Original Article

Comprehensive analysis of HOX family genes in endometrial cancer

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Background: Endometrial cancer (EC) is one of the most prevalent malignancies in the female population. Homeoboxes (HOXs) are a large family of transcription factors that have a variety of functions in biological processes (BPs), including developmental differentiation, and their dysregulated expression has been implicated in tumorigenesis. However, the involvement of HOXs in EC has received little attention. Thus, we aimed to identify the potential role of HOXs in EC from a multi-omics perspective through bioinformatics analysis.

Methods: We obtained transcriptome, mutation, and methylation data and the corresponding clinical data for normal and tumor tissues from The Cancer Genome Atlas (TCGA) database. Abnormal expression of HOXs in EC was identified via differential analysis, and the diagnostic value of HOXs in EC was assessed with the receiver operating characteristic (ROC) method. Univariate and multivariate Cox regression models were employed to evaluate the predictive efficacy of HOXs in EC. Methylation and mutation analyses revealed epigenetic and genetic sequence alterations in HOXs. Single-sample gene set enrichment analysis (ssGSEA) was used to explore the altered immune microenvironment in EC. Moreover, the gene activity and pathway enrichment of downstream key HOX genes were revealed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis in EC.

Results: HOXs were found to be linked to the growth of EC and potentially playing a role in establishing the tumor immune microenvironment in patients with EC. *HOXB9* was found to be a vital prognostic molecule in patients with EC and is expected to contribute to a novel treatment approach.

Conclusions: We used bioinformatics techniques to clarify the potential role of HOXs from a multi-omics perspective, and our findings provide a foundation for future investigations into the molecular mechanisms of HOXs in EC.

Keywords: Endometrial cancer; homeobox (HOX); *HOXB9*; methylation; immune microenvironment

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Introduction

Endometrial cancer (or endometrial carcinoma; EC) is the fourth most frequent cancer in women, and its incidence, which varies by region (higher in North America and Europe), is increasing (1-3). Surgery in combination with additional treatments (e.g., radiotherapy and/or chemotherapy) is currently the standard option for patients with EC (4,5). However, the prognosis of these patients remains inconsistent, with that of certain individuals with recurring or severe tumors (stage III and IV) being poor (6).

As an encouraging treatment modality, targeted therapy offers promise for patients with poor sensitivity to standard therapies (7). The identification of novel biomarkers and the molecular investigation of related mechanisms can help to improve the impact of targeted therapy on the prognoses of patients with EC (8,9).

The homeobox (*HOX*) gene family contains 39 genes encoding homologous transcription factors and is separated into four categories (*HOXA*, *HOXB*, *HOXC*, and *HOXD*) on chromosomes 7, 17, 12, and 2, respectively (10). The HOX transcription factor family participates in multiple biological processes (BPs), including cell growth, differentiation, apoptosis, angiogenesis, etc. The abnormal transcription of HOX genes has been found to be associated with the abnormal growth of malignancies (11). DNA methylation is a key epigenetic mark regulating HOX gene expression, and its dysregulation contributes to HOX-mediated diseases, including cancer (12). Previous studies have shown that *HOXA5* is critically involved in the proliferation, differentiation, and apoptosis of cancer cells (13,14). In cancer, the protein products encoded by HOX genes can function as both transcriptional activators and repressors. *HOXB9* expression correlates with prognosis, immune infiltration, and response to immunotherapy across multiple cancer types (15). CUT HOX genes are involved in the development, differentiation, and disease through transcriptional regulation (16). The expression of HOX gene family members is critical for maintaining the environmental balance in normal adult tissues and can inform tumor diagnosis and treatment (10,17). Abnormal transcription of HOX genes has been observed in a wide range of tumors (which encompasses gynecological tumors such as those of the breasts and ovaries) (18); however, a comprehensive evaluation of the transcription and clinical relevance of HOX family genes in EC has not been performed.

Therefore, we obtained the transcriptome, mutation, and methylation data of normal and tumor tissues, along with the related medical data, from The Cancer Genome Atlas (TCGA) database for follow-up studies. Abnormal expression of HOXs in EC was identified through differential analysis. The therapeutic utility of HOXs in EC was evaluated using receiver operating characteristic (ROC) analysis, while the predictive significance of four HOX clusters in EC was evaluated using univariate and multivariate Cox regression analyses. Methylation and mutation methods implied epigenetic and genetic sequence alterations in HOXs. Furthermore, we identified the altered immune microenvironment in patients with EC using single-sample gene set enrichment analysis (ssGSEA) and characterized the relationship between HOXs and the establishment of the immune microenvironment. In addition, the gene function and pathway enrichment of downstream key HOX genes were investigated using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment studies in EC. In conclusion, we used bioinformatics techniques to examine the prospective function of HOXs from a multi-omics perspective in order to establish a foundation for further investigations into the molecular pathways of HOXs related to EC. By clarifying HOX-immunological links, these findings may inform biomarker-guided therapies and subclass-stratified treatment strategies for heterogeneous EC cases. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2146/rc).

Highlight box

Key findings

- Homeoboxes (HOXs) are linked to the growth of endometrial cancer (EC). *HOXB9* is a vital prognostic molecule in patients with EC.

What is known and what is new?

- The differential expression analysis revealed an up-regulation of *HOXB* family genes, particularly *HOXB9*, *HOXB8*, and *HOXB13*, in endometrial cancer tissues. Conversely, most of the gene groups belonging to *HOXA*, *HOXC*, and *HOXD* were found to be down-regulated.
- Certain *HOX* genes, such as *HOXD3*, *HOXD8*, *HOXD4*, *HOXB13*, and *HOXB9*, exhibit significant diagnostic potential and can be distinguished from normal and tumor samples based on RNA sequencing data.

What is the implication, and what should change now?

- *HOXB9* emerges as a pivotal prognostic factor in patients with endometrial cancer, thus holding immense potential as a novel targeted therapeutic pathway.
- The analysis of immune cell abundance revealed a positive correlation between *HOXB* genes and immunoregulatory cells, potentially influencing the establishment of the endometrial cancer immune microenvironment.

Methods

Data source

Using the UCSC Xena platform (http://xena.ucsc.

Table 1 Patient information

Variable	TCGA-UCEC, n (%)
Age (years)	
≥55	452 (81.7)
<55	98 (17.7)
NA	3 (0.6)
Clinical stage	
I	345 (62.4)
II	52 (9.4)
III	127 (23.0)
IV	29 (5.2)
Histologic grade	
G1	99 (17.9)
G2	122 (22.1)
G3	332 (60.0)

TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; NA, not applicable (missing or not collected).

edu/) (18), we downloaded the gene expression RNA sequencing (RNA-seq) data [high-throughput sequencing data fragments per kilobase of transcript per million mapped reads (HTSeq-FPKM)] for TCGA-uterine corpus endometrial carcinoma (UCEC) with the data storage unit format of log 2 (FPKM + 1). Methylation data were acquired simultaneously on the Illumina Human Methylation 450 platform, and annotation information was derived from the Gene Expression Omnibus (GEO) database (19) GPL13534 platform (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL13534). Somatic mutations were selected for MuTect2 variant aggregation and masking. The source of the copy number variation (CNV) data was the Genomic Identification of Significant Targets In Cancer (GISTIC) copy number dataset. The corresponding patient clinical information (age, clinical grade, etc.) is summarized in *Table 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Expression of HOXs

Data from 39 HOX genes were obtained, and the correlations between each of them at the RNA-seq level were calculated using the "Hmisc" R package (The R Foundation for Statistical Computing, version 4.0.2). Differences in expression levels between normal and tumor samples were calculated with the "limma" R package, and the results were displayed using the "ggplot2" R package.

Diagnostic and prognostic value of HOXs

In our analysis, we utilized the "pROC" package in R to assess the diagnostic performance of HOXs in differentiating between tumor and normal samples. This involved generating ROC curves, from which we calculated the area under the curve (AUC) to evaluate the sensitivity and specificity of HOX gene expression profiles. Employing the "survival" and "survminer" R packages, we conducted univariate and multivariate Cox regression analyses of the HOXA/B/C/D gene clusters to investigate the predictive ability of HOXs in patients with EC. Kaplan-Meier survival curves were also plotted to visually represent the survival probabilities over time, stratified by the expression levels of significant HOX genes.

Methylation, mutation, and CNV of HOXs

The correlation of HOX expression and methylation site [methylation level of cytosine-phosphate-guanine (CpG)] was analyzed with the "Hmisc" R package, methylation levels in normal and tumor tissues were determined with "limma" R package, and the mutation and CNV data were visualized after statistical curation with the "ggplot2" R package.

The correlation of HOXs and the immune microenvironment

ImmuCellAI (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) (20) can calculate the abundance of immune cells in each sample (including 18 T-cell subtypes and six other immune cell subtypes) from RNA-seq data. We visualized the results with the "ggplot2" and "ggsignif" R packages. Moreover, we assessed the correlation between HOX expression and the abundance of infiltrating immune cells.

GO and KEGG enrichment analyses based on HOXB9

On the basis of the average *HOXB9* production value, we split the tumor cases into high- and low-expression categories. After identifying the subgroups depending

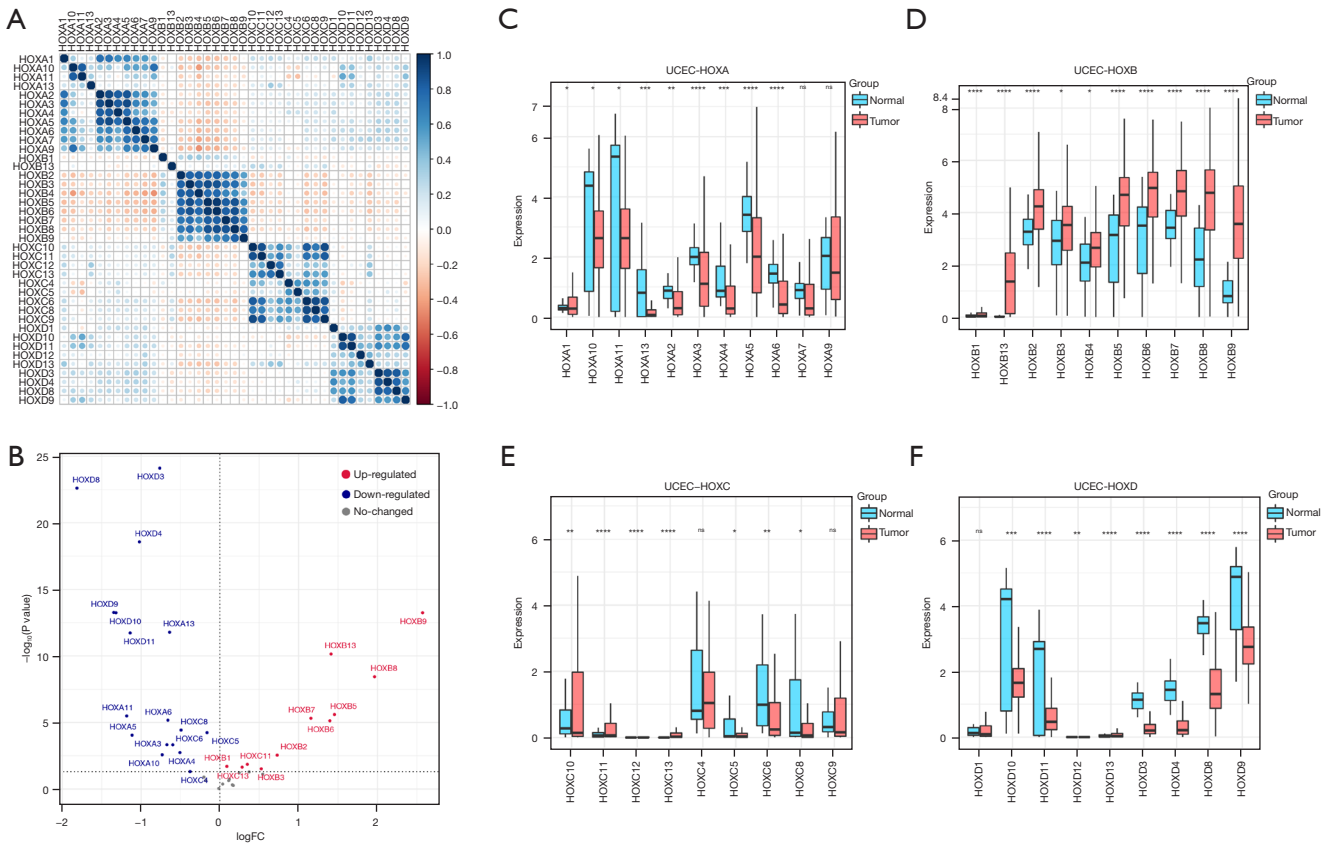


Figure 1 HOX gene expression status. (A) Heatmap of expression correlations (blue indicates a positive correlation; red indicates a negative correlation). (B) Volcano map of HOX differential analysis. (C) Box plot of HOXA. (D) Box plot of HOXB. (E) Box plot of HOXC. (F) Box plot of HOXD. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not statistically significant. HOX, homeobox; FC, fold change.

on *HOXB9* expression, we applied differential analysis to identify the differentially expressed genes (DEGs). We conducted GO and KEGG enrichment analyses of DEGs using the "clusterProfiler" R package to uncover the functions and pathways enriched downstream of *HOXB9*.

Statistical analysis

R v.3.6.1 software was used for statistical evaluation and visualization. Insufficient survival data and other medical data were indicated as not applicable (NA). Pearson correlation analysis was performed to determine correlations between two groups. We conducted Kaplan-Meier analysis to examine the overall survival (OS) time period in the two groups. Furthermore, both univariate and multivariate Cox regression analyses were conducted to evaluate the genes' predictive ability. The *t*-test was adopted for comparisons between two groups, while the analysis of variance was used for evaluations involving multiple categories. P<0.05 was regarded as statistically significant.

Results

The expression of HOXs in EC

We obtained relevant clinical information for 553 patients with EC (*Table 1*) and their transcriptomic data from 35 normal samples and 547 tumor samples. In *Figure 1A*, the heatmap of the transcription levels of 39 *HOX* genes shows a positive correlation between the expression level of each gene in the *HOXA*, *HOXB*, *HOXC*, and *HOXD* clusters. There was a notable negative correlation between the HOXB cluster and other clusters, especially the *HOXA* cluster. *Figure 1B* shows the results of the "limma" R package differential analysis, and a total of 28 differentially expressed (DE) HOXs were identified (11 genes were upregulated, and 17 genes were downregulated). *Figure 1C-1F*

show the box plots of the expression levels of the *HOXA*, *HOXB*, *HOXC*, and *HOXD* clusters between normal and tumor samples. The results showed that *HOXB* cluster genes, especially *HOXB9*, *HOXB8*, and *HOXB13*, were mostly significantly upregulated in EC tumor samples. The majority of the *HOXA*, *HOXC*, and *HOXD* genes were downregulated, especially the *HOXD* cluster. The boxplots of *HOX* expression and clinical traits (age, clinical stage, and pathological grade) are shown in *Figure 2A-2C*.

Diagnostic and prognostic value of HOX genes

We assessed the sensitivity and specificity of HOXs in distinguishing between normal and tumor samples by drawing ROC curves (*Figure 3A-3D*). The top 10 genes with the highest AUC values were *HOXD3* (0.928), *HOXD8* (0.926), *HOXD4* (0.922), *HOXB13* (0.893), *HOXB9* (0.869), *HOXB8* (0.796), *HOXC13* (0.789), *HOXB7* (0.788), *HOXB5* (0.78), and *HOXD9* (0.774). Univariate Cox regression analysis of the *HOXA* cluster (*Figure 4A*) indicated *HOXA1/2/4/5/6/7/12* as risk factors; the multivariate results (*Figure 4B*) indicated *HOXA11* as a protective factor and *HOXA13* as a risk factor. Both univariate and multivariate analyses of the *HOXB* cluster (*Figure 4C*,*4D*) indicated *HOXB6* as a potential protective factor and *HOXB9* as a risk factor. The univariate Cox results of the *HOXC* cluster indicated *HOXC5* as risk factor (*Figure 4E*), and the multivariate results (*Figure 4F*) indicated *HOXC11* and *HOXC9* as protective factors. The univariate results of the *HOXD* cluster (*Figure 4G*) indicated *HOXD1*, *HOXD4*, and *HOXD13* as risk factors; however, they were not statistically significant in the multivariate Cox analysis (*Figure 4H*). Among the *HOX* genes, those found statistically significant in both univariate and multivariate Cox analyses were *HOXA13*, *HOXB6*, and *HOXB9*, and their Kaplan-Meier survival curves are shown in *Figure 5A-5C*.

Methylation of HOXs

The difference in HOX gene CpGs between normal and tumor samples was analyzed with the "limma" R package, which could clarify the CpGs that were upregulated, downregulated, or not statistically different (*Figure 6A-6D*). The *HOXA*, *HOXC*, and *HOXD* gene clusters had more upregulated CpGs, while the *HOXB* gene cluster had more downregulated CpGs. *HOXB9* was highly expressed in tumor tissues according to the previous analysis and was shown to be a risk factor in the univariate and multivariate Cox regression analyses. The heatmap of *HOXB9* with its CpG correlation is shown in *Figure 7A*, and sites with point |correlation coefficient| >0.4 methylation levels between normal and tumor tissues are shown in *Figure 7B-7E*. The findings indicated that cg12370791, cg10633363, and cg13643585 were inversely correlated with *HOXB9* gene expression levels, and methylation levels were lower in tumor tissue (but the difference of cg13643585 was not statistically significant); moreover, cg14240300 was positively correlated with *HOXB9*, and methylation levels were significantly increased in tumor tissue.

Mutations and CNVs of HOXs

As shown in *Figure 8A*, missense mutations and 3' untranslated region (UTR) mutations were found to be the most common types of mutations in HOX family genes in EC. Nucleotide alterations were often C>T, G>A, and C>A (*Figure 8B*). *Figure 8C* shows the proportion of patients with each HOX mutant type, with *HOXC10* (8.3%), *HOXA7* (7.92%), *HOXA1* (7.55%), *HOXC6* (6.42%), and *HOXB3* (6.42%) having the highest mutation rates. The CNV of HOXs is shown in the percentage accumulation map in *Figure 8D*. The top five ranked genes in terms of CNVs were *HOXB1*, *HOXB3*, *HOXB2*, *HOXB4*, and *HOXB6*.

HOXs and immune cell infiltration abundance

Using ImmuCellAI, we calculated the abundance of 24 immune cell types in each sample and the difference between normal and EC samples, as shown in *Figure 9A*,*9B*. *Figure 9C* shows the association between the abundance of 24 immune cells, with a high positive correlation between immunoregulatory cells such as natural regulatory cells (nTregs), induced regulatory cells (iTregs), T helper 1 (Th1) cells, and T helper 2 (Th2) cells. There was an obvious positive correlation between HOXB cluster genes and immune regulatory cells [e.g., T follicular helper (Tfh) cells, nTregs, iTregs, CD4-naïve T cells], implicating that they were involved in the development of the EC immunological microenvironment (*Figure 9D*).

HOXB9 gene-based GO and KEGG enrichment studies

In accordance with the mean value of *HOXB9* gene expression, we divided the EC samples into high- and low-expression groups for differential analysis and analyzed the GO and KEGG enrichment analysis results using the

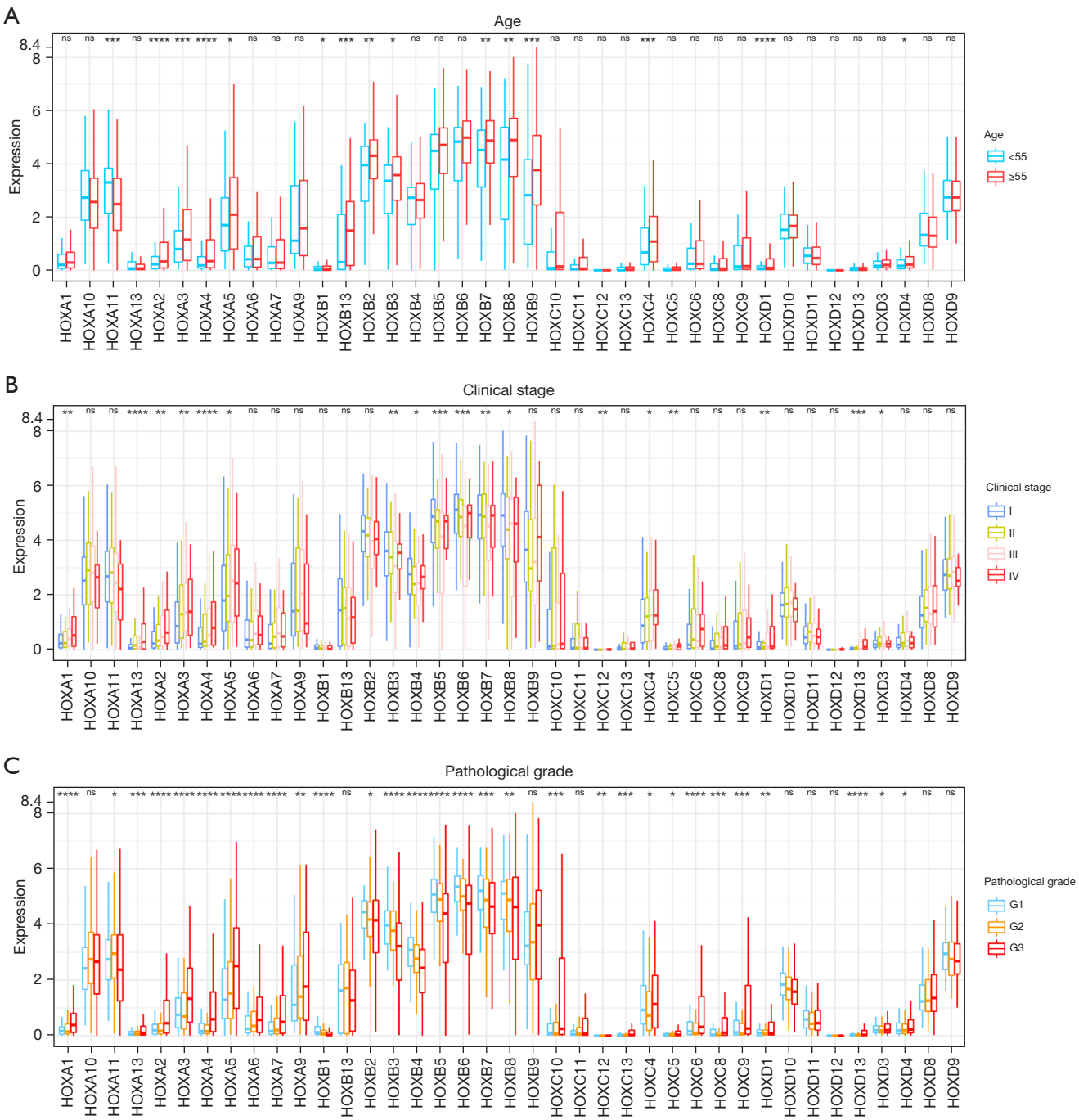


Figure 2 Correlation of HOX expression with clinical traits. (A) Correlation of HOX expression with age, (B) clinical stage, and (C) pathological grade. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not statistically significant. HOX, homeobox.

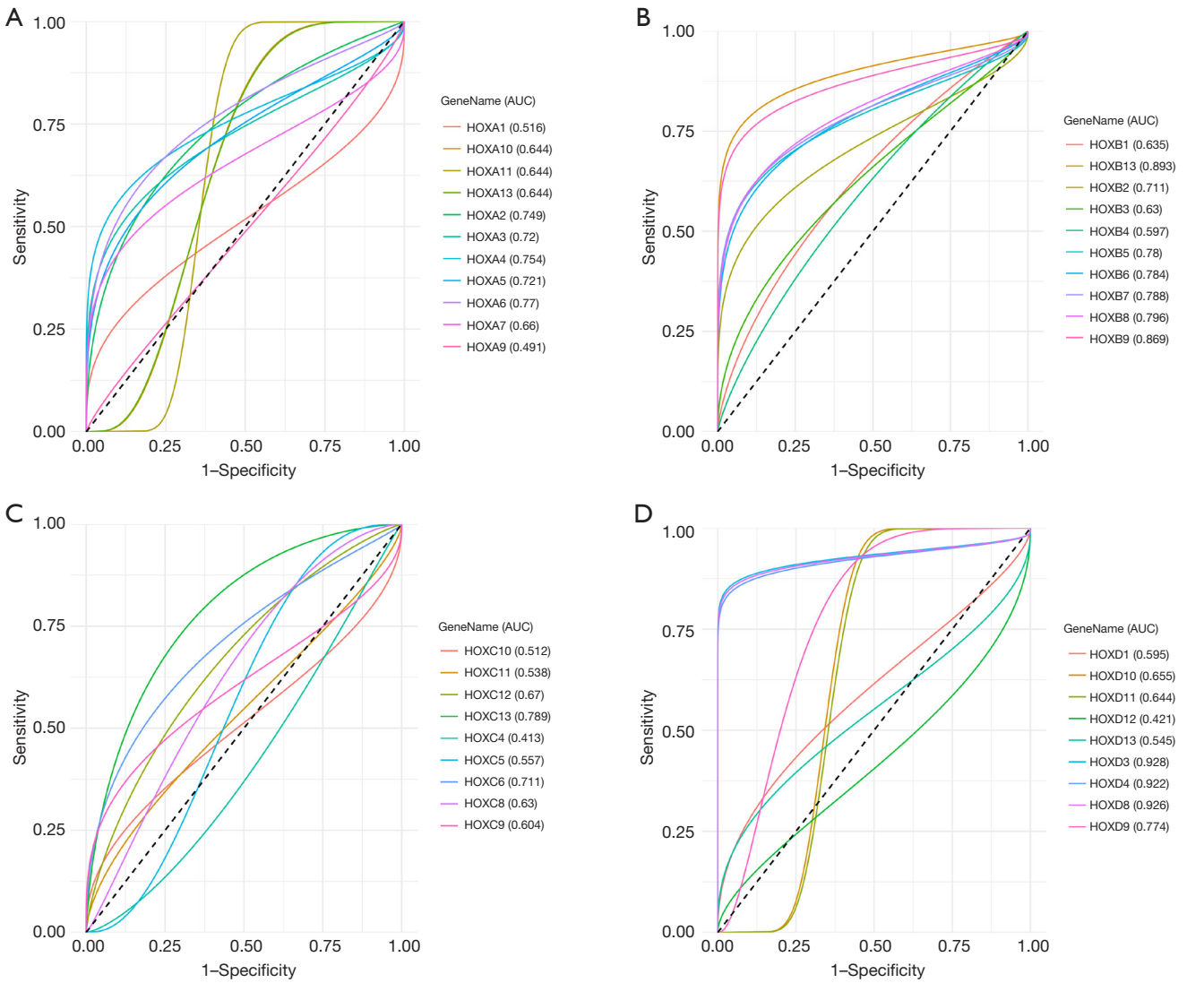
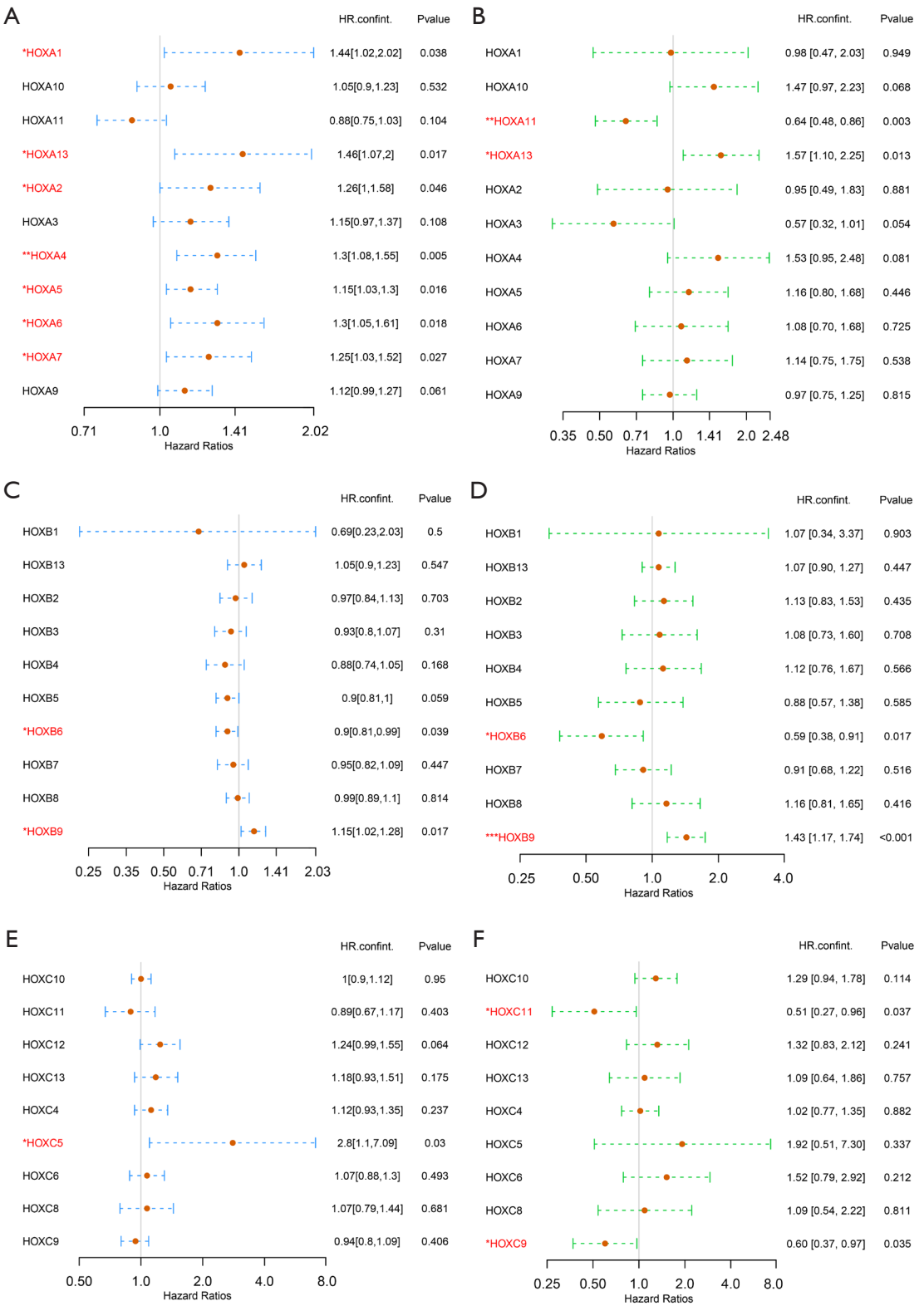


Figure 3 Diagnostic value of HOXs. ROC curves of (A) HOXA, (B) HOXB, (C), HOXC, and (D) HOXD. HOX, homeobox; AUC, area under the curve; ROC, receiver operating characteristic.

"clusterProfiler" R package. The BP results showed that *HOXB9* downstream genes were enriched in BPs such as DNA chromosome segregation and repair (*Figure 10A*). The cellular component (CC) results (*Figure 10B*) showed that the downstream gene products were mostly located on chromosomes when they performed their functions. The molecular function (MF) results showed (*Figure 10C*) the enrichment of downstream genes for MFs of helicase activation and DNA replication region binding. KEGG enrichment analysis (*Figure 10D*) showed that downstream genes were related to DNA replication, the cell cycle, RNA transport, and other pathways. *HOXB9* may further affect the EC phenotype by affecting the cell cycle and DNA replication, among other processes.

Discussion

HOXs are a group of evolutionarily conserved transcription factors that participate in embryonic growth and stem cell (SC) differentiation and are dysregulated in various tumors (12,21-23). In this study, we evaluated the expression landscape of HOXs in EC, and the results showed that all cluster *HOXB* genes (including *HOXB9*, *HOXB13*, and *HOXB8*) had increased expression in EC samples, and



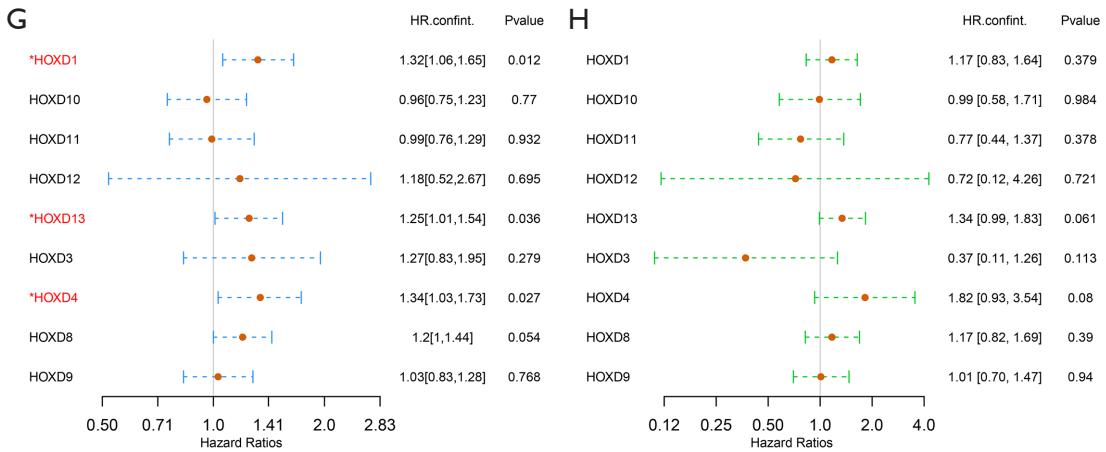


Figure 4 Prognostic value of HOXs. (A) HOXA univariate Cox analysis and (B) HOXA multivariate Cox analysis. (C) HOXB univariate Cox analysis and (D) HOXB multivariate Cox analysis. (E) HOXC univariate Cox analysis and (F) HOXC multivariate Cox analysis. (G) HOXD univariate Cox analysis and (H) HOXD multivariate Cox analysis. *, P<0.05; **, P<0.01; ***, P<0.001. HOX, homeobox; HR, hazard ratio.

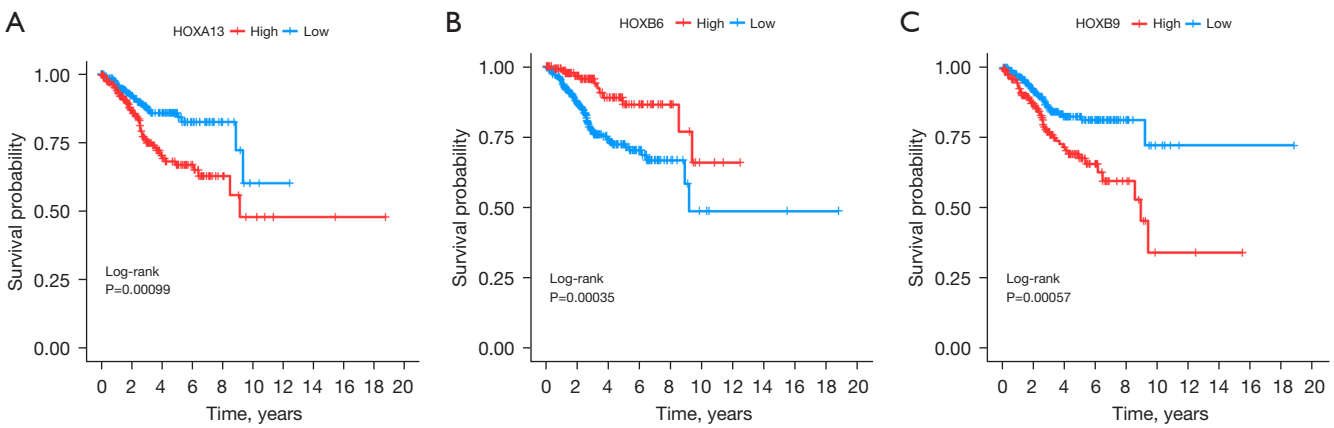


Figure 5 Kaplan-Meier plots. (A) *HOXA13*, (B) *HOXB6*, and (C) *HOXB9*.

most cluster *HOXA*, *HOXC* and *HOXD* genes were mostly downregulated. Some HOXs (e.g., *HOXD3*, *HOXD8*, *HOXD4*, *HOXB13*, and *HOXB9*) demonstrated excellent diagnostic value and could distinguish normal from EC samples according the RNA-seq data. Through univariate and multivariate Cox regression analyses, we found that *HOXA13* and *HOXB9* may be risk factors and that *HOXB6* may be a protective factor. Among these, *HOXB9* was upregulated in EC and was found to be a risk factor. Importantly, ECs demonstrate significant inter-tumor heterogeneity, encompassing four molecular subtypes with distinct mutational landscapes and clinical behaviors. Integrating dysregulated HOX expression patterns, like *HOXB9* overexpression, with these known EC subclasses could provide valuable insights into disease heterogeneity and help improve subclass-directed management. Overall, the CpGs of *HOXA*, *HOXD*, and *HOXC* cluster genes had mostly upregulated methylation levels in EC samples, while those of *HOXB* cluster genes had downregulated methylation levels, with most CpGs being negatively correlated with gene expression levels. cg12370791 and cg10633363 were negatively correlated with the expression level of *HOXB9*; meanwhile, cg14240300 was positively correlated with *HOXB9*, exhibiting obvious alterations in EC, and may thus be the epigenetic regulatory site of the methylation of *HOXB9*. The genes with the top five

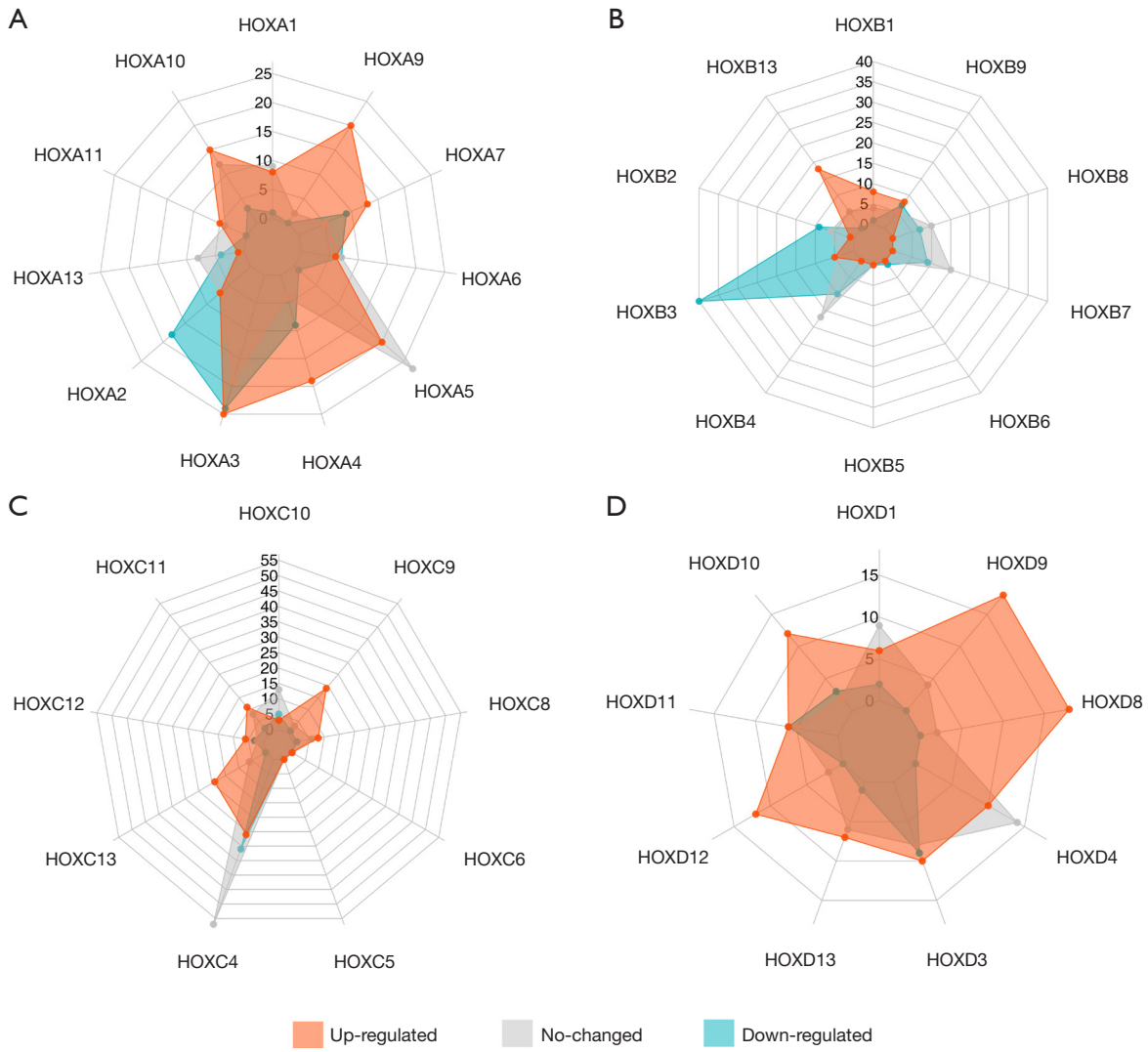


Figure 6 Methylation differences in HOXs. (A) HOXA, (B) HOXB, (C) HOXC, and (D) HOXD. HOX, homeobox.

somatic HOX mutation rates were *HOXC10*, *HOXA7*, *HOXA1*, *HOXC6*, and *HOXB3*. *HOXB* cluster genes showed multiple copy number increases at the copy number level. In addition, we examined the abundance of immune cells through ssGSEA, and the outcomes indicated a clear positive link between *HOXB* cluster genes (e.g., *HOXB1*, *HOXB3*, and *HOXB5*) and immunological negative regulatory cells (e.g., Tfh cells, nTregs, and iTregs,). We further explored the GO and KEGG enrichment results of downstream *HOXB9* genes to identify the relevant molecular mechanisms. The results indicated that *HOXB9* may affect the EC cell phenotype by controlling cell cycle and DNA replication. Furthermore, our approach in examining HOX gene dysregulation in the context of EC molecular subtypes offers a nuanced understanding of the genetic underpinnings in EC. This perspective not only enhances our grasp of the diverse molecular mechanisms but also opens avenues for developing more targeted and effective therapeutic strategies.

Our study demonstrated the utility of bioinformatics approaches to systematically mine altered HOX family genes in EC. Through integrated transcriptomic, epigenetic and clinical survival analyses, we identified several core HOX isoforms, like *HOXB9*, *HOXA13* and *HOXB6*, as potential prognostic biomarkers in EC. Moreover, by applying bioinformatics tools like ssGSEA, we characterized relationships between aberrant HOX expression and immune infiltration patterns in the EC

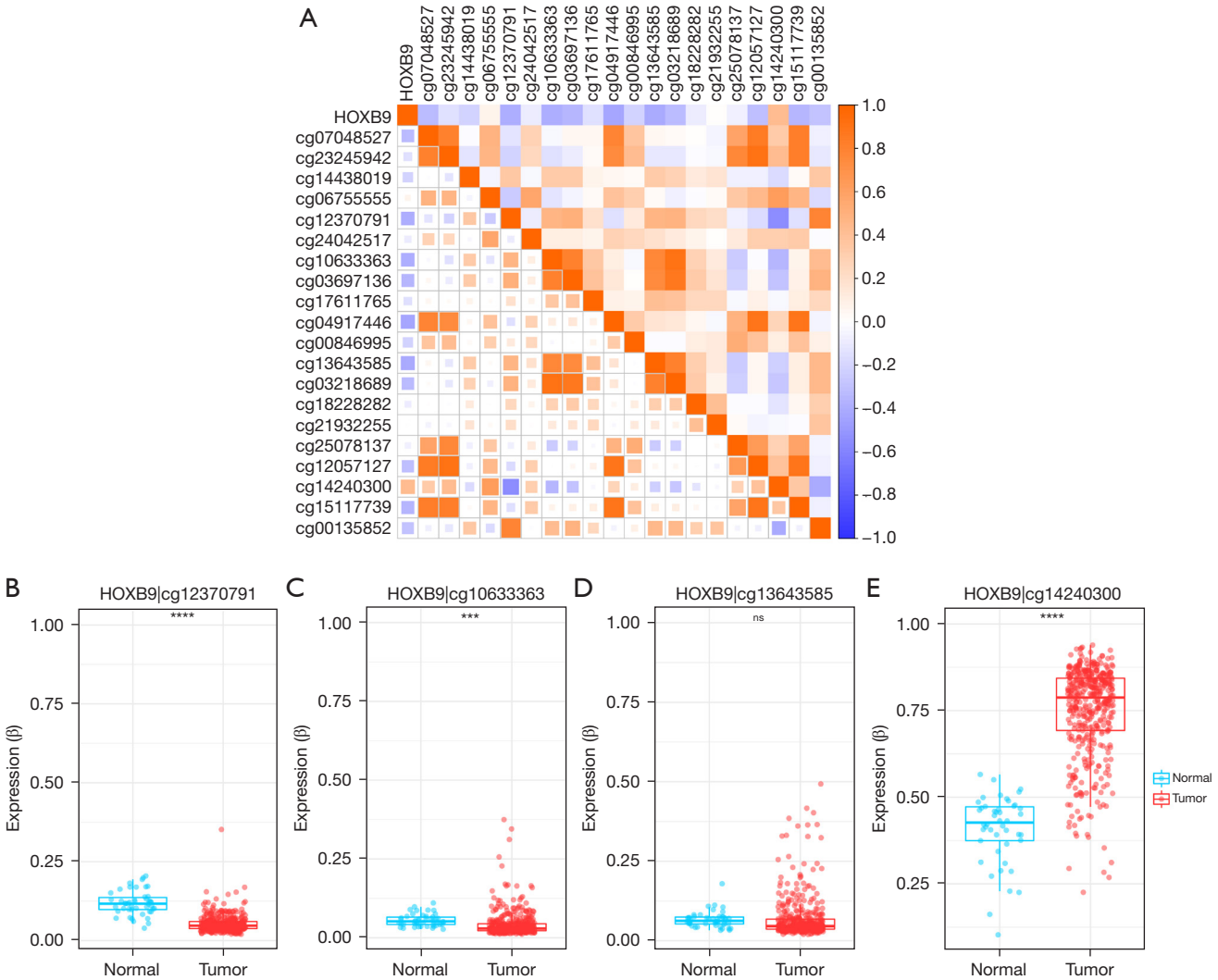


Figure 7 *HOXB9* and CpG heatmaps. (A) Heatmap of the correlation of *HOXB9* with CpG (red indicates a positive correlation; blue indicates a negative correlation). (B) *HOXB9*|cg12370791. (C) *HOXB9*|cg 10633363. (D) *HOXB9*|cg13643585. (E) *HOXB9*|cg14240300. ***, P<0.001; ****, P<0.0001; ns, not statistically significant. CpG, cytosine-phosphate-guanine.

tumor microenvironment. For instance, HOXB cluster overexpression was found to be associated with enhanced infiltration of immunomodulatory cell types like Tregs and Tfh cells. Our findings provide a valuable insight for future prognostic predictions and immunological research in EC. The *HOXB* cluster genes were significantly upregulated in EC. Methylation is an important epigenetic transcriptional regulation pattern of HOXs (24), and further studying *HOX* key CpG sites and developing novel drug-targeted epigenetic modifications may contribute to precision medicine (25). Among the HOXs, *HOXB9* is strongly linked to the prognosis of patients with EC and might be considered an indication of risk. *HOXB9* has been extensively investigated in a wide range of malignancies [such as colon cancer (26) and liver cancer (27)], but it is rarely studied in EC. The CpG sites of *HOXB9*, cg12370791, cg10633363, and cg14240300 may be potential therapeutic targets. Recent studies also indicate potential associations between specific HOX genes with microsatellite status and immunotherapy responses across cancers (28,29), warranting investigations into similar links in EC.

SCs are abundant in the endometrium (30). It has been reported that HOXs are involved in the SC differentiation process, and dysregulated HOXs may lead to the transition

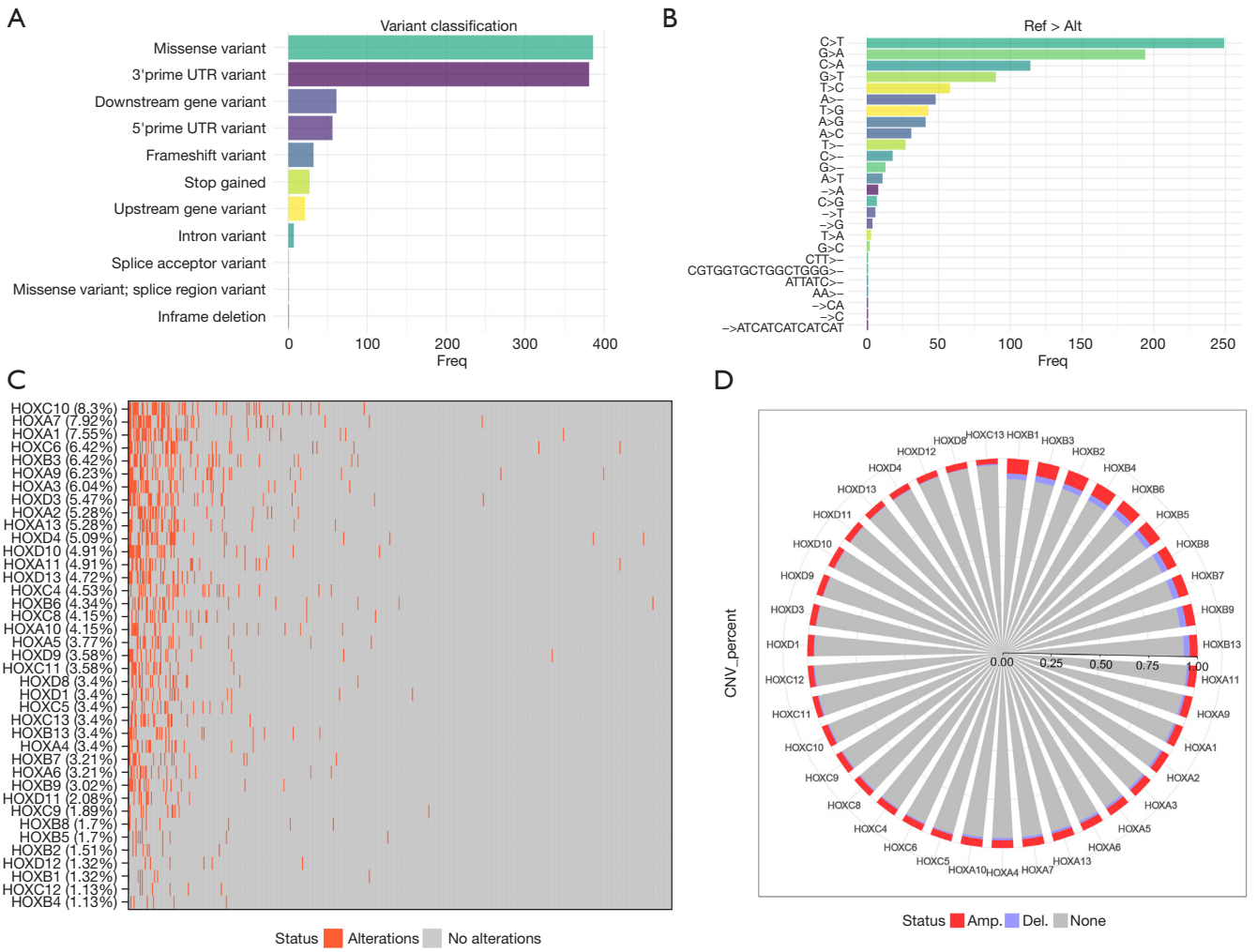


Figure 8 HOX gene mutations and copy number variations. (A) Bar plot of mutation type. (B) Bar plot of nucleotide change. (C) Mutation waterfall map. (D) Copy number variation percentage accumulation map. UTR, untranslated region; Ref, reference; Alt, alternate; Freq, frequency; CNV, copy number variation; Apm., amplification; Del., deletion; HOX, homeobox.

from SCs to EC cells. Interestingly, HOXs also affect hematopoietic SC differentiation [specific HOX genes are required for SC maturation into distinct blood cell types (24)]. Carè *et al.* (31) reported that the *HOXB* cluster gene underlies T-lymphocyte activation.

Key novelty is the multi-omics profiling of HOX dysregulation in EC. Notably, links were discovered between HOXB genes and immune cell infiltration. Limitations of this study are the retrospective dataset reliance and lack of validation experiments or mechanistic insights. Critical future steps include validating *HOXB9* as a prognostic biomarker and investigating pathways by which HOXB gene modules may coordinate immune microenvironment changes to open immunotherapy opportunities.

In our study, the *HOXB* cluster was shown to be positively correlated with certain types of immunomodulatory cells (e.g., iTregs, nTregs, and Tfh cells), suggesting that *HOXB* cluster genes may affect the establishment of the EC immune microenvironment. Exploring the underpinning mechanisms can open new avenues to improve EC immunotherapy.

Conclusions

We comprehensively analyzed HOX expression,

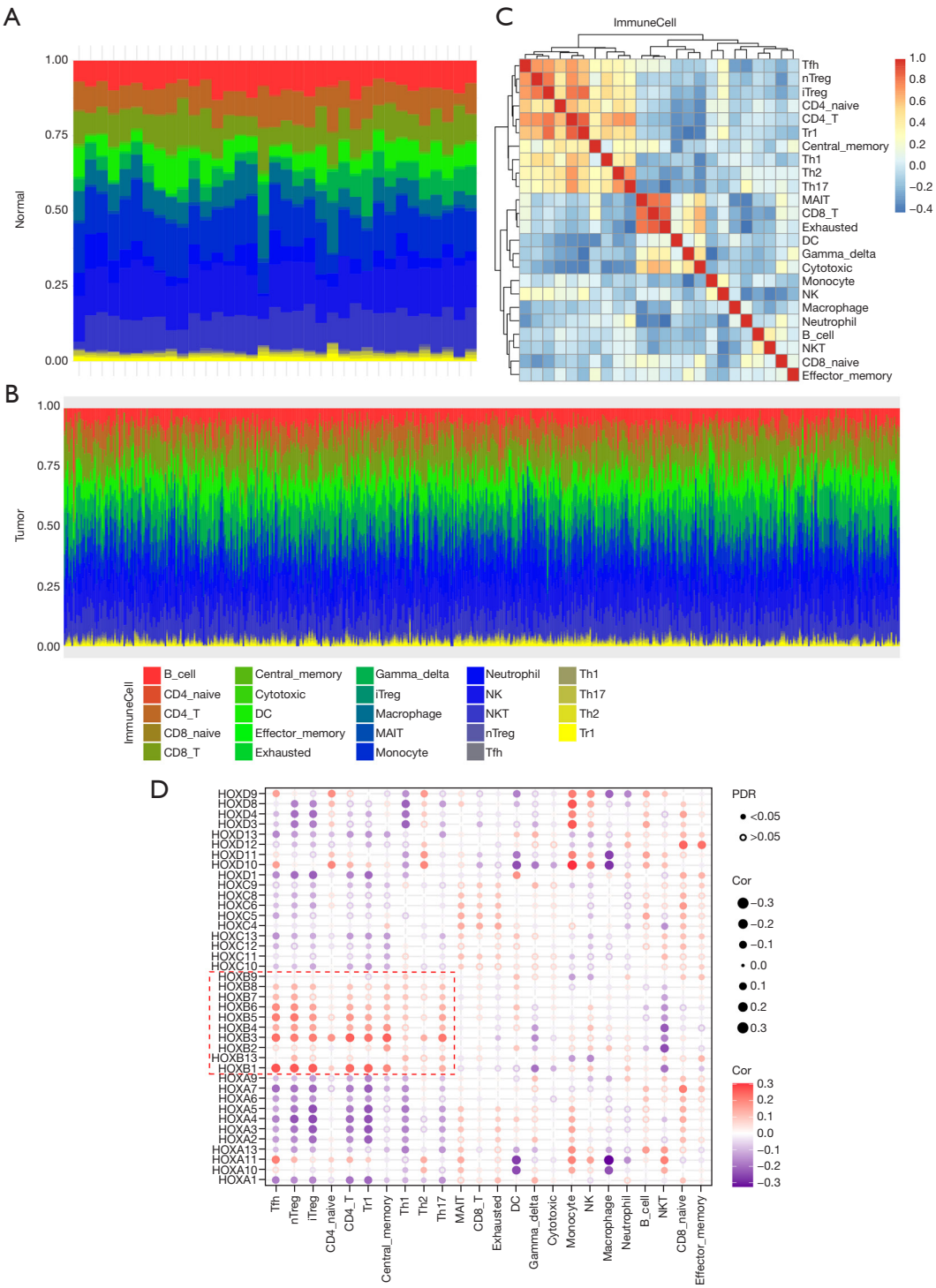


Figure 9 Analysis of HOXs and their association with the immune microenvironment. (A) Percentage of immune cell infiltration in normal samples. (B) Percentage of immune cell infiltration in endometrial cancer samples. (C) Heatmap of immune cell correlation (red indicates a positive correlation; blue indicates a negative correlation). (D) Correlation map of HOX expression and immune cell abundance. The red box indicates immune cells positively correlated with HOXB. FDR, false discovery rate; Cor, correlation; HOX, homeobox.

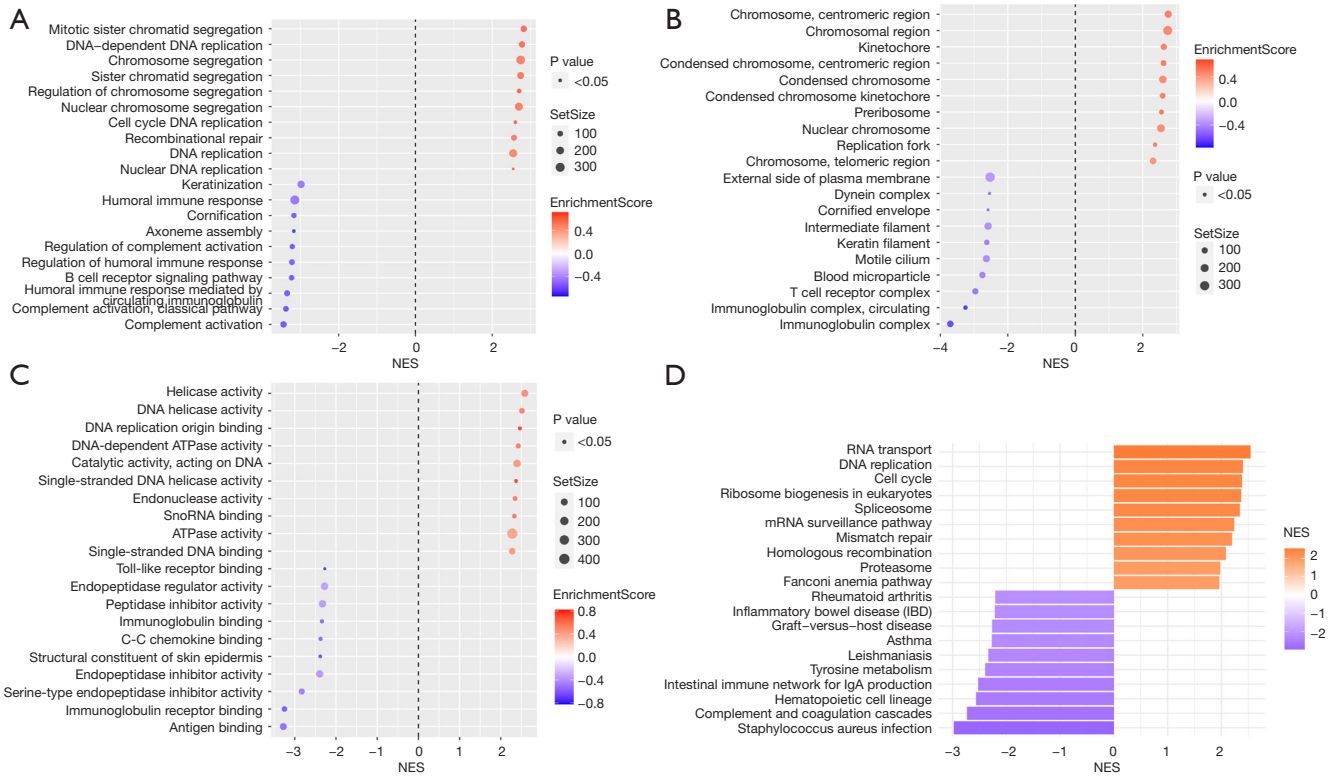


Figure 10 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis results for *HOXB9*. (A) Biological process. (B) Cell component. (C) Molecular function. (D) Kyoto Encyclopedia of Genes and Genomes. NES, normalized enrichment score.

methylation, mutation, and CNV patterns in EC and identified *HOXB9* in the *HOXB* cluster as a key prognostic molecule in patients with EC, which may serve as a novel therapeutic target. Our study contributes to the further investigation of the molecular mechanisms of HOXs in EC.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2146/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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