The Clinical Significance of Forkhead Box M1 in Esophageal Squamous Cell Carcinoma Tissues: A Study Based on In-house Immunohistochemistry, RNA-sequencing, and Data Mining

Wan-Ying Huang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Jing-Xiao Li
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Zhi-Guang Huang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Rong-Quan He
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Shang-Wei Chen
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Guo-Sheng Li
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Dong-Ming Li
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Yu-Lu Tang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Deng Tang
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Jiang-Hui Zeng
The Third Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Hua-Fu Zhou
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Jun Liu
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Zong-Wang Fu
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region
Gang Chen (✉ chengang@gxmu.edu.cn)
The First Affiliated Hospital of Guangxi Medical University, Guangxi Zhuang Autonomous Region

Research Article

Keywords: Esophageal squamous cell, Biomarkers, Clinical cancer research, Cell cycle

Posted Date: January 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1199894/v1

Abstract

Background

Esophageal squamous cell carcinoma (ESCC) ranks the sixth in mortality rates in cancers due to a lack of a specific target of diagnosis and treatment in the early stages. Although Forkhead box M1 (FOXM1) has been reported to be differentially expressed in ESCC, its clinical role and function in ESCC remained unclarified.

Methods

Data from our hospital and public databases (n = 1906) were combined to estimate how FOXM1 overexpression showed its discriminatory ability between ESCC and non-ESCC esophageal tissues. Downstream targets of FOXM1 were predicted by using Cistrome database. Functional enrichment analyses were performed to explore the potential signaling pathways related to FOXM1 in ESCC. Based on the available clinical parameters, we investigated the prognosis potential of FOXM1 and its targets.

Results

The pooled standard mean difference (SMD) for FOXM1 is 2.62 (95% CI: 2.08–3.16), indicating that FOXM1 is upregulated in ESCC. FOXM1 has an extremely high discrimination potential in ESCC because the area under the curve (AUC) of the summary receiver operating characteristic curve (sROC) is 0.99 (95% CI: 0.97–0.99). A total of 168 downstream targets were identified, and nine hub genes were screened from them. We found that FOXM1 and its targets were significantly enriched in the cell cycle. Additionally, the correlation between FOXM1 and clinical parameters had not been observed, except for age.

Conclusions

FOXM1 is upregulated in ESCC and has an extremely high discrimination potential in ESCC.

1. Introduction

Esophageal cancer (ESCA) is considered one of the most common neoplasms worldwide, ranking the sixth among cancer-associated causes of death (1),(2). Esophageal squamous cell carcinoma (ESCC) is the most common histological type of ESCA worldwide (3),(4),(5). Comparing with other countries, China has the highest ESCC incidence rate and accounts for more than half of the total cases in the world (6). Due to the lack of specific clinical symptoms, ESCC in early stages is usually ignored by patients (7),(8),(9). Sadly, with high metastasis and recurrence rates, the prognosis of ESCC patients in advanced stages is extremely awful (10),(11),(12). To date, surgery remains the first choice for treating ESCC in early stages and has a considerable effect, but therapists do not obtain optimistic efficacy whether surgery or other therapy methods (13),(14),(15),(16),(17). The latest practice guidelines recommended postoperative neoadjuvant chemotherapy as the first choice for patients with advanced ESCC, but according to the

statistics, the 5-year survival rate of advanced patients who accepted neoadjuvant chemotherapy remains less than 40% (18). Thus, exploring the molecular markers related to the occurrence, development, and prognosis of ESCC, and then applying them to the early diagnosis and target therapy of ESCC is of great significance for the clinical treatment and prognosis amelioration of ESCC.

Forkhead box M1 (FOXM1) is a typical transcription factor associated with cell proliferation. As a member of the Forkhead box family, a conserved wing helix DNA-binding domain comprising 100 amino acids characterizes FOXM1 (19). FOXM1 is considered to participate in cell cycle regulations, which are reflected in G1/S and G2/M transitions, mitosis, and the cytokinesis process (20),(21). The previous evidences showed that FOXM1 is upregulated in liver cancer, lung cancer, gastric cancer, and many human malignant tumors, which is accosiated with tumor growth, metastasis, angiogenesis, and prognosis (22),(23),(24),(25),(26). Similarly, some researchers have reported the biological functions of FOXM1 in ESCC. For example, a report written by Su et al. indicated that upregulated GACAT3 could regulate the expression of miR-149 and then regulate the expression of FOXM1 in ESCC. They also reported that upregulated FOXM1 plays an oncogene role and promotes ESCC metastasis (27). In a study that included 115 ESCC patients, Cheng et al. reported that the expression of FOXM1 has a significantly negative correlation with the survival time of patients and promotes the proliferation of ESCC cells by participating in the formation of Vasculogenic Mimicry (28). After analyzing 186 ESCC tissues, Xiao et al. found that FOXM1 is upregulated in ESCC and significantly associated with tumor sizes and differentiation (29). Unfortunately, some limitations of the researches about FOXM1 in ESCC are obvious: the existing research had included small sample sizes, and most of them were single-center researches; the expression of FOXM1 in patients with different clinical parameters has not been fully clarified. In addition, the authors have not found any reports on the downstream target genes or signal pathways of differentially expressed transcription factor FOXM1 regulating the development of ESCC. Therefore, we herein aimed to fill the above gaps.

In the presented study, the authors wanted to explore the expression, potential signaling pathway, and prognostic role of transcription factor FOXM1 in ESCC. Thus, the mRNA-sequencing data and microarray chips from our hospital alongside 1542 samples from public databases were included and analyzed to reveal the clinical significance and potential mechanism of FOXM1 in ESCC.

2. Methods

The flow chart of this study is displayed in Supplementary Figure 1.

2.1 The detection of FOXM1 protein expression based on immunohistochemistry (IHC)

We obtained three tissue microarrays (ESC242, ESC1503, and ESC1504) from Fanpu, Inc. (Guilin, P.R. China), which comprised 162 non-cancerous esophagus tissues and 162 ESCC tissues. FOXM1 was detected with recombinant anti-FOXM1 antibody (ab207298), then we fixed, stained and examined the IHC chips. Before knowing the sample in advance, we randomly selected 10 visual fields with a 400X optical microscope and calculated the percentages of tumor cells with FOXM1 positive expression.

Finally, the mean percentages of the above tumor cells were estimated according to the previous studies (16),(30),(31),(32).

2.2 Clinical samples and RNA sequencing

From August 2019 to December 2019, we obtained eight pairs of ESCC and paracancerous tissues from the First Affiliated Hospital of Guangxi Medical University, and none of the mentioned tissues received chemotherapy or radiotherapy before resection. These samples were then subjected to mRNA sequencing. Finally, mRNA-sequencing data of about 30,000 genes was obtained. In order to make more researchers benefit from our data, we also uploaded the mRNA-sequencing data to gene expression omnibus (GEO) database (accession number: GSE164158)(33).

2.3 Clinical significance of FOXM1 in ESCC

Before the subsequent analysis, we collected ESCC series from public databases, including the cancer genome atlas (TCGA), genotype-tissue expression (GTEx), GEO, ArrayExpress, Oncomine and sequence read archive (SRA). “Esophageal squamous cell carcinoma” was used as the search keyword. The included series should meet the following conditions: (1) Tissues or body fluid were collected from homo sapiens; (2) the series should comprise microarrays or RNA sequencing; (3) contains at least three pairs of ESCC and adjacent tissues or can be merged with series on the same sequencing platform; and (4) contains the expression data of FOXM1. The flow chart of the data filtering process for the series is shown in Supplementary Figure 2. All the included series were performed a $\log_2(x + 1)$ conversion and merged according to their platforms.

The authors extracted the FOXM1 expression data and assessed the differences in FOXM1 expression between ESCC and adjacent tissues through student’s t-tests in IBM SPSS 23.0. Scatter plots and receiver operating characteristic (ROC) curves were plotted using Graphpad Prism 8.0 to show the expression and discrimination potential of FOXM1 in every series. Moreover, the standardized mean difference (SMD), funnel plot, and a summary receiver operating characteristic curve (sROC) was analyzed through Stata 14.0. Then, patients’ clinical information from the TCGA dataset, in-house sequencing, and IHC were collected, and correlation analysis between the expression of FOXM1 and different clinical parameters were performed.

2.4 Identification of FOXM1 differentially co-expressed genes (DCEGs)

2.4.1 Identification of FOXM1 differentially expressed genes (DEGs)

Based on 60 collectable public datasets and in-house sequencing data including eight pairs of ESCC and normal tissues, we respectively evaluated the SMD of all genes. When lower 95% CI > 0 and SMD > 0, the genes are considered upregulated in ESCC. The genes would be identified as DEGs when they met both of the conditions.

2.4.2 Identification of FOXM1 co-expressed genes (CEGs)

As mentioned earlier, 13 series were merged and normalized from 60 public datasets. Then, the authors calculated Pearson's r-values between FOXM1 and other genes. We screened the genes with $r > 0.3$ and $p < 0.05$ and then made an interaction among 13 series, and when the gene appeared seven or more times in 13 series, it would be identified as CEG.

An interaction was made between DEGs and CEGs, and the DCEGs of FOXM1 were identified and performed in the subsequent study.

2.5 Screening of transcription factor FOXM1 targets

We pretended that the differentially expressed transcription factor FOXM1 would affect the differential expression of downstream genes in ESCC and then promote the proliferation and invasion of ESCC cells. To find out the potential downstream genes regulated by FOXM1, we downloaded 40 gene lists with FOXM1 putative targets from Cistrome DataBase (CistromeDB) and screened genes with a score ≥ 0.5, and then we interacted them with FOXM1 DCEGs. We considered that the obtained genes have the greatest potential to be FOXM1 targets in ESCC.

2.6 Exploration of FOXM1 potential mechanisms in regulating ESCC

To reveal the potential signal pathways of differentially expressed FOXM1 in regulating ESCC development, a clusterProfiler package supported by R v3.6.2 was used to predict signal pathways in gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Then, we listed the genes enriched in the top three KEGG terms and entered them into the search tool to retrieve interacting genes (STRING) to structure the protein–protein interaction (PPI) network as previously reported (34),(35),(36),(37). Next, we downloaded the PPI network analysis result and screened the top nine significant hub genes through degree conversion; the CytoHubba module achieved this step in cytoscape v3.7.1. Finally, the correlations between FOXM1 and its hub genes were estimated, and the binding peaks between FOXM1 and its hub genes were found in cistromeDB.

2.7 Prognosis analysis of FOXM1 and hub genes

We wanted to investigate the correlation between the expression level of FOXM1 and patients' prognosis. As mentioned earlier, we collected patients' clinical information from TCGA databases, so we performed a survival analysis for samples with high and low FOXM1 expression. The same operation was applied to the nine hub genes to explore whether the hub genes also influenced patients' prognosis.

2.8 Statistic analysis

Herein, we performed $\log_2(x+1)$ conversion on all the mRNA expression data from collected published series. Student's t-test was performed by IBM SPSS 23.0 and we showed the mean and standard deviation (SD). The scatter plots and ROC curves were plotted by Graphpad Prism 8. Stata 14.0 was applied to plot forest plot of SMD, funnel plot and sROC curve, which represent the expression level, publication bias and discrimination potential of FOXM1 in ESCC. R v3.6.2 was used to calculate and plot

the correlation between FOXM1 and its targets. In addition, $p < 0.05$ was considered statistically significant.

3. Results

3.1 Clinical significance of FOXM1 in ESCC

IHC results indicated that the FOXM1 protein is overexpressed in ESCC tissues, but we did not observe the FOXM1 protein expressed in normal tissues (Figure 1). The mRNA-sequencing data from our hospital also indicated that FOXM1 is significantly upregulated in ESCC ($p < 0.05$) (Figure 2A). According to the public data, FOXM1 was significantly upregulated in ESCC cells, and the same results were also observed in every public series (Figure 2B-N). To visualize the results, a forest plot is shown in Figure 3A, which indicates that FOXM1 was upregulated in ESCC (SMD = 2.62, 95% CI: 2.08–3.16). No publication bias was observed (Figure 3B). Moreover, an sROC was plotted to explain ROC curves synthetically, and it indicated that FOXM1 had an extremely high potential to discriminate ESCC patients from healthy people due to AUC = 0.99 (95% CI: 0.97–0.99) (Figure 3C). With high sensitivity and specificity (comprehensive sensitivity = 0.95, comprehensive specificity = 0.96), FOXM1 has the potential for clinical application (Figure 3D). After that, the relationship between FOXM1 expression and the patients' clinical parameters was also discussed. According to sequencing data from the TCGA database, we found that the expression level of FOXM1 was significantly different in the group and age of patients ($p < 0.05$) (Table 2). However, although our clinical and IHC samples also present differential FOXM1 expression in different groups, differential expression between the two age groups was not observed (Supplementary Tables 1 and 2).

3.2 Identification of FOXM1 downstream targets

After screening, 984 DEGs and 2142 CEGs of FOXM1 in ESCC were identified from 61 series. Based on cistromeDB, 1841 putative targets were screened. We interacted with the above three terms (DEGs, CEGs and putative targets), and then 168 targets were identified and used for the follow-up research (Figure 4A).

3.3 Enrichment analysis

First, 168 targets were used for enrichment analysis. The GO analysis results indicated that FOXM1 targets significantly enriched in mitotic nuclear division, kinetochore, and ATP binding, and the KEGG analysis results indicated that the above targets significantly enriched in cell cycle, progesterone-mediated oocyte maturation, and oocyte meiosis (Figure 4B-D and Figure 5A). Then, the genes enriched in the mentioned top 3 KEGG signaling pathways were entered into STRING and structured a PPI network (Figure 5B). Based on degree conversion, nine hub genes were identified, including polo-like kinase 1 (PLK1), cyclin-dependent kinase 1 (CDK1), cyclin-dependent kinase 2 (CDK2), mitotic arrest deficient 2 like 1 (MAD2L1), cyclin B1 (CCNB1), BUB1 mitotic checkpoint serine/threonine kinase (BUB1), cell division cycle 20 (CDC20), cyclin B2 (CCNB2), and cell division cycle 25A (CDC25A) (Figure 5C).

3.4 The relationship between FOXM1 and its hub genes

We extracted the expression data from the aforementioned datasets and explored the correlations between FOXM1 and its hub genes. Obviously, not only was FOXM1 significantly associated with other hub genes, but also there were significant correlations among the hub genes that were observed (Figure 6). To verify that FOXM1 has targeted regulatory effects on hub genes, we explored the binding sites of hub genes in cistromDB and found binding peaks between FOXM1 and the transcription initiation points of hub genes (Figure 7).

The survival status and survival time of the TCGA patients were collected. After performing survival analysis of FOXM1 and its nine downstream targets, we found that there is no significant correlations between survival time and the mentioned genes ($p > 0.05$) (Figure 8).

4. Discussion

By analyzing the eight pairs of clinical samples from our hospital, IHC data from 174 patients, and 1542 ESCC mRNA-sequencing data from public databases, this study solved the following problems: First, the expression of FOXM1 is upregulated in ESCC and has an extremely high discrimination potential in ESCC; second, the downstream target genes of FOXM1 were predicted and verified; and finally the potential signaling pathways of FOXM1 in regulating ESCC were also indicated. To our knowledge, we indicated the expression of FOXM1 is upregulated in ESCC and has a great ability to discriminate ESCC from healthy cells for the first time, these results were based on the largest sample size at present. Moreover, not only the mRNA expression of FOXM1 were revealed, we also explored the protein expression of FOXM1 in ESCC, and obtained a same result.

In previous researches, FOXM1 was considered differentially expressed in ESCC. Many researchers, including Yuan, Pedro, and Song et al., have reported that FOXM1 is upregulated in ESCC based on samples from their hospitals (38),(39),(40). However, studies have shown an interesting view. Takata et al. detected the expression level of FOXM1 protein in 174 ESCC tissues and found that only 54% of ESCC samples showed positive FOXM1 expression; although they did not detect FOXM1 expression in normal tissues, the results showed that only half of the tissues were positive and FOXM1 was not considered differentially expressed in ESCC (41). Obviously, most of the researches had similar results to ours. This study indicated that the expression of FOXM1 is significantly upregulated in ESCC and that it has a very high discriminated effect between ESCC and healthy cells. Nevertheless, the expression level of FOXM1 in ESCC remains controversial; although we have larger samples, more data remain to be collected and analyzed in the future. Additionally, many studies have indicated the correlations between FOXM1 expression and patients' clinical parameters. Cheng et al. performed a correlation analysis between the expression of FOXM1 and the prognosis of patients; the results showed that FOXM1 expression is negatively correlated with patients' survival time (28). Nevertheless, there was no significant correlation between FOXM1 expression and patients' survival time observed in our results. It is noteworthy that Hui et al. observed that FOXM1 expression is associated with the pathological stages of patients, which is also

inconsistent with our results (42). In data from the TCGA database, we observed that FOXM1 expression is only significantly related to patients' ages, and other clinical parameters do not show positive results. To date, the relationship between FOXM1 and patients' prognosis/clinical parameters remains controversial, and the clinical information that we could collect is limited. Thus, it is essential to conduct further research with larger sample sizes and prognostic information.

As a transcription factor, transcriptional regulation of FOXM1 has been reported in multiple types of cancers. Yang et al. indicated that FOXM1 can bind with the FKH sequence located on the HMMR promoter region and activate the transcription initiation of HMMR, and the expression of HMMR promotes metastasis of bladder cancer cells through the Wnt/β-catenin signaling pathway (43). Liu et al. observed that FOXM1 plays a regulatory role in tumor proliferation, invasion, chemotherapy resistance, and genomic mutation by regulating downstream ovarian cancer-related genes (44). In a study focus on pancreatic cancer, Wang et al. thought that upregulated FOXM1 will activate the production of miRNA-552 and influence the expression of DACH1, PCDH10, and SMAD4, which promotes the development of pancreatic cancer (45). Moreover, the transcriptional regulation of FOXM1 has also been reported in head and neck cancer, prostatic cancer, breast cancer, hepatocellular carcinoma, and other cancers (46),(47),(48),(49). In our results, due to the lack of research on the transcriptional regulation of FOXM1 in ESCC, we could only infer the downstream target genes of FOXM1 from 40 putative target lists of FOXM1 in other tumors. Finally, 168 genes were identified as FOXM1 downstream targets.

In this study, nine hub genes were identified. According to the existing literature, most of the hub genes are related to FOXM1 in various cancer types. Wang et al. reported that FOXM1 and PLK1 are risk factors for breast cancer, and FOXM1 might play a regulatory role in PLK1 transcription (48). In Qi's opinions, FOXM1 was identified as a hub gene in lung cancer, and differentially expressed FOXM1 regulates CDK1, CCNB2, and CDC20 through upregulated SBF2-antisense RNA 1 (50). In a study on liver cancer, Wu et al. indicated that FOXM1 inhibits the production of thyroid hormones and the expression of CDK2 (51). Li et al. found that there is a great binding site between FOXM1 and the promoter of CCNB1, and downregulated CCNB1 inhibits the expression of FOXM1 in cervical squamous cell carcinoma (52). Yu et al. indicated that FOXM1 can bind with the promoter region of BUB1 and then participate in the radio-resistance of glioblastoma (53). In addition, CDC25A was also considered an important gene in the FOXM1-miR-21-5p-CDC25A axis, which regulates the development of acute lymphoblastic leukemia (54). Unfortunately, we did not find any study on the relationship between FOXM1 and MAD2L1; we will continue to pay attention to relevant progress.

Furthermore, to our knowledge, some researchers have also reported the potential molecular mechanisms of FOXM1 in ESCC. Yuan et al. thought that upregulated FOXM1 can eliminate the inhibitory effect of miRNA-134 and relates proteins on the proliferation and invasion of ESCC cells and then promote the development of ESCC (38). Pedro et al. indicated that overexpressed FOXM1 regulates AKT phosphorylation by participating in the PIK3/AKT signaling pathway, which can promote the proliferation of ESCC. This regulatory effect is related to the poor prognosis of patients (39). As mentioned earlier, the downstream targets of FOXM1 were applied to predict potential signaling pathways. In this study, we

indicated that the downstream targets of FOXM1 are significantly enriched in the cell cycle, which has not been reported in ESCC. After searching literature, the following researches supported our conclusions would be showed. Lee et al. found that downregulated FOXM1 arrests the cell cycle in hepatocellular carcinoma by inhibiting the expression of downstream targets AURKA and PLK1 (55). Researchers have also reported that panobinostat induces the G2/M cell cycle arrest in gastric metastatic cancer cells, which is based on the downregulation of FOXM1 (56). Furthermore, Shi et al. thought that lncRNA OSER1-AS1 targets FOXM1 expression and then promotes cell apoptosis and cell cycle arrest in lung adenocarcinoma cells (57). Interestingly, Pedro et al. reported that FOXM1 not only regulates ESCC cell proliferation in G1/S and G2/M phase but also plays a regulatory role in the M phase (58). The authors found that many other researchers' results are similar to ours; FOXM1 downstream targets, including PLK1 and CDK1, are probably the key targets of FOXM1 in regulating the cell cycle (55),(20).

This study revealed the expression of FOXM1 mRNA and protein in ESCC using 1906 samples. We showed the potential regulatory mechanisms of FOXM1. However, some limitations are obvious: First, all the samples were collected from tissues of homo sapiens, and we could not know the expression level of FOXM1 in body fluids; second, as a controversial topic, only a few clinical parameters and patient prognosis information were collected in this study, and we hope to clarify the relationship between FOXM1 and clinical parameters more objectively in the future; finally, all the analysis on the potential mechanisms is still based on bioinformatics, and further experiments in vivo and in vitro are needed to verify our conclusions.

5. Conclusion

Overall, based on clinical samples from our hospital and public databases, we reported that FOXM1 was significantly upregulated in ESCC in large sample sizes for the first time, and that upregulated FOXM1 had an extremely high potential to discriminate ESCC from healthy cells. Furthermore, the downstream targets of FOXM1 and the potential signaling pathways for regulating ESCC were also predicted. We filled the deficiency of previous studies about FOXM1 in ESCC, and proposed that FOXM1 might be an important target for the diagnosis and treatment of ESCC.

Declarations

Compete interests

All authors declare no conflicts of interest in this paper.

Ethical approval statement

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Review Committee of the First Affiliated Hospital of Guangxi Medical University (2019-KY-E-149). Written informed consent was obtained from eligible patients before registration.

Consent for publication

All of the authors had consented to publication requirements.

Data availability statement

All of the public data could be collected from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/), Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) ArrayExpress (https://www.ebi.ac.uk/arrayexpress) Oncomine (https://www.oncomine.org) and sequence read archive (SRA, https://www.ncbi.nlm.nih.gov/sra) databases, the accession numbers were shown on Table 1. The in-house RNA-sequencing data of the clinical cases from our hospital were deposited in the GEO repository, accession number (GSE164158).

Funding statement

The present study was supported by the Guangxi Medical High-level Key Talents Training "139" Program (2020), the Guangxi Higher Education Undergraduate Teaching Reform Project (2020JGA146, 2021JGA142), the Guangxi Educational Science Planning Key Project (2021B167), the Guangxi Medical University Training Program for Distinguished Young Scholars (2017), the College Student Innovation and Entrepreneurship Training Program Project (202110598153) and the Guangxi Medical University Future Academic Stars Project (WLXSZX21109).

Author contribution statement

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work

Acknowledgement

Herein, we are grateful to Guangxi Key Laboratory of medical pathology for its technical supports.

References

1. Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2020;70(4):313.

2. Yang Y, Hong P, Xu W, He Q, Li B. Advances in targeted therapy for esophageal cancer. Signal transduction and targeted therapy. 2020;5(1):229.

3. Gao H, Shen W, Zhang X, Xu J, Li Y, Li S, et al. Preliminary analysis of the benefits of different irradiation types on patients with postoperative locoregional recurrence of esophageal cell squamous

carcinoma. Annals of palliative medicine. 2021;10(9):9467-79.

4. Guo X, Meng X, Liu R. Prognostic value of microvessel density in esophageal squamous cell carcinoma-a systematic review and meta-analysis. Pathology, research and practice. 2021;227:153644.

5. Zhang X, Lu N, Wang L, Wang Y, Li M, Zhou Y, et al. Recent advances of mA methylation modification in esophageal squamous cell carcinoma. Cancer cell international. 2021;21(1):421.

6. Arnold M, Ferlay J, van Berge Henegouwen M, Soerjomataram I. Global burden of oesophageal and gastric cancer by histology and subsite in 2018. Gut. 2020;69(9):1564-71.

7. Zhang W, Yan C, Zhang T, Chen X, Dong J, Zhao J, et al. Addition of camrelizumab to docetaxel, cisplatin, and radiation therapy in patients with locally advanced esophageal squamous cell carcinoma: a phase 1b study. Oncoimmunology. 2021;10(1):1971418.

8. Visaggi P, Barberio B, Ghisa M, Ribolsi M, Savarino V, Fassan M, et al. Modern Diagnosis of Early Esophageal Cancer: From Blood Biomarkers to Advanced Endoscopy and Artificial Intelligence. Cancers. 2021;13(13).

9. Yuan Z, Wang X, Geng X, Li Y, Mu J, Tan F, et al. Liquid biopsy for esophageal cancer: Is detection of circulating cell-free DNA as a biomarker feasible? Cancer communications (London, England). 2021;41(1):3-15.

10. Chetwood J, Garg P, Finch P, Gordon M. Systematic review: the etiology of esophageal squamous cell carcinoma in low-income settings. Expert review of gastroenterology & hepatology. 2019;13(1):71-88.

11. Yamamoto Y, Kadota T, Yoda Y, Hori K, Hatogai K, Kojima T, et al. Review of early endoscopic findings in patients with local recurrence after definitive chemoradiotherapy for esophageal squamous cell carcinoma. Esophagus : official journal of the Japan Esophageal Society. 2020;17(4):433-9.

12. Wang Y, Lu Y, Xu W, Wang Y, Wu Y, Che G. Prognostic value of osteopontin expression in esophageal squamous cell carcinoma: A meta-analysis. Pathology, research and practice. 2019;215(10):152571.

13. Bull A, Pucher P, Lagergren J, Gossage J. Chest drainage after oesophageal resection: A systematic review. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus. 2021.

14. Paiboonrungruang C, Simpson E, Xiong Z, Huang C, Li J, Li Y, et al. Development of targeted therapy of NRF2 esophageal squamous cell carcinoma. Cellular signalling. 2021:110105.

15. Puhr H, Preusser M, Ilhan-Mutlu A. Immunotherapy for Esophageal Cancers: What Is Practice Changing in 2021? Cancers. 2021;13(18).

16. Zhang H, Si J, Yue J, Ma S. The mechanisms and reversal strategies of tumor radioresistance in esophageal squamous cell carcinoma. Journal of cancer research and clinical oncology. 2021;147(5):1275-86.

17. Jin Z, Chen D, Chen M, Wang C, Zhang B, Zhang J, et al. (Neo)adjuvant Chemoradiotherapy is Beneficial to the Long-term Survival of Locally Advanced Esophageal Squamous Cell Carcinoma: A Network Meta-analysis. World journal of surgery. 2021.

18. Thakur B, Devkota M, Chaudhary M. Management of Locally Advanced Esophageal Cancer. JNMA; journal of the Nepal Medical Association. 2021;59(236):409-16.

19. Chen L, Wei Q, Bi S, Xie S. Maternal Embryonic Leucine Zipper Kinase Promotes Tumor Growth and Metastasis via Stimulating FOXM1 Signaling in Esophageal Squamous Cell Carcinoma. Frontiers in oncology. 2020;10:10.

20. Pal-Ghosh R, Xue D, Warburton R, Hill N, Polgar P, Wilson J. CDC2 Is an Important Driver of Vascular Smooth Muscle Cell Proliferation via FOXM1 and PLK1 in Pulmonary Arterial Hypertension. International journal of molecular sciences. 2021;22(13).

21. Li Y, Wu F, Tan Q, Guo M, Ma P, Wang X, et al. The multifaceted roles of FOXM1 in pulmonary disease. Cell communication and signaling : CCS. 2019;17(1):35.

22. Wei Y, Wang Z, Zong Y, Deng D, Chen P, Lu J. LncRNA MFI2-AS1 promotes HCC progression and metastasis by acting as a competing endogenous RNA of miR-134 to upregulate FOXM1 expression. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;125:109890.

23. Zhao P, Li T, Wang Y, Wang Y, Gu Q, Li Z. LncRNA MYCNOS promotes glioblastoma cell proliferation by regulating miR-216b/FOXM1 axis. Metabolic brain disease. 2021;36(6):1185-9.

24. Goodman A, Mahmud W, Buckingham L. Gene variant profiles and tumor metabolic activity as measured by FOXM1 expression and glucose uptake in lung adenocarcinoma. Journal of pathology and translational medicine. 2020;54(3):237-45.

25. Ai C, Zhang J, Lian S, Ma J, Győrffy B, Qian Z, et al. FOXM1 functions collaboratively with PLAU to promote gastric cancer progression. Journal of Cancer. 2020;11(4):788-94.

26. Kalathil D, John S, Nair A. FOXM1 and Cancer: Faulty Cellular Signaling Derails Homeostasis. Frontiers in oncology. 2020;10:626836.

27. Su M, Tang J, Zhang B, Yang D, Wu Z, Wu J, et al. LncRNA GACAT3 promotes esophageal squamous cell carcinoma progression through regulation of miR-149/FOXM1. Cancer cell international. 2021;21(1):478.

28. Cheng L, Wang Q, Tao X, Qin Y, Wu Q, Zheng D, et al. FOXM 1 induces Vasculogenic mimicry in esophageal cancer through β-catenin /Tcf4 signaling. Diagnostic pathology. 2020;15(1):14.

29. Xiao Z, Jia Y, Jiang W, Wang Z, Zhang Z, Gao Y. FOXM1: A potential indicator to predict lymphatic metastatic recurrence in stage IIA esophageal squamous cell carcinoma. Thoracic cancer. 2018;9(8):997-1004.

30. Li G, Yang L, Chen G, Huang S, Fang Y, Huang W, et al. Laryngeal Squamous Cell Carcinoma: Clinical Significance and Potential Mechanism of Cell Division Cycle 45. Cancer biotherapy & radiopharmaceuticals. 2021.

31. Huang W, Chen G, Chen S, Dang Y, Deng Y, Zhou H, et al. The Indication of Poor Prognosis by High Expression of ENO1 in Squamous Cell Carcinoma of the Lung. Journal of oncology. 2021;2021:9910962.

32. He R, Li J, Du X, Dang Y, Yang L, Huang Z, et al. LPCAT1 overexpression promotes the progression of hepatocellular carcinoma. Cancer cell international. 2021;21(1):442.

33. Chen S, Zhou H, Zhang H, He R, Huang Z, Dang Y, et al. PTTG1The Clinical Significance and Potential Molecular Mechanism of in Esophageal Squamous Cell Carcinoma. Frontiers in genetics. 2020;11:583085.

34. Li R, Chen G, Dang Y, He R, Liu A, Ma J, et al. Expression and Clinical Significance of BCL2 Interacting Protein 3 Like in Multiple Myeloma. Technology in cancer research & treatment. 2021;20:15330338211024551.

35. Gao L, Pang Y, Guo X, Zeng J, Tang Z, Xiong D, et al. Polo like kinase 1 expression in cervical cancer tissues generated from multiple detection methods. PeerJ. 2020;8:e10458.

36. Yang D, Huang W, Chen G, Chen S, Yang J, He R, et al. Clinical significance of transcription factor RUNX2 in lung adenocarcinoma and its latent transcriptional regulating mechanism. Computational biology and chemistry. 2020;89:107383.

37. Pang Y, Li J, Gao L, Yang X, Dang Y, Lai Z, et al. The clinical value and potential molecular mechanism of the downregulation of MAOA in hepatocellular carcinoma tissues. Cancer medicine. 2020;9(21):8004-19.

38. Yuan Y, Wang Q, Cao F, Han B, Xu L. MiRNA-134 suppresses esophageal squamous cell carcinoma progression by targeting FOXM1. International journal of clinical and experimental pathology. 2019;12(6):2130-8.

39. Nicolau-Neto P, Da Costa N, de Souza Santos P, Gonzaga I, Ferreira M, Guaraldi S, et al. FOXM1Esophageal squamous cell carcinoma transcriptome reveals the effect of on patient outcome through novel PIK3R3 mediated activation of PI3K signaling pathway. Oncotarget. 2018;9(24):16634-47.

40. Song L, Wang X, Feng Z. Overexpression of FOXM1 as a target for malignant progression of esophageal squamous cell carcinoma. Oncology letters. 2018;15(4):5910-4.

41. Takata A, Takiguchi S, Okada K, Takahashi T, Kurokawa Y, Yamasaki M, et al. Clinicopathological and prognostic significance of FOXM1 expression in esophageal squamous cell carcinoma. Anticancer research. 2014;34(5):2427-32.

42. Hui M, Chan K, Luk J, Lee N, Chung Y, Cheung L, et al. Cytoplasmic Forkhead box M1 (FoxM1) in esophageal squamous cell carcinoma significantly correlates with pathological disease stage. World journal of surgery. 2012;36(1):90-7.

43. Yang D, Ma Y, Zhao P, Ma J, He C. HMMR is a downstream target of FOXM1 in enhancing proliferation and partial epithelial-to-mesenchymal transition of bladder cancer cells. Experimental cell research. 2021:112860.

44. Liu C, Barger C, Karpf A. FOXM1: A Multifunctional Oncoprotein and Emerging Therapeutic Target in Ovarian Cancer. Cancers. 2021;13(12).

45. Wang X, Dou N, Wang J, Zhang Y, Li Y, Gao Y. FOXM1-induced miR-552 expression contributes to pancreatic cancer progression by targeting multiple tumor suppressor genes. International journal of biological sciences. 2021;17(4):915-25.

46. Ma X, Zhang H, Li Q, Schiferle E, Qin Y, Xiao S, et al. viaFOXM1 Promotes Head and Neck Squamous Cell Carcinoma Activation of the Linc-ROR/LMO4/AKT/PI3K Axis. Frontiers in oncology. 2021;11:658712.

47. Kaochar S, Rusin A, Foley C, Rajapakshe K, Robertson M, Skapura D, et al. Inhibition of GATA2 in prostate cancer by a clinically available small molecule. Endocrine-related cancer. 2021.

48. Wang Q, Zhong W, Deng L, Lin Q, Lin Y, Liu H, et al. The Expression and Prognostic Value of SUMO1-Activating Enzyme Subunit 1 and Its Potential Mechanism in Triple-Negative Breast Cancer. Frontiers in cell and developmental biology. 2021;9:729211.

49. Yoshida T, Ohe C, Ikeda J, Atsumi N, Saito R, Taniguchi H, et al. Integration of NRP1, RGS5, and FOXM1 expression, and tumour necrosis, as a postoperative prognostic classifier based on molecular subtypes of clear cell renal cell carcinoma. The journal of pathology Clinical research. 2021.

50. Qi H, Wang L, Zhang X, Sun W, Liu J. LncRNA SBF2-AS1 inhibits apoptosis and promotes proliferation in lung cancer cell via regulating FOXM1. Journal of BUON : official journal of the Balkan Union of Oncology. 2020;25(4):1761-70.

51. Wu C, Yeh C, Lin K. Thyroid hormones suppress FOXM1 expression to reduce liver cancer progression. Oncology reports. 2020;44(4):1686-98.

52. Li S, Liu N, Piao J, Meng F, Li Y. CCNB1 Expedites the Progression of Cervical Squamous Cell Carcinoma via the Regulation by FOXM1. OncoTargets and therapy. 2020;13:12383-95.

53. Yu H, Zhang S, Ibrahim A, Deng Z, Wang M. Serine/threonine kinase BUB1 promotes proliferation and radio-resistance in glioblastoma. Pathology, research and practice. 2019;215(8):152508.

54. Xia M, Zhang Q, Luo M, Li P, Wang Y, Lei Q, et al. Regulatory network analysis reveals the oncogenesis roles of feed-forward loops and therapeutic target in T-cell acute lymphoblastic leukemia. BMC medical genomics. 2019;12(1):8.

55. Lee H, Chu K, Moon E, Quan F. Histone Deacetylase Inhibitor-Induced CDKN2B and CDKN2D Contribute to G2/M Cell Cycle Arrest Incurred by Oxidative Stress in Hepatocellular Carcinoma Cells via Forkhead Box M1 Suppression. Journal of Cancer. 2021;12(17):5086-98.

56. Lee N, Kim D, Jin H, Meng R, Chae O, Kim S, et al. Inactivation of the Akt/FOXM1 Signaling Pathway by Panobinostat Suppresses the Proliferation and Metastasis of Gastric Cancer Cells. International journal of molecular sciences. 2021;22(11).

57. Shi T, Sun W, Shi Y, Wang Q, Yan Z, Zhang M. LncRNA OSER1-AS1 interacts with miR-612/FOXM1 axis to modulate gefitinib resistance of lung adenocarcinoma. American journal of translational research. 2021;13(3):1365-76.

58. Nicolau-Neto P, Palumbo A, De Martino M, Esposito F, de Almeida Simão T, Fusco A, et al. UBE2C Is a Transcriptional Target of the Cell Cycle Regulator FOXM1. Genes. 2018;9(4).

Tables

Table 1. Basic information of datasets of esophageal squamous cell carcinoma.

Platform	Study	ESCC	Non-ESCC	Platform	Study	ESCC	Non-ESCC
GPL13287	GSE70409	17	17	GPL13607	GSE45350	4	4
GPL16956	GSE89102	5	5	GPL571	GSE36223	0	23
GPL18109	GSE53624	119	119		GSE39491	0	40
GPL9052	GSE119436	4	4		GSE53892	3	0
GPL13497	GSE97558	3	0		GSE38129	30	30
	GSE45168	5	5		GSE29001	21	24
GPL570	GSE3526	0	4		GSE20347	17	17
	GSE161533	28	56		GSE44021	73	73
	GSE77861	7	7	GPL16791	GSE111011	7	7
	GSE100942	4	5		GSE156651	0	4
	GSE26886	9	19		GSE103356	0	6
	GSE33810	2	1		GSE116272	0	4
	GSE17351	5	5		GSE113341	0	10
	GSE7307	0	4		GSE113777	0	1
	GSE19472	0	2	GPL96	GSE1420	0	8
	GSE148247	0	3		GSE13083	0	7
	GSE146808	3	0		GSE52138	1	2
	GSE45670	0	10		GSE44021	34	34
	GSE17353	0	4		GSE23400	53	53
	GSE63941	22	0	GPL20301	GSE149609	17	10
	GSE13378	8	0		GSE142556	2	0
	GSE67508	8	0	GPL20795	GSE164158	8	8
	GSE86013	2	0		GSE128914	3	0
	GSE27424	0	6		GSE124514	2	0
	GSE69925	274	0		GSE128913	3	0
	GSE44021	6	6	GPL5175	GSE75241	15	15
	GSE32701	20	0		GSE49292	0	3
	GSE35975	1	0		GSE65013	0	6

	GSE11373	1	0		GSE75243	2	0
TCGA-GTEx	TCGA	81	2	In-house	-	8	8
	GTEx	0	291				

Note: ESCC, esophageal squamous cell carcinoma; TCGA, The Cancer Genome Atlas; GTEx, The Genotype-Tissue Expression.

Table 2. Relationship between FOXM1 expression of RNA-sequencing dataset and clinicopathological parameters of ESCC patients.

Clinical parameters	Case(n)	FOXM1 expression		T-test	
		Mean	SD	T-value	P-value
Group					
ESCC	81	5.941	0.917	-16.312	0.000***
Non-ESCC	293	4.178	0.619		
Gender					
Male	69	6.311	1.029	1.018	0.312
Female	12	5.987	0.940		
Race†					
Asian	37	6.406	1.018	1.44	0.243
Black or African American	5	6.697	0.674		
White	37	6.074	1.058		
Age (years)					
< 60	48	6.595	0.988	3.836	0.000***
≥ 60	33	5.779	0.866		
Clinical T stage†					
T1	5	5.912	1.593	0.396	0.757
T2	8	6.306	1.627		
T3	19	6.509	0.849		
T4	3	6.602	0.351		
N stage†					
N0	14	6.243	1.551	0.258	0.775
N1	12	6.388	0.724		
N2	2	6.897	0.708		
M stage					
M0	31	6.333	1.167	-0.758	0.454
M1	4	6.791	0.793		
Clinical stage†					
II	18	6.370	1.362	0.245	0.784

III	11	6.457	0.479
IV	4	6.791	0.793

Note: ESCC, esophageal squamous cell carcinoma; Non-ESCC, control group without esophageal squamous cell carcinoma samples; SD, standard deviation. Race†, Clinical T stage†, Clinical stage†, N stage†, Clinical stage† were analyzed by single factor ANOVA, and the rest were analyzed by student's T test. *, p<0.05; **, p<0.01; ***, p<0.001.

Figures

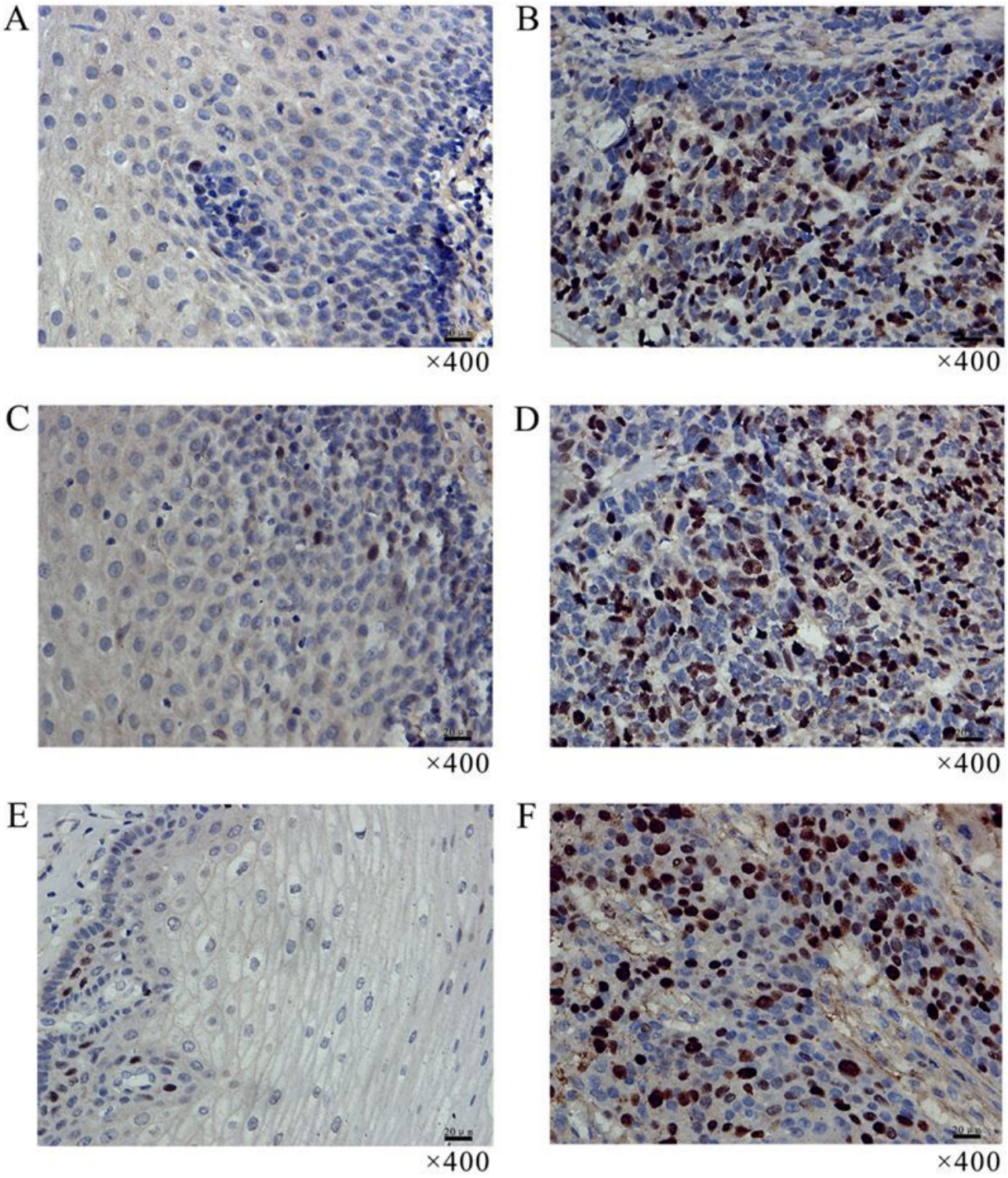

Figure 1

Immunohistochemistry of FOXM1. (A, C, E): Normal esophageal tissue. B, D, F: Esophageal squamous cell carcinoma. Magnification, ×400. Note: FOXM1, Forkhead box M1

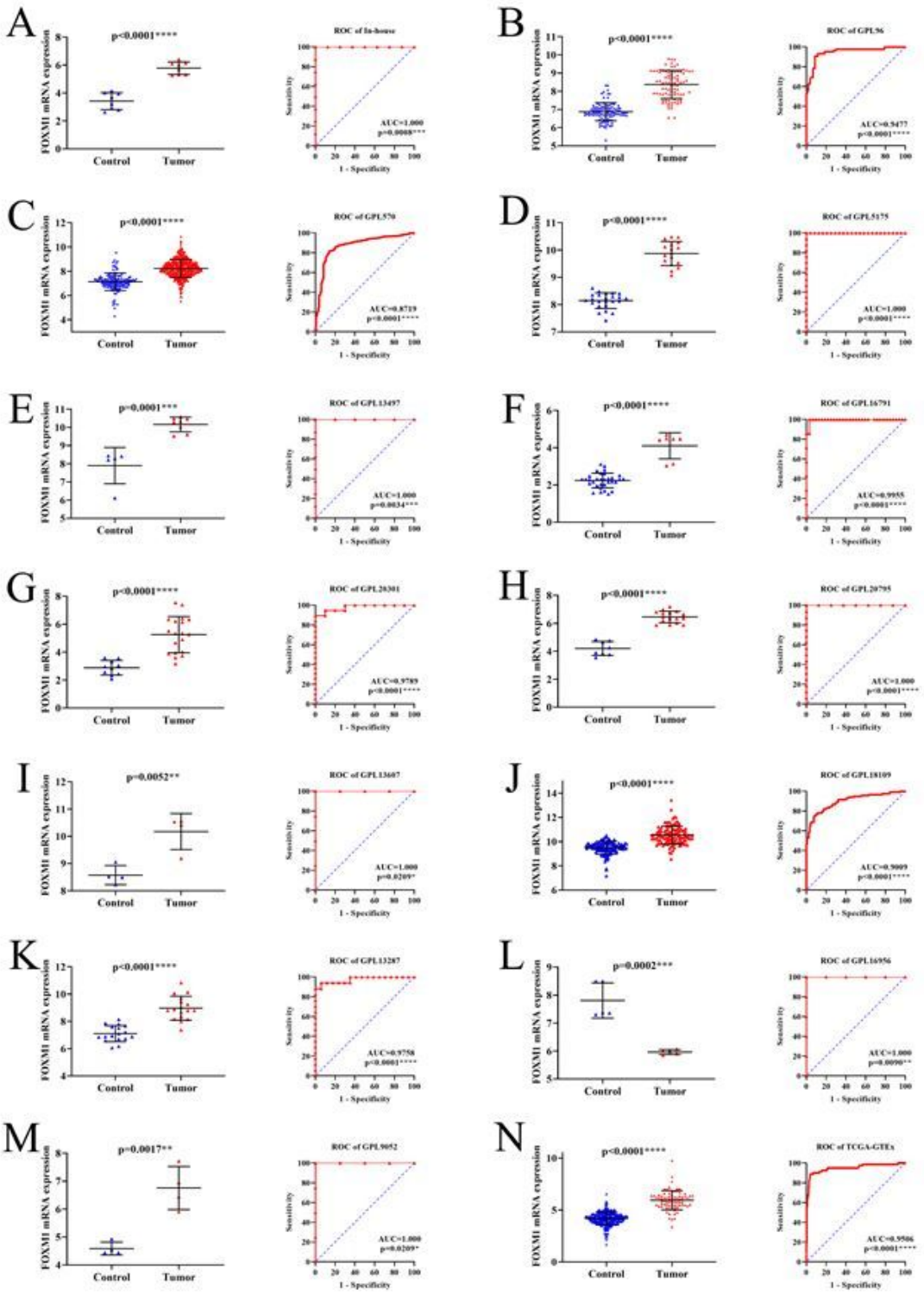

Figure 2

Expression level of FOXM1 in esophageal squamous cell carcinoma datasets. Scatter plots showed the differential expression of FOXM1 in esophageal squamous cell carcinoma and normal esophageal tissue, and ROC plots showed the ability of FOXM1 to distinguish esophageal squamous cell carcinoma from normal esophageal tissue. In the case of AUC > 0.5, the closer AUC is to 1, the stronger the ability of FOXM1 to distinguish esophageal squamous cell carcinoma from normal esophageal tissue. A: In-house

sequencing data; B: GPL96; C: GPL570; D: GPL5175; E GPL13497; F: GPL16791; G: GPL20301; H: GPL20795; I: GPL136-7; J: GPL18109; K: GPL13287; L: GPL16956; M: GPL9052; N: TCGA-GTEx.

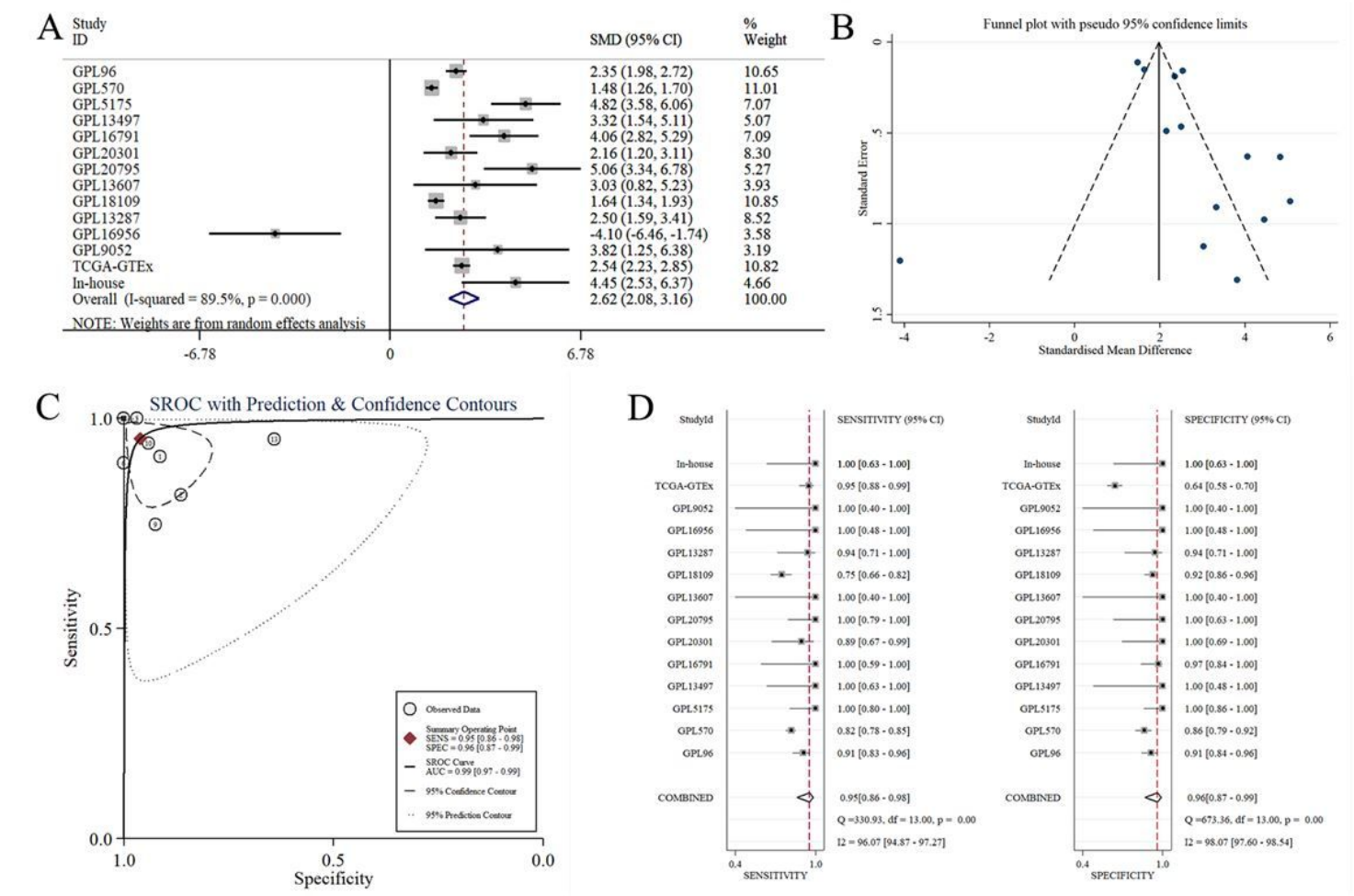

Figure 3

Integrated analysis of FOXM1 expression. A: Forest plot, SMD = 2.62 [2.08–3.16]. B: Funnel plot. C: SROC plot, AUC = 0.99 [0.97–0.99]. D: Plot of comprehensive sensitivity and comprehensive specificity, comprehensive sensitivity = 0.95 [0.86–0.98], comprehensive specificity = 0.96 [0.87–0.99]. Note: SD, standard deviation; SMD, standardized mean difference; CI, confidence interval; FOXM1, Forkhead box M1; SROC, summary receiver operator characteristic curve; AUC, area under the curve.

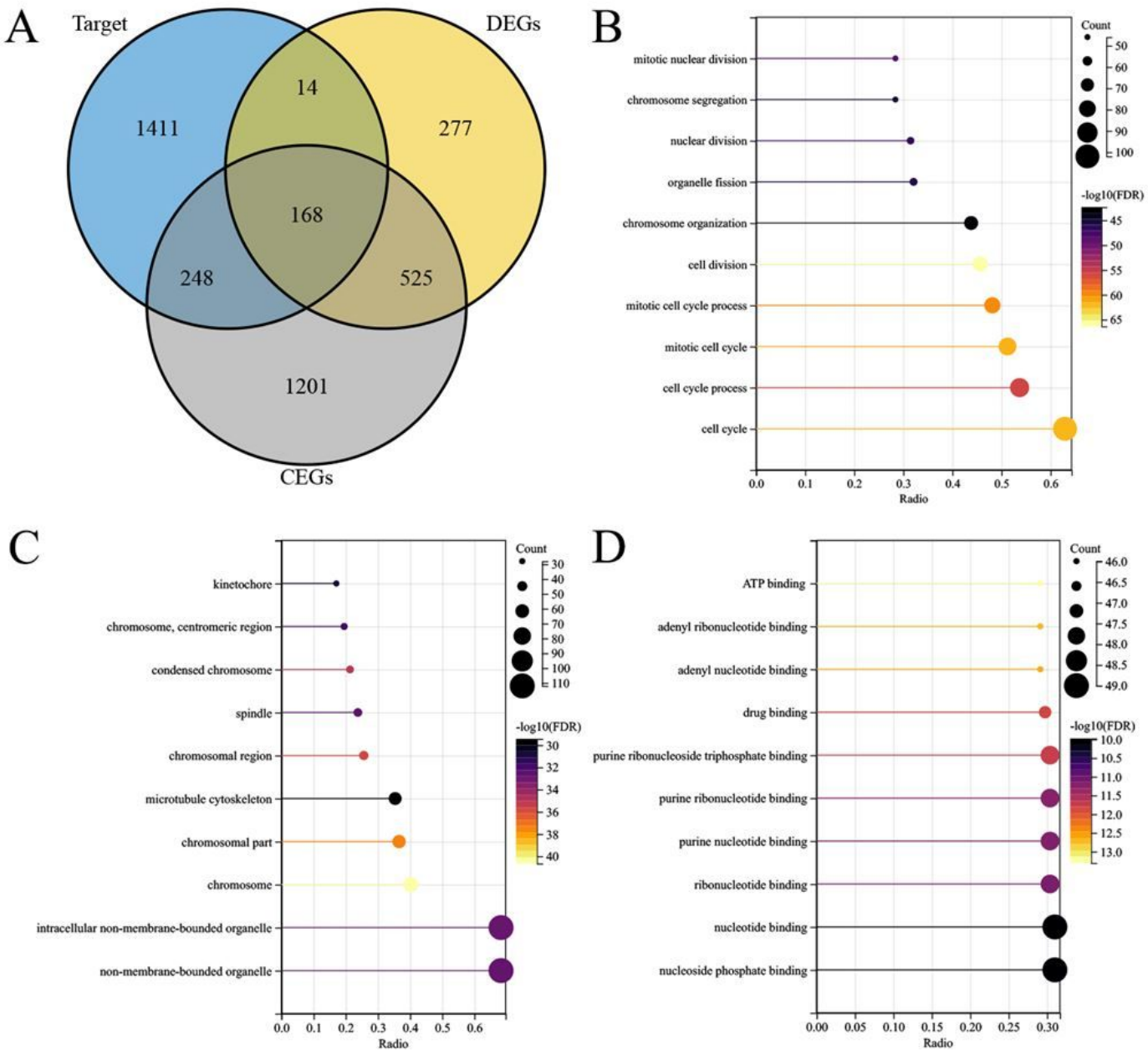

Figure 4

Bioinformatics analysis of the molecular mechanism of FOXM1 in esophageal squamous cell carcinoma. A: Venny diagram. B: Tableau diagram of biological process. C: Tableau diagram of a cellular component. D: Tableau diagram of molecular function. Note: DEGs, differentially expressed genes; CEGs, co-expressed genes.

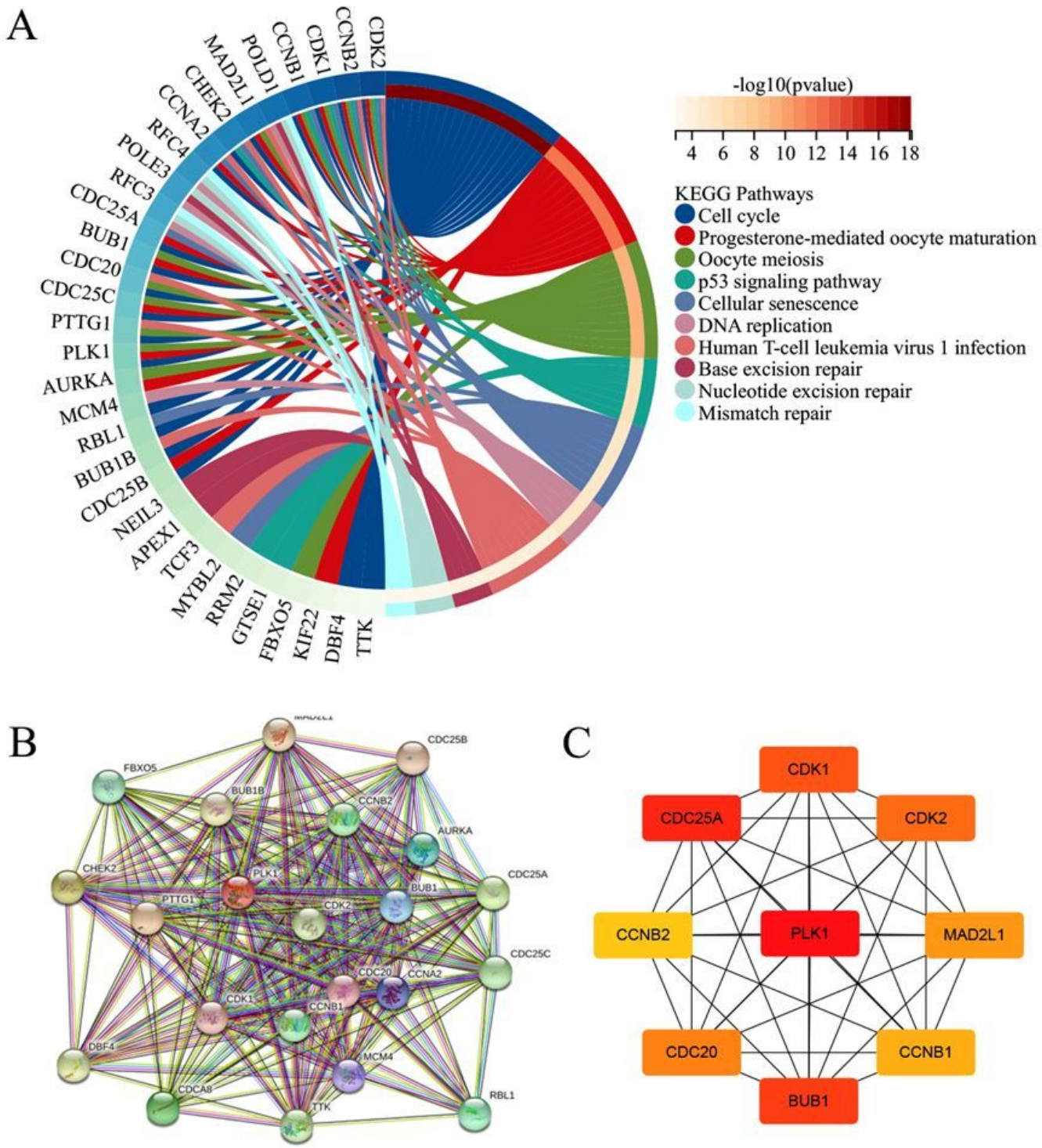

Figure 5

Enrichment analysis of FOXM1 DCEGs in esophageal squamous cell carcinoma. A: Kyoto Encyclopedia of Genes and Genomes enrichment analysis. B: PPI networks showed the connection among the DCEGs. C: The top nine hub genes were screened out. DCEGs, differentially co-expressed genes; PPI, protein–protein interaction.

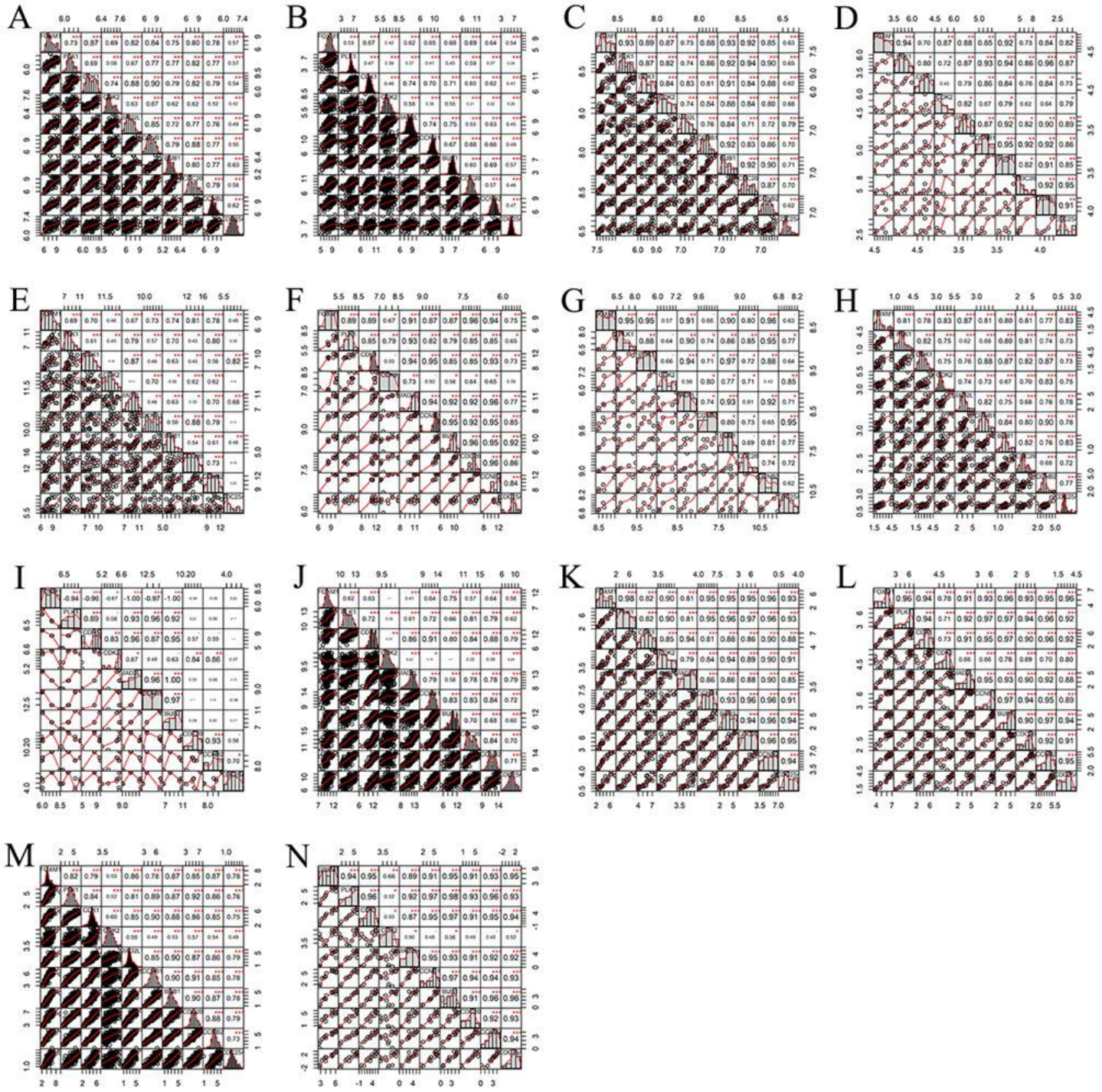

Figure 6

The correlations between FOXM1 and its hub genes based on the collected data sets. A: GPL96; B: GPL570; C: GPL5175; D: GPL9052; E: GPL13287; F: GPL13497; G: GPL13607; H: GPL16791; I: GPL16956; J: GPL18109; K: GPL20301; L: GPL20795; M: TCGA-GTEx; N: In-house sequencing data.

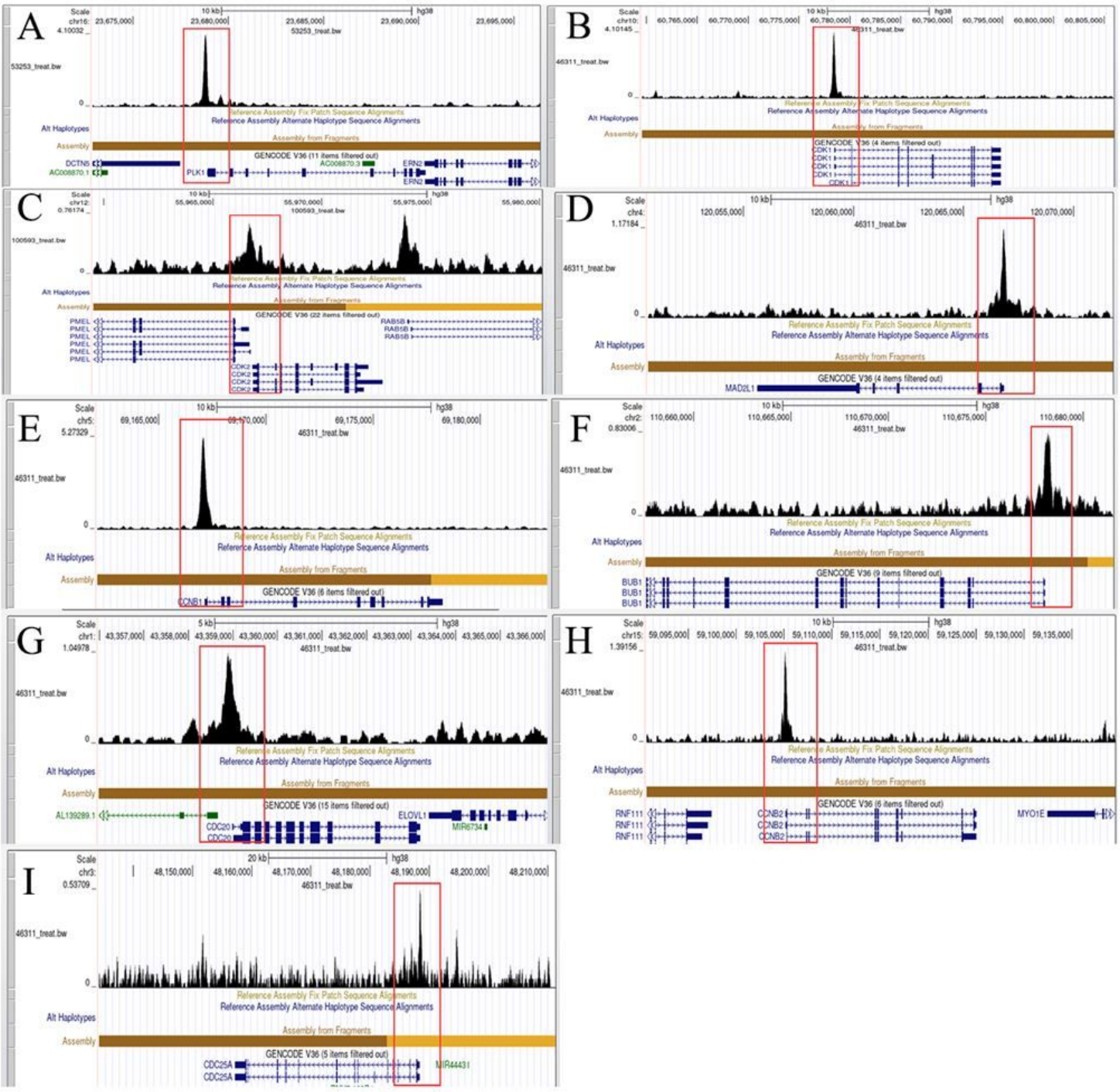

Figure 7

There are binding peaks between FOXM1 and its hub genes. A: PLK1; B: CDK1; C: CDK2; D: MAD2L1. E: CCNB1. F: BUB1. G: CDC20. H: CCNB2. I: CDC25A

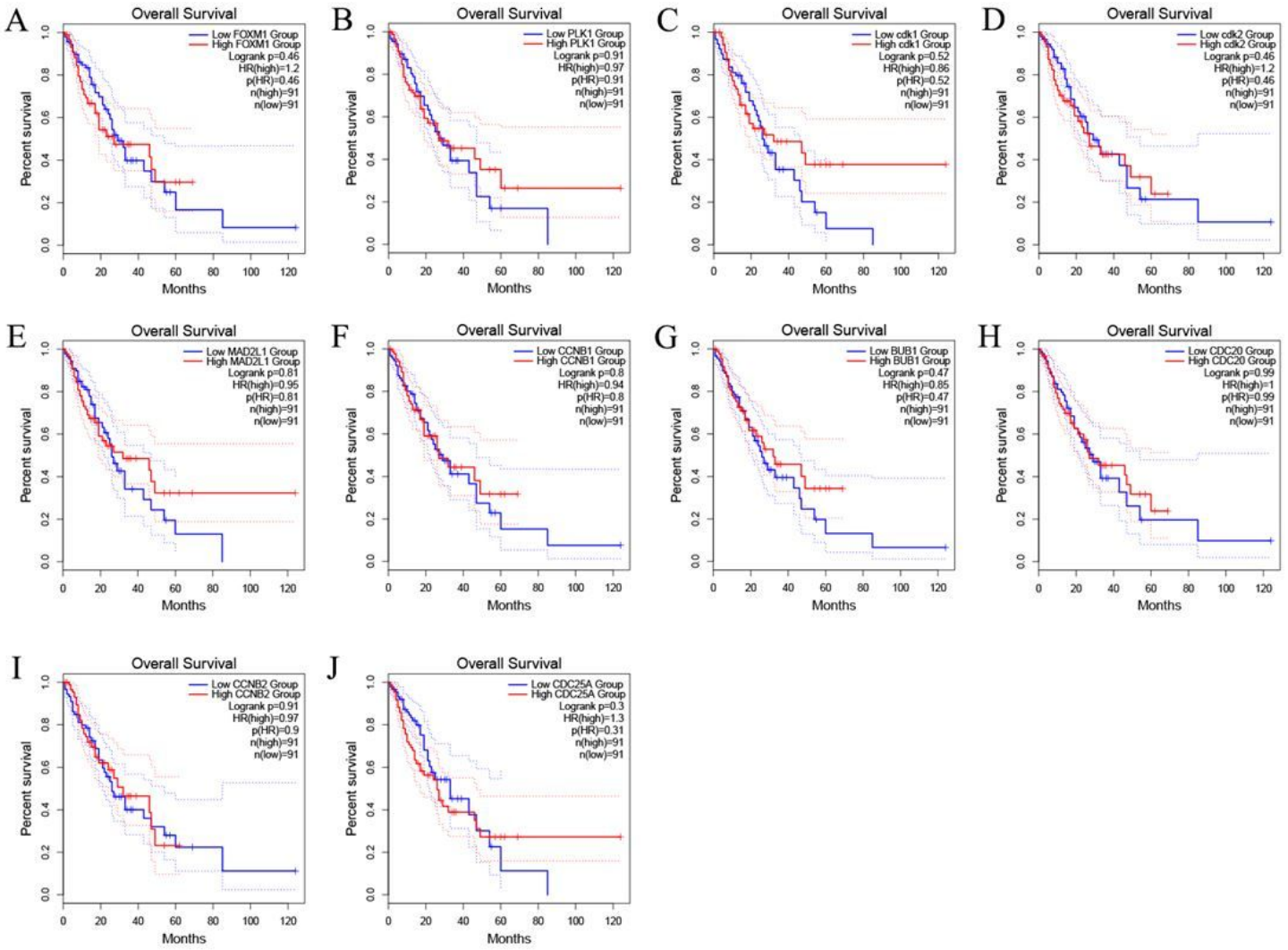

Figure 8

Prognosis analysis of patients with esophageal squamous cell carcinoma in RNA sequencing database. A: FOXM1; B: PLK1; C: CDK1; D: CDK2; E: MAD2L1; F: CCNB1; G: BUB1; H: CDC20; I: CCNB2; J: CDC25A; P < 0.05* statistical significance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure2.pdf
- Supplementaryfigure1.pdf
- Supplementarytable2.doc
- Supplementarytable1.doc