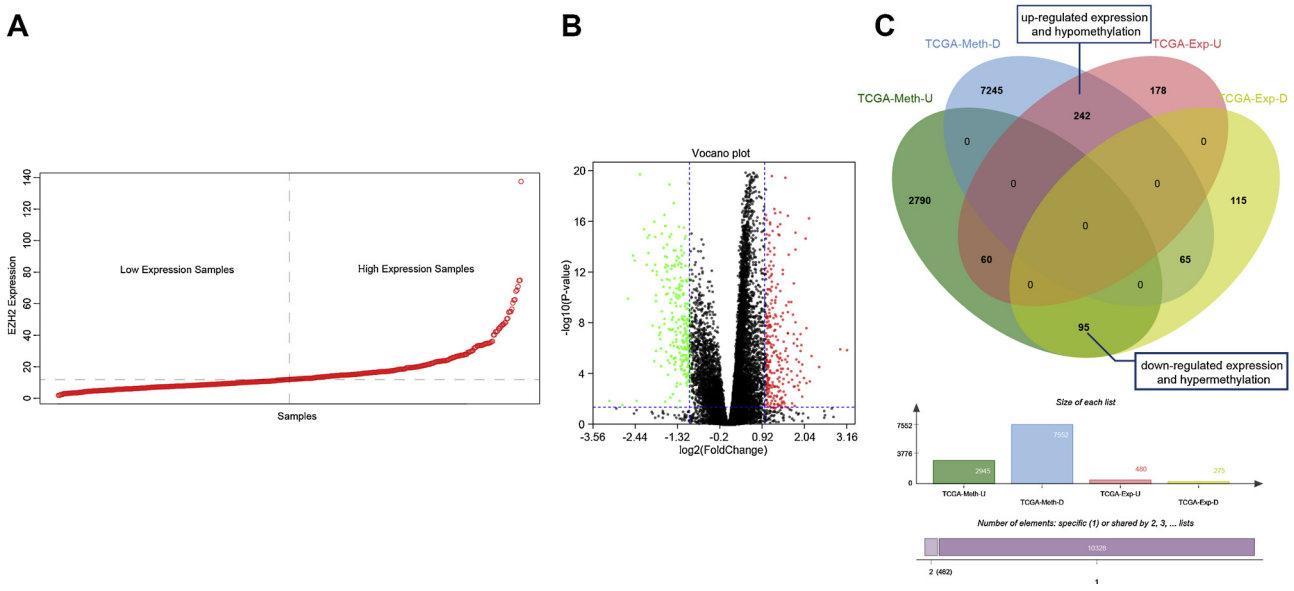


**Fig. 1.** A total of 337 genes were identified as EZH2 downstream regulation candidate genes in TCGA.
Note: A, classification of RNA-Seq and 450 K methylation data of 453 patients with lung adenocarcinoma in TCGA database according to EZH2 expression; B, volcano map of differentially expressed genes between the EZH2 overexpression and EZH2 downregulation groups; C, changes in methylation and expression caused by EZH2 in TCGA database, and the screening of genes that showed the opposite trend of methylation and expression changes; TCGA, The Cancer Genome Atlas; EZH2, enhancer of zeste homologue 2.

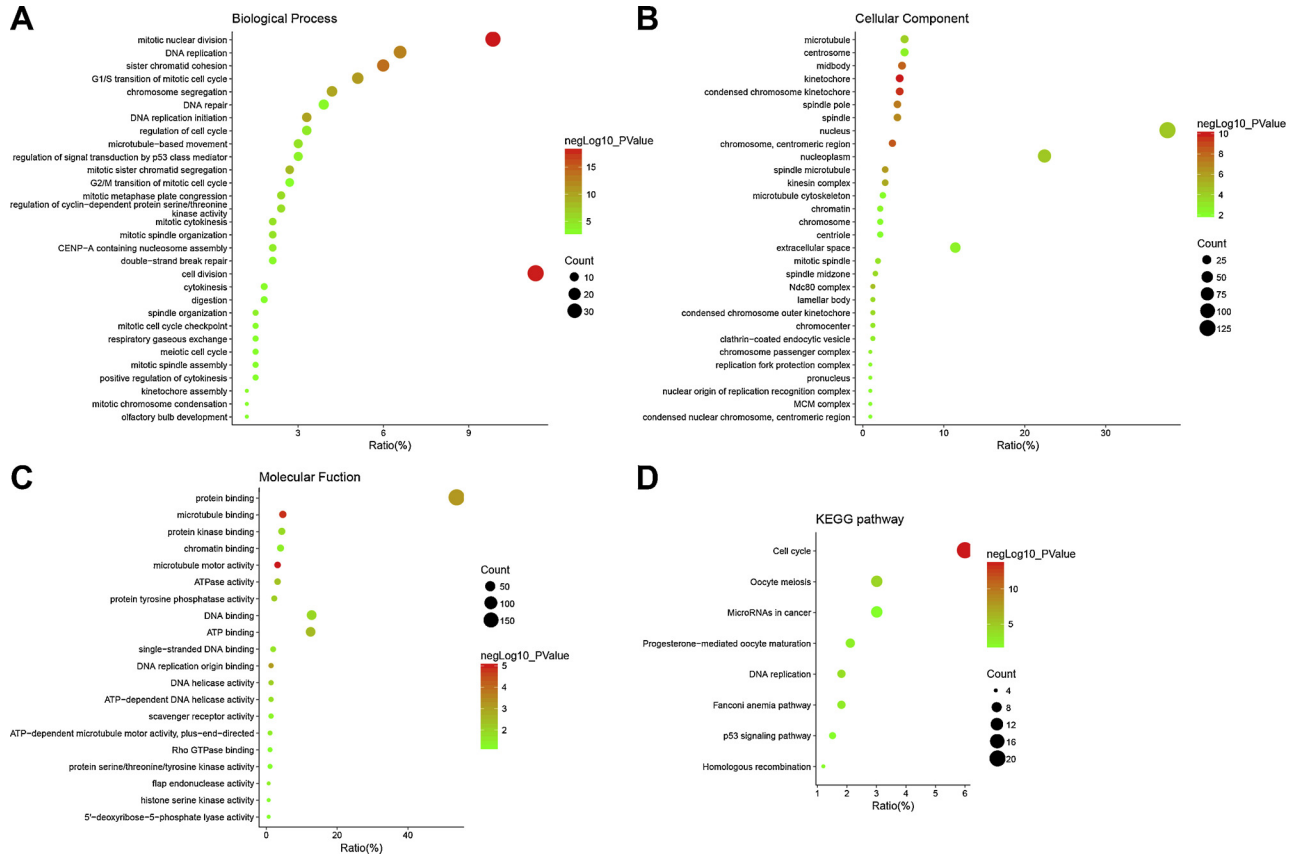


**Fig. 2.** GO and KEGG functional analyses showed that functions of EZH2 downstream candidate genes are closely related to the progression of lung adenocarcinoma.
Note: A–D refer to the enrichment results of Biological Process (BP), Cellular Component (CC), Molecular Function (MF), and KEGG enrichment of EZH2 downstream candidate genes; the ordinate represents the enriched GO/KEGG entries; the abscissa refers to the ratio of genes enriched in entries; the colour of the circle represents the *p* value, and the size of the colour refers to the number of genes enriched in the same entry; EZH2, enhancer of zeste homologue 2.

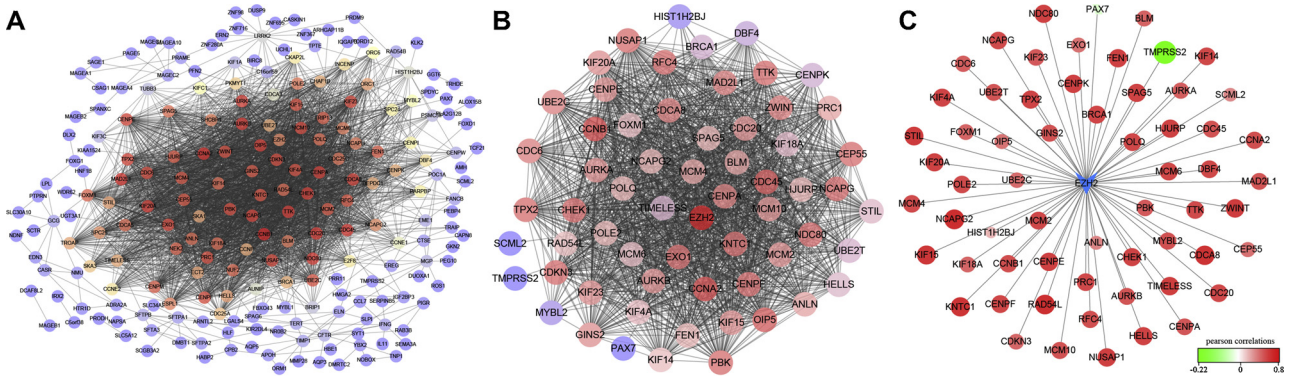


**Fig. 3.** Correlations of EZH2 with its downstream regulation candidate genes.
Note: A, PPI network of EZH2 and its downstream candidate genes. The colour of the gene refers to its association with other genes; red indicates a strong association, while purple indicates a weak association; B, PPI subnetwork of genes that interact with EZH2. The colour of the gene refers to its association with other genes; red indicates a strong association, while purple indicates a weak association; C, network of downstream candidate genes driven by EZH2. The blue arrow represents EZH2, and the circle refers to the downstream candidate genes that interact with EZH2. The colour of the circle refers to the association of genes with EZH2; red indicates a positive correlation, while green indicates a negative correlation; PPI, protein-protein interaction; EZH2, enhancer of zeste homologue 2.

lung adenocarcinoma expression and methylation profiles in TCGA were processed by GO and KEGG functional analyses. The results of Biological Process (BP) (Fig. 2A), Cellular Component (CC) (Fig. 2B), Molecular Function (MF) (Fig. 2C), and KEGG pathway (Fig. 2D) enrichment were obtained. EZH2 downstream regulation candidate genes were enriched in several bioprocesses, including mitotic nuclear division, DNA replication, cell division, and G1/S transition of the mitotic cell cycle. KEGG enrichment revealed the enriched entries as cell cycle and DNA replication, suggesting that the functions of genes involved in lung adenocarcinoma are associated with cell proliferation. DNA repair and the p53 signalling pathway were also clearly enriched entries, indicating that the candidate genes correlate with DNA repair. Cell proliferation and DNA repair could affect the progression of lung adenocarcinoma. The functional analysis revealed that the functions of EZH2 downstream candidate genes are closely related to the progression of lung adenocarcinoma.

### 3.3. Correlations between EZH2 and its downstream candidate genes

The correlations between EZH2 and its 337 downstream candidate genes were calculated, and the Pearson correlation coefficients of candidate genes and EZH2 were obtained (Supplemental Table 3). The interaction network of 337 genes was obtained using the String database to construct the PPI network of EZH2 downstream candidate genes (Fig. 3A). To analyse the association among genes that interact with EZH2, the subnetwork of EZH2-interacting genes was further extracted (Fig. 3B). A total of 61 genes interact with EZH2, and the interacting network of these genes is close. Combined with the expression correlation and gene interaction of EZH2 with candidate genes, the network of downstream candidate genes driven by EZH2 was constructed (Fig. 3C). In the interaction map, EZH2 expression was negatively related to PAX7 and TMPRSS2 but positively associated with other genes.

### 3.4. A total of 222 genes show opposite trends of methylation and expression changes

The differentially expressed genes of lung adenocarcinoma were screened from GSE27262 chip data according to $adj.P.Val < 0.05$ and $|\log FC| > 1$ and the volcano map of differentially expressed genes (Fig. 4A). A total of 1480 genes were obtained, among which 908 were downregulated and 572 were upregulated. The heat maps of the first 100 differentially expressed genes were drawn (Fig. 4B). Information regarding the differential methylation screen from GSE66836 is shown in Supplemental Table 4. There were 3678 genes with upregulated methylation and 2742 genes with downregulated methylation. The expression and methylation differences of lung adenocarcinoma genes were compared, and a Venn map was drawn (Fig. 4C). In lung adenocarcinoma tissues, 174 genes exhibited upregulated methylation but downregulated expression, and 48 genes exhibited upregulated expression but downregulated methylation. The trends of methylation and expression changes in the 222 genes were opposite.

### 3.5. Higher CEP55 level in lung adenocarcinoma tissues

Sixty-one lung adenocarcinoma genes that showed opposite trends of methylation and expression changes in the TCGA database were screened in the EZH2 overexpression and EZH2 downregulation groups and were determined to interact with EZH2. According to expression and methylation chip data in the GEO database, 222 genes showed opposite trends of methylation and expression changes. After comparing the results between TCGA and GEO databases (Fig. 5A), CEP55 was the only gene shared between the two databases. CEP55 expression between the EZH2 overexpression and EZH2 downregulation groups was compared after classification according to EZH2 expression in TCGA expression profile. CEP55 expression in the EZH2 overexpression group was higher than that in the EZH2 downregulation group (Fig. 5B). The change in CEP55 expression in GSE27262 chip data is shown in Fig. 5C, which revealed that the CEP55 level was higher in lung adenocarcinoma tissues than adjacent normal tissues.

### 3.6. CEP55 expression and methylation are positively associated with EZH2 expression and methylation

TCGA data analysis tools UALCAN and MethHC were employed to verify the changes in EZH2 and CEP55 expression and methylation in lung adenocarcinoma. The changes in EZH2 and CEP55 expression and methylation in TCGA database are shown in Fig. 6. Compared with adjacent normal tissues, EZH2 exhibited upregulated expression (Fig. 6A) and downregulated methylation (Fig. 6C) in lung adenocarcinoma tissues, and the changes in CEP55 expression (Fig. 6B) and methylation (Fig. 6D) were consistent with EZH2. In TCGA database, the expression and methylation of EZH2 and CEP55 displayed obvious changes. CEP55 exhibited high expression and low methylation in lung adenocarcinoma tissues. The changes in CEP55 in lung adenocarcinoma in TCGA and GEO databases were consistent.

### 3.7. EZH2 expression is positively related to CEP55 expression

Expression data of EZH2 and CEP55 were extracted from expression profiles in TCGA database and GSE27262 to examine the correlation

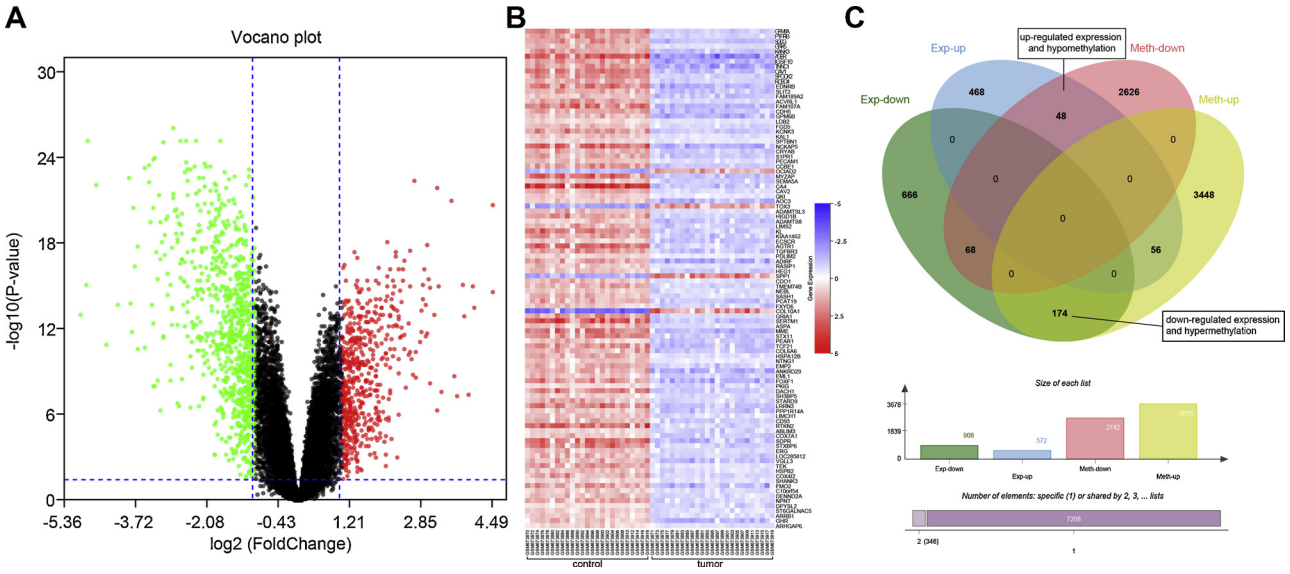


**Fig. 4.** A total of 222 genes that show opposite trends of methylation and expression changes.
Note: A, volcano map of differentially expressed genes in GSE27262; B, heat map of the first ten differentially expressed genes in GSE27262. The ordinate represents the number of samples, and the abscissa refers to the differentially expressed genes. The histogram on the right side represents the differential genes, and each rectangle represents a sample expression value; C, methylation and expression changes in lung adenocarcinoma genes and the trends of methylation and expression changes in 222 genes were opposite.

between EZH2 and CEP55 expression. In TCGA database, EZH2 expression is positively correlated with CEP55 expression (r = 0.4926, $p < 0.0001$) (Fig. 7A). EZH2 expression is also positively correlated with CEP55 expression in GSE27262 chip data (r = 0.7888, $p < 0.0001$) (Fig. 7B).

### 3.8. A higher CEP55 level is related to a poor prognosis of lung adenocarcinoma

TCGA online analysis tool UALCAN was used to analyse the effects of EZH2 and CEP55 expression on the survival of patients with lung adenocarcinoma. The survival curves for EZH2 (Fig. 8A) and CEP55 (Fig. 8B) expression with lung adenocarcinoma were constructed according to gene expression. In the survival curve with EZH2 (Fig. 8A), EZH2 expression exerted no significant effect on the survival conditions of patients with lung adenocarcinoma ($p = 0.8$). However, according to the survival curve with CEP55 (Fig. 8B), CEP55 expression is associated with the survival rate of patients with lung adenocarcinoma. A higher CEP55 level represented a low survival rate, suggesting a poor prognosis ($p = 0.0022$). The analysis of survival curves for EZH2 and EP55 indicated that differential CEP55 expression has a greater impact on the prognosis of lung adenocarcinoma.

## 4. Discussion

Genes with expression and methylation differences caused by

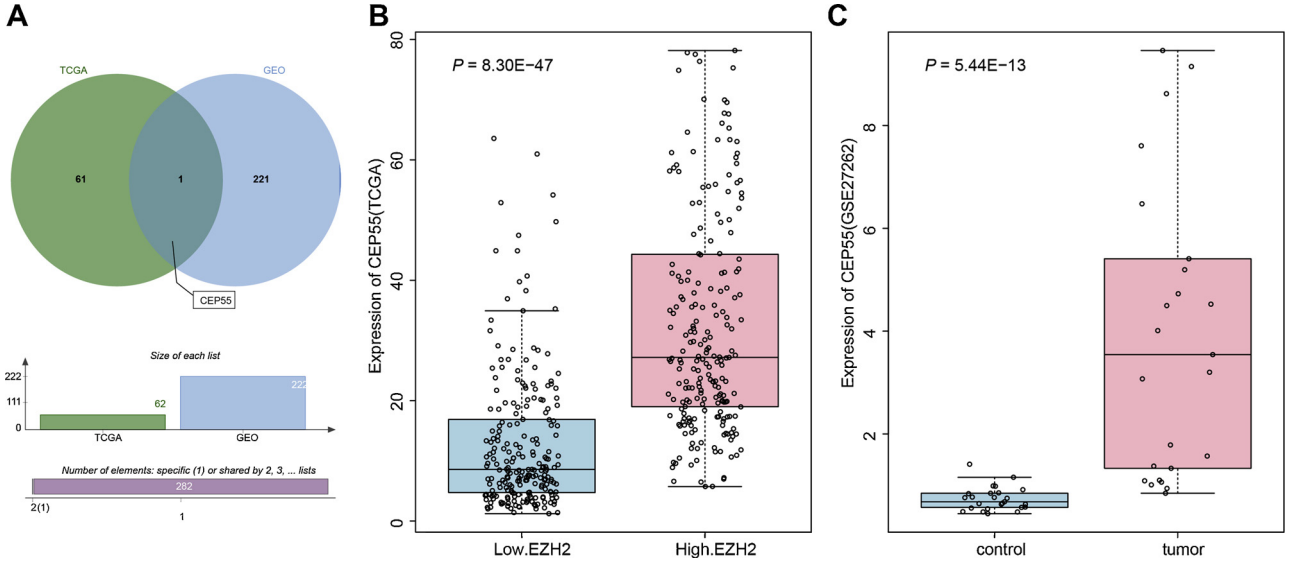


**Fig. 5.** Lung adenocarcinoma tissues showed a higher CEP55 level.
Note: A, EZH2 downstream regulation candidate genes in TCGA database and genes that were differentially methylated and expressed in lung adenocarcinoma in GEO chip data, where CEP55 is a common gene; B, CEP55 expression change in the EZH2 overexpression and EZH2 downregulation groups in TCGA database; C, differential expression of CEP55 in lung adenocarcinoma in GEO chip data, GSE27262; TCGA, The Cancer Genome Atlas; EZH2, enhancer of zeste homologue 2; GEO, Gene Expression Omnibus; CEP55, centrosomal protein 55.

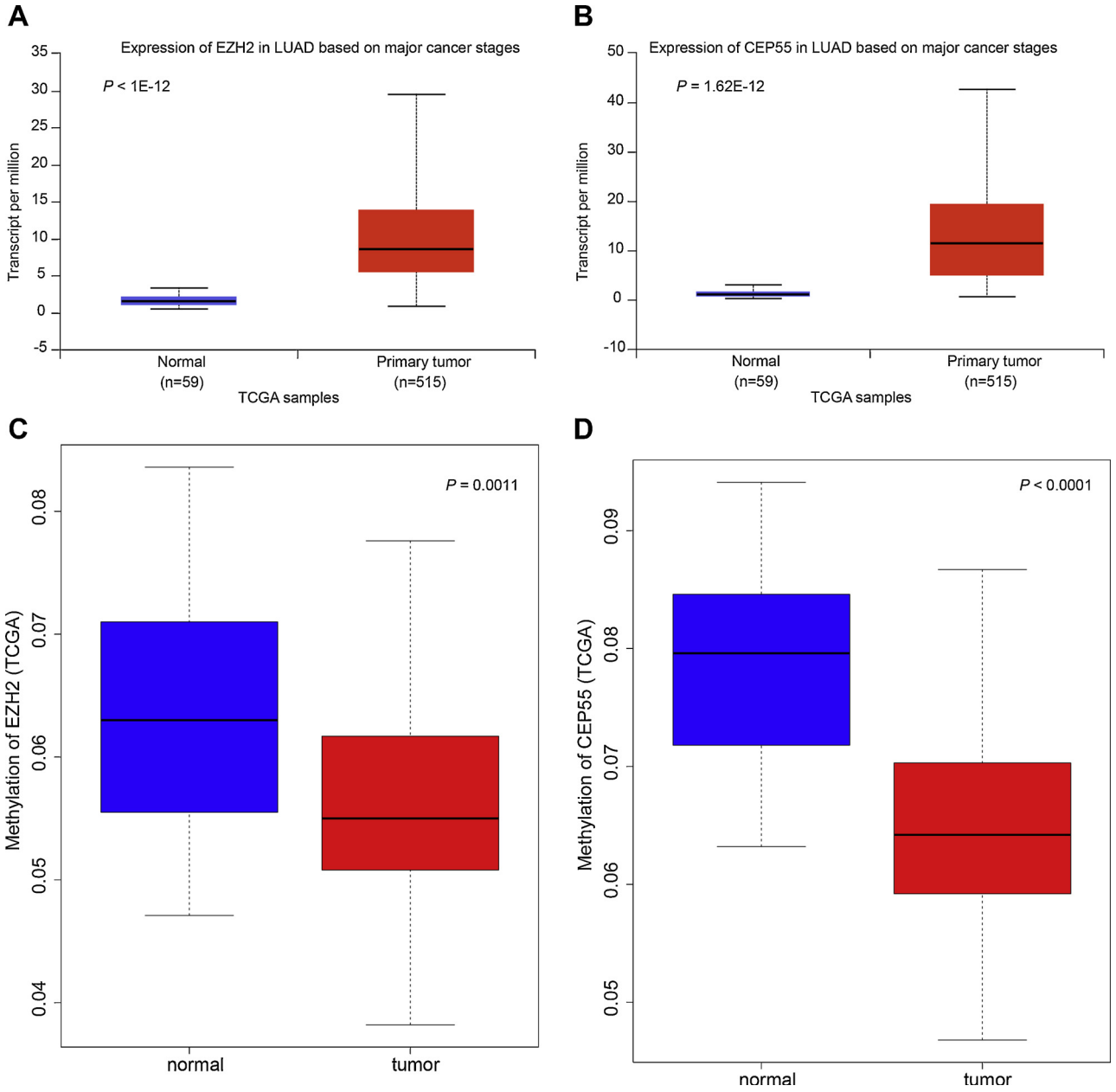


**Fig. 6.** CEP55 expression and methylation are positively associated with EZH2 expression and methylation.
Note: A, EZH2 expression change in lung adenocarcinoma; B, CEP55 expression change in lung adenocarcinoma; C, EZH2 methylation change in lung adenocarcinoma; D, CEP55 methylation change in lung adenocarcinoma; EZH2, enhancer of zeste homologue 2; CEP55, centrosomal protein 55.

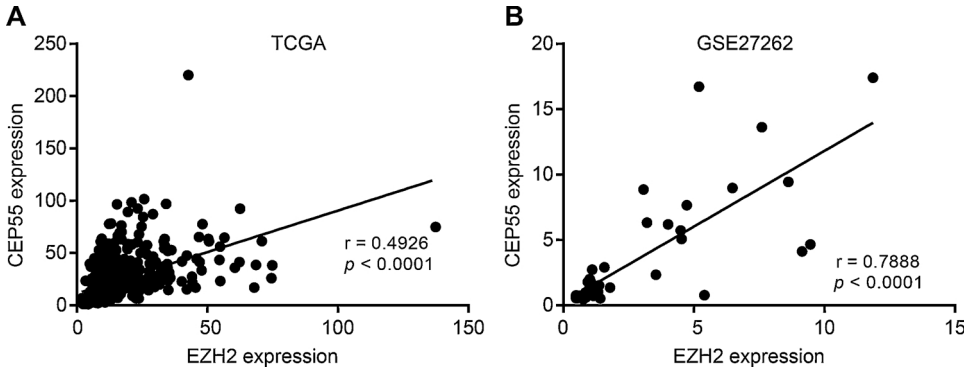


**Fig. 7.** EZH2 expression is positively related to CEP55 expression.
Note: A, correlation between EZH2 and CEP55 expression in TCGA; B, correlation between EZH2 CEP55 expression in GSE27262; TCGA, The Cancer Genome Atlas; EZH2, enhancer of zeste homologue 2; CEP55, centrosomal protein 55.

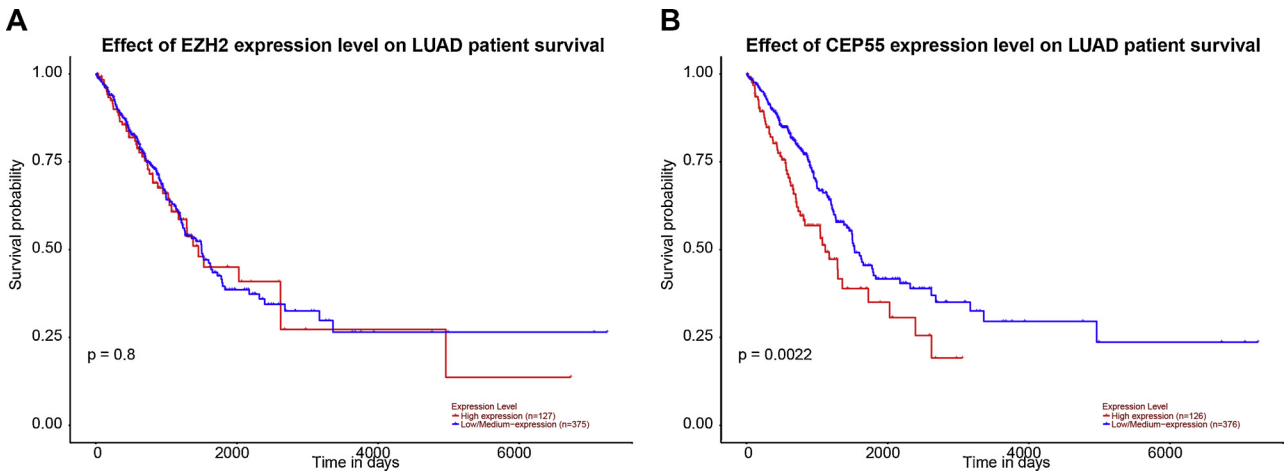


**Fig. 8.** Survival curves for EZH2 and CEP55 in lung adenocarcinoma.
Note: survival curves depicting survival probity in the two sets of patients with lung adenocarcinoma by EZH2 (A) or CEP55 (B) expression (low and high); EZH2, enhancer of zeste homologue 2; CEP55, centrosomal protein 55.

changes in EZH2 expression were screened from lung adenocarcinoma methylation and expression profiles in TCGA database and considered EZH2 downstream regulation candidate genes. The methylation level affects the expression level and the methylation level is negatively correlated with the expression level [3,49]. According to classification by EZH2 expression, 337 genes showed opposite trends of expression and methylation changes, and 61 genes interact with EZH2, representing EZH2 downstream regulation candidate genes. Subsequently, the changes in EZH2 methylation and expression were verified according to lung adenocarcinoma methylation and expression in the GEO database. In TCGA database, EZH2 expression led to increased CEP55 expression but decreased methylation. In the GEO database, CEP55 expression was downregulated, while its methylation was upregulated. Therefore, CEP55 was identified as the EZH2 downstream regulation gene.

According to TCGA database analysis, CEP55 differs in methylation and expression between the EZH2 overexpression and EZH2 downregulation groups. MethHC and UALCAN were adopted to further verify differences in the expression and methylation of CEP55 and EZH2 in lung adenocarcinoma tissues (TCGA database). Accumulating reports have paid increasing attention to the effects of the aberrant expression of EZH2 on lung cancer, even on lung adenocarcinoma [11,27,30,36]. At present, several studies have demonstrated that CEP55 is aberrantly expressed in various cancers, such as bladder cancer [40] and head and neck squamous cell carcinoma (HNSCC) [46]. Furthermore, CEP55 overexpression is regarded as an event in the early stage of cancer, and CEP55 is activated in precancerous and cancerous tissues in colon cancer [37]. However, few studies have focused on the role of CEP55 methylation differences in cancer, and changes in CEP55 methylation and expression in lung adenocarcinoma and its potential molecular mechanism remain poorly understood.

The EZH2 overexpression group exhibited upregulated CEP55 expression but downregulated methylation, suggesting that changes in EZH2 expression in lung adenocarcinoma result in differences of CEP55 methylation and expression. Moreover, according to TCGA database and GSE27262 chip data, CEP55 expression is positively correlated with EZH2 expression, indicating that differential CEP55 expression in lung adenocarcinoma is related to EZH2 expression. As an epigenetic modifying factor, EZH2 promotes cancer by inhibiting the expression of tumour suppressor genes. For instance, EZH2 suppresses the expression of the antiangiogenic factor vasohibin 1 (VASH1) by elevating its methylation to promote tumour angiogenesis [29]. In addition, EZH2 affects cancer by activating oncogenes. EZH2 activates Ephrin-B2 expression by binding to the Ephrin-B2 promoter region to enhance tumour angiogenesis [16]. EZH2 expression increases the RAF1 gene copy number to activate the RAF1-ERK-β-catenin signalling pathway to promote the progression of breast cancer [8]. Consistent with our results, EZH2 overexpression affects lung adenocarcinoma by upregulating CEP55 expression.

Moreover, according to the functional analysis, CEP55 was enriched in the entry of biological processes related to mitosis of mitotic nuclear division, mitotic metaphase plate congression, and mitotic cytokinesis. CEP55 is a type of mitotic phosphoprotein that plays a vital role in cell division. In the last stage of cell division, the physical separation of two daughter cells is achieved [43]. Therefore, CEP55 may affect the progression of cancer by altering the biological behaviour of cancer cells.

To understand the potential of the differential expression of EZH2 and CEP55 acting as biomarkers for the prognosis of lung adenocarcinoma, survival curves for EZH2 and CEP55 expression were constructed. Changes in EZH2 expression could not accurately predict the prognosis of patients with lung adenocarcinoma. However, a previous study proved that a higher EZH2 expression level is associated with relapse-free and overall survival of patients with lung adenocarcinoma [4]. Cao et al. revealed that EZH2 is upregulated (compared with normal tissues) in non-small cell lung cancer (NSCLC) tissues and is an independent factor of a poor prognosis of these patients. However, interestingly, EZH2 expression itself in tumour tissues is not able to predict the prognosis of patients with NSCLC [6]. It remains to be determined whether EZH2 is a prognostic factor of lung adenocarcinoma in a large-scale analysis. Moreover, we also found that higher CEP55 expression represents a poorer prognosis of patients with lung adenocarcinoma, suggesting that CEP55 could serve as a prognostic factor for patients with lung adenocarcinoma. Several studies have revealed that CEP55 overexpression is considered a potential adverse prognostic factor for multiple cancers, including epithelial ovarian cancer [52], oesophageal squamous cancer [24], and pancreatic cancer [34]. Moreover, after radical resection in primary lung adenocarcinoma, CEP55 exhibited some prognostic significance [23]. We conclude that CEP55 is correlated with the prognosis of patients with lung adenocarcinoma and CEP55 expression and methylation are regulated by EZH2, providing a new target for clinical research and the treatment of lung adenocarcinoma.

## 5. Conclusion

Our results revealed that CEP55 is overexpressed and its methylation is decreased in lung adenocarcinoma, and the two factors are related to the prognosis of lung adenocarcinoma. Differences in EZH2

expression in lung adenocarcinoma result in changes in CEP55 expression and methylation, and CEP55 is a downstream regulatory gene of EZH2. Therefore, we believe that CEP55 is regulated by EZH2 and correlates with the prognosis of lung adenocarcinoma, providing a greater understanding of the regulatory mechanism of epigenetic modifying factors in lung adenocarcinoma and a theoretical basis for clinical studies of lung adenocarcinoma. This study predicted the downstream molecules regulated by EZH2 in lung adenocarcinoma through bioinformatics analysis. The conclusions should be verified in future studies, and it is of great significance to investigate and clinically verify the regulatory mechanism of EZH2 on CEP55.

## Competing interests

The authors have declared that no competing interests exist.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2018.11.016.

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