Screening of tumor grade-related mRNAs and IncRNAs for Esophagus Squamous Cell Carcinoma

Xin Gao¹,² | Qian Liu² | Xue Chen¹ | Shaoping Chen² | Jianmei Yang² | Qiang Liu² | Yufeng Cheng¹

¹Department of Radiotherapy, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China
²Department of Oncology, Dongying People's Hospital, Dongying, China

Correspondence
Dr. Yufeng Cheng, Department of Radiotherapy, Qilu Hospital, Cheeloo College of Medicine, Shandong University, No. 107, Wenhua Xi Road, Jinan 250012, China.
Email: yufengcheng_doctor@163.com

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Abstract
Background: The goal of our study was to screen tumor grade-related IncRNAs and mRNAs to reveal the underlying molecular mechanism of esophagus squamous cell carcinoma (ESCC).

Methods: The IncRNA and mRNA sequencing data were obtained from The Cancer Genome Atlas (TCGA). Tumor grade correlation analysis of IncRNAs and mRNAs was executed, followed by the functional enrichment analysis of all tumor grade-related mRNAs. The differentially expression mRNAs (DEmRNAs) and differentially expressed IncRNAs (DEIncRNAs) were obtained. PPI network and DEmRNA-DElncRNA interaction analysis were constructed. The functional annotation of the DEmRNAs co-expressed with DEIncRNAs was performed. The expression levels of the candidate genes were validated using qRT-PCR.

Results: A total of 1864 tumor grade-related mRNAs (846 positively related and 1018 negatively related) and 552 tumor grade-related IncRNAs (331 positively related and 221 negatively related) were obtained. The top 10 significantly grade-related mRNAs and IncRNAs included CA12, FABP4, DECR1, BAIAP2, IL1RAPL2, PPARD, LAD1, TSPAN10, LDOC1, ZNF853, RP11-25G10.2, RP11-557H15.3, RP11-521D12.5, CHKB-AS1, RP11-219B4.3, CH17-335B8.4, RP11-99 J16-4.2, CTB-111H14.1, ADNP-AS1, and JHDM1D-AS1. SFN, IL1RAPL2, and RP11-25G10.2 were overlapped from grade 1, grade 2, and grade 3. PPI network showed that top 10 proteins with higher degrees, including GNAI1, RAP2B, GNAZ, SHH, ADCY1, PRKAR2B, SH3GL1, GNA15, and ARRB1. A DEIncRNAs-nearby DEmRNAs network was constructed to obtain hub IncRNAs including ADAMTS9-AS2, RP11-210 M15.2, RP11-13 K12.1, ZBED3-AS1, and RP11-25G10.2. Except for RP11-25G10.2, ADAMTS9-AS1, ZBED3-AS1, SFN, ATP1A2, and GNA15 were consistent with our TCGA analysis.

Conclusions: Alterations of DEmRNAs and DEIncRNAs may provide key insights into the molecular mechanisms of ESCC.

Keywords
DEIncRNAs, DEmRNAs, esophagus squamous cell carcinoma, The Cancer Genome Atlas (TCGA), tumor grade
1 | INTRODUCTION

Esophageal cancer is one of the most common malignancies worldwide. The incidence of esophageal cancer is threefold higher in men than in women. The predominant histological types of esophageal cancer are adenocarcinoma and squamous cell carcinoma. Esophageal squamous cell carcinoma (ESCC) is one of aggressive squamous cell carcinomas with high incidence. The high mortality rate has been attributed to late diagnosis and poor treatment response, among others. The World Health Organization ESCC histological tumor classification is as follows: grade 1 (well differentiated), grade 2 (moderately differentiated), and grade 3 (lowly differentiated). Therefore, screening of tumor grade-related mRNAs and lncRNAs in ESCC is key issue to the treatment of ESCC patients.

Long non-coding RNA (lncRNA), of more than 200 nucleotides in length, can regulate gene expression during several biological processes. Cumulative reports of abnormal lncRNA expression have shown that lncRNA may be used as a new independent biomarker for the early diagnosis, prognosis, and metastasis prediction of various cancer types. HOTAIR is a well-known lncRNA whose expression level strongly predicts the metastatic and survival of different cancer types, including ESCC. However, the potential molecular mechanisms of ESCC remain unclear.

Here, we aimed to investigate the differentially expressed mRNAs (DEmRNAs) and differentially expressed lncRNAs (DElncRNAs) associated with the different tumor grades including grade 1, grade 2, and grade 3. We first performed a tumor grade correlation analysis of lncRNAs and mRNAs expression data in patients with ESCC using the Cancer Genome Atlas (TCGA). Then, the screening of DEmRNAs and DElncRNAs between different tumor grades was performed, and this was followed by the DElncRNAs-target DEmRNAs interactions network. The qRT-PCR was applied to validate the expression of candidate gene. Our study identified potentially key mRNAs and lncRNAs in ESCC and provided a basis for further understanding the mechanisms of ESCC and the predictive performance of lncRNAs.

2 | METHODS

2.1 | Integrated profiles in TCGA

The TCGA data portal included the data of 185 patients with Esophagus Squamous Carcinoma, including clinical data of 185 patients. RNA sequencing data of 164 patients up to 24 May 2018. In this study, ESCC patients were allocated to grade 1, grade 2, and grade 3 groups in accordance with the recorded tumor grade. The lncRNA and mRNA gene expression profiles and clinical data of ESCC were downloaded from TCGA.

2.2 | Identification of tumor grade-related mRNAs and lncRNAs

The TCGA datasets were downloaded and transformed from Fragments Per Kilobase Million (FPKM) data into Transcripts Per Kilobase Million (TPM) data. Here, we used log2 of the TPM value to measure of mRNA and lncRNA expression level. The undetectable lncRNAs and mRNAs (with read count value =0 in more than 20% ESCC case) were filtered and deleted. The linear by linear association test was applied to analyze the correlation of the expression of mRNAs and lncRNAs with tumor grade by using the lbl.test function of the coin package in R-3.3.3. p<0.05 was defined as the criteria of statistical significance.

2.3 | Screening of differentially expressed mRNAs (DEmRNAs) and lncRNAs (DElncRNAs) related to tumor grade

The difference in the expression of grading degree related lncRNAs and mRNAs for the different clinical tumor grades (grade 1 vs. grade 2, grade 1 vs. grade 3, grade 2 vs. grade 3) was performed. The Tukey’s Honest Significant Difference test was used for multiple comparisons between different clinical tumor grades. The p < 0.05 was considered as statistical significance. The overlapped DElncRNA and DEmRNA for all clinical tumor grades were also analyzed.

2.4 | Functional annotation

Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using GeneCoDis3 (http://genecodis.cnb.csic.es/analysis). FDR <0.05 was considered to show statistically significant differences.

2.5 | Protein-protein interaction (PPI) network construction

A total of 332 DEmRNAs (after overlapping for grade 1 vs. grade 2 and grade 1 vs. grade 3) in ESCC were used to construct the PPI network by using String (https://string-db.org/) and Cytoscape 3.5.0 (http://www.cytoscape.org/). The nodes and edges represent the proteins and interactions between the two proteins, respectively.

2.6 | DEmRNA-DElncRNA interaction analysis

The correlation between DElncRNAs and DEmRNAs was analyzed by the Spearman correlation coefficient. DElncRNA-DEmRNA pairs
with $p<0.05$ and $|r|>0.6$ were served as significant mRNA-IncRNA co-expression pairs. The Cytoscape 3.5.0 (http://www.cytoscape.org/) was used to build the DElncRNA-DEmRNA co-expression network.

2.7 | qRT-PCR Confirmation

Six genes (RP11-25G10.2, ADAMTS9-AS1, ZBED3-AS1, SFN, ATP1A2, and GNA15) were selected as candidate genes. Eighteen tissue samples were obtained, including nine samples of ESCC para-cancer, two tumor samples of grade 3 ESCC, and seven tumor samples of grade 2 ESCC. This study was approved by the ethics institute of our hospital. The signed informed consent was obtained from all the participants.

Total RNA was isolated using a TRizol kit (Tiangen, China). A Fast Quant RT Kit (Tiangen, China) was utilized to obtain the complementary DNA. Using Super Real PreMix Plus SYBR Green (Tiangen, China), quantitative real-time PCR was generated using the LightCycler 96. Relative gene expression was analyzed by the $2^{-\Delta\Delta Ct}$ method.

2.8 | Validation in a published GEO dataset (GSE53625)

The expression patterns of the expression of top 10 upregulated and top 10 downregulated mRNAs and IncRNAs were validated using the GSE53625 dataset. The Gene Expression Omnibus (GEO) dataset GSE53625 dataset was downloaded from the GEO (https://www.ncbi.nlm.nih.gov/geo/). The GSE53625 dataset was published and the tissue sample consisted of 179 normal controls, 32 grade 1 ESCC patients, 98 grade 2 patients, and 49 grade 3 patients.

3 | RESULTS

3.1 | Tumor grade-related mRNAs and IncRNAs

A total of 1864 tumor grade-related mRNAs (846 positively related and 1018 negatively related) and 552 tumor grade-related IncRNAs (331 positively related and 221 negatively related) were identified. The top 10 significantly grade-related mRNAs, including CA12, FABP4, DECR1, BAIAP2, IL1RAPL2, PPARD, LAD1, TSPAN10, LDOC1, and ZNF853, were listed in Figure 1 and Table 1. The top 10 significantly grade-related IncRNAs, including RP11-25G10.2, RP11-557H15.3, RP11-521D12.5, CHKB-AS1, RP11-219B4.3, CH17-335B8.4, RP11-99 J16-A.2, CTA-111H14.1, ADNP-AS1, and JHDM1D-AS1, were shown in Figure 2 and Table 2. Besides, the heat map of top 100 tumor grade-related mRNAs and IncRNAs was shown in Figure 3.
3: 186 DEmRNAs and 46 DElncRNAs between grade 2 and grade 3. It is worth mentioning that two overlapped DEmRNAs including SFN and IL1RAPL2 were found between grade 1, grade 2, and grade 3. It is noted that one overlapped DElncRNAs including RP11-25G10.2 was identified between grade 1, grade 2, and grade 3. The overlap of DEmRNAs and DElncRNAs correlated with the tumor grade of ESCC between the grade 1 and grade 2, between the grade 1 and grade 3, and between the grade 2 and grade 3 were displayed in Figure 4.

### 3.3 Functional annotation of tumor grade degree related mRNAs

A total of 332 DEmRNAs (after overlapping for grade 1 vs. grade 2 and grade 1 vs. grade 3) in ESCC were used to perform the GO and KEGG enrichment analysis. According to GO enrichment analysis, signal transduction (FDR=1.57E-09), protein phosphorylation (FDR=6.20E-05), plasma membrane (FDR=1.75E-13), and nucleotide binding (FDR=4.54E-05) were significantly enriched GO terms. The KEGG pathway enrichment analysis displayed that Inositol phosphate metabolism (FDR=4.39E-06), Gastric acid secretion (FDR=0.000134781), Phosphatidylinositol signaling system (FDR=0.00134861), Pathways in cancer (FDR=0.00852066), and Salivary secretion (FDR=0.00999689) were four significantly enriched pathways. The top 15 most significantly enriched GO and KEGG pathways of mRNAs were shown in Figure 5.

### 3.4 PPI network construction

A total of 332 DEmRNAs (after overlapping for grade 1 vs. grade 2 and grade 1 vs. grade 3) in ESCC were used to construct the PPI network.
network construction. The PPI network was consisted of 153 nodes and 274 edges (Figure 6). The top 10 proteins with higher degrees, including GNAI1 (degree =18), RAP2B (degree =14), GNAZ (degree =13), SHH (degree =12), ADCY1 (degree =12), PRKAR2B (degree =12), SH3GL1 (degree =12), GNA15 (degree =11), and ARRB1 (degree =11).

### 3.5 DEmRNA-DElncRNA interaction analysis

The 87 DElncRNAs (after overlapping for grade 1 vs. grade 2 and grade 1 vs. grade 3) and 332 DEmRNAs (after overlapping of grade 1 vs. grade 2 and grade 1 vs. grade 3) were used to build the DEmRNA-DElncRNA interaction analysis. A total of 456 DElncRNA-DEmRNA co-expression pairs including 55 lncRNAs and 175 mRNAs were identified with absolute value of the Spearman correlation coefficient \(|r|>0.6\) and \(p\)-value<0.05. The co-expressed DElncRNA-DEmRNA network was shown in the Figure 7. Among which, ADAMTS9-AS2 (degree=95), RP11-210 M15.2 (degree=61), RP11-13 K12.1 (degree=39), ZBED3-AS1 (degree=30), and RP11-25G10.2 (degree=17) were lncRNAs with higher degrees.

Validation of qRT-PCR.

As shown in Figure 8, RP11-25G10.2, SFN, and GNA15 were downregulated and ADAMTS9-AS1, ZBED3-AS1, and ATP1A2 were upregulated in the grade 2 ESCC compared with grade 3 ESCC. Except for RP11-25G10.2, the other five genes were consistent with our TCGA analysis.

### 3.6 Validation in GSE53625

As presented in Figure 9 and Figure 10, the expression of eight DEmRNAs (CA12, FABP4, BAIAP2, PPARD, LAD1, TSPAN10, LDOC1, and ZNF853) and three DElncRNAs (CHKB-AS1, RP11-219B4.3, and JHDM1D-AS1) was consistent with our TCGA results, generally. This validation may enhance the credibility of our results in integrated analysis. Further confirmation and experiments with larger sample sizes will be conducted in our following study.

| IncRNA     | Mean_G1 | Mean_G2 | Mean_G3 | PValue | Association |
|------------|---------|---------|---------|--------|-------------|
| RP11-25G10.2 | 0.7837  | 2.0551  | 2.9416  | 1.39E−05 | Positive    |
| RP11-557H15.3 | 8.3860  | 6.5275  | 5.0147  | 1.88E−05 | Negative    |
| RP11-521D12.5 | 7.2330  | 6.3871  | 5.2905  | 7.08E−05 | Negative    |
| CHKB-AS1     | 6.8058  | 6.4156  | 5.8739  | 1.33E−04 | Negative    |
| RP11-219B4.3 | 1.6527  | 2.4740  | 3.4196  | 2.47E−04 | Positive    |
| CH17-335B8.4 | 4.3742  | 4.0931  | 2.7438  | 3.52E−04 | Negative    |
| RP11-9916_A.2 | 0.7723  | 1.4853  | 2.0785  | 6.05E−04 | Positive    |
| CTB-111H14.1 | 1.9260  | 2.8209  | 3.0849  | 6.93E−04 | Positive    |
| ADNP-AS1     | 6.5575  | 6.9455  | 7.1321  | 7.49E−04 | Positive    |
| JHDM1D-AS1   | 10.1829 | 9.6409  | 8.7570  | 7.73E−04 | Negative    |
ESCC has a higher incidence among blacks and Asians, accounting for 70% of esophageal cancers. Although its diagnosis and treatment have advanced recent years, ESCC ranks among the fourth leading cause of cancer-related death. In this study, two differentially expressed mRNAs (SFN and IL1RAPL2) and one common differentially expressed lncRNAs (RP11-25G10.2) overlapped for ESCC in grade 1, grade 2, and grade 3.

In the PPI network, GNAI1, RAP2B, GNAZ, SHH, ADCY1, PRKAR2B, SH3GL1, GNA15, and ARRB1 were top 10 proteins with higher degrees. GNAI1, G protein subunit alpha 1, is differentially expressed mRNA in pancreatic ductal adenocarcinoma, and plays a key role in the pathogenesis of pancreatic ductal adenocarcinoma. GNAI1 was associated with the tumor grade of ESCC and significantly enriched in pathways of cancer. ADCY1 is highly expressed in multidrug-resistant in ESCC cell lines and facilitated ESCC cells resistance to most chemotherapy drugs.

**FIGURE 4** Venn diagram showing the overlap of DE mRNAs and DE lncRNAs for the tumor grade of ESCC between the grade 1 and grade 2, between the grade 1 and grade 3 and between the grade 2 and grade 3. Numbers represent the number of mRNAs and lncRNAs.

**FIGURE 5** The top 15 significant enrichment GO terms and KEGG pathways of DE mRNAs. The x-axis shows -log FDR and the y-axis shows GO terms or KEGG pathways. (A) Biological processes. (B) Cellular components. (C) Molecular functions. (D) KEGG pathways.

### DISCUSSION

In the PPI network, GNAI1, RAP2B, GNAZ, SHH, ADCY1, PRKAR2B, SH3GL1, GNA15, and ARRB1 were top 10 proteins with higher degrees. GNAI1, G protein subunit alpha 1, is differentially expressed mRNA in pancreatic ductal adenocarcinoma, and plays a key role in the pathogenesis of pancreatic ductal adenocarcinoma. GNAI1 was associated with the tumor grade of ESCC and significantly enriched in pathways of cancer. ADCY1 is highly expressed in multidrug-resistant in ESCC cell lines and facilitated ESCC cells resistance to most chemotherapy drugs.
and ADCY1 were DEIncRNAs related to ESCC grading. GNAI1 and ADCY1 were significantly enriched pathway of Gastric acid secretion. Therefore, we hypothesized that GNAI1 and ADCY1 may play pivotal roles in ESCC by regulating signaling pathway of Gastric acid secretion.

GNA15, a G protein subunit alpha 15, is differentially expressed mRNA in human squamous cell carcinoma and pancreatic ductal adenocarcinoma.\textsuperscript{16,17} It has been reported that GNA15 is negatively correlated with the tumor grade of ESCC patients.\textsuperscript{14} In the current study, GNA15 was negatively correlated with the tumor grade of ESCC. The interaction network analysis results showed that GNA15 was co-expressed with RP11-210 M15.2 and ZBED3-AS. Hence, we further hypothesized that P11-210 M15.2 and ZBED3-AS regulate GNA15, which may be crucial to ESCC tumor grading.

\textbf{FIGURE 6} The PPI network. Ellipses represent nodes and lines represent edges. Red and green represent positive correlation and negative correlation with tumor grade, respectively.
Phospholipase C beta 4 (PLCB4) is overexpressed in primary gastrointestinal stromal tumors, and it is a novel overexpressed enzyme that regulates lipid catabolism, promotes cell proliferation, and independently confers a worse prognosis.\(^\text{18}\) PLCB4 hotspot mutation is similar to a gain-of-function mutation leading to the activation of the same signaling pathway, promoting uveal melanoma tumorigenesis.\(^\text{19}\) In the present study, PLCB4 was positively correlated with tumor grade in patients with ESCC. The KEGG results showed that PLCB4 was a significantly enriched pathway of salivary secretion. The interaction network analysis results showed that PLCB4 was co-expressed with RP11-210 M15.2. Therefore, we presumed that RP11-210 M15.2 may be involved in ESCC tumor grade by regulating the salivary secretion pathway.

Two DEmRNAs (SFN and IL1RAPL2) and one overlapped DElncRNAs (RP11-25G10.2) overlapped for grade 1, grade 2, and grade 3. Stratifin (SFN) is originally identified as a p53-inducible gene that is responsive to DNA-damaging agents.\(^\text{20}\) SFN is a differentially expressed gene related to human lung adenocarcinoma cell proliferation, and SFN may facilitate lung tumor development and progression.\(^\text{21}\) SFN promoter methylation may be associated with the carcinogenesis of breast cancer and that the use of SFN promoter methylation may represent a useful
blood-based biomarker for the clinical diagnosis of breast cancer. Hepatocellular carcinoma-secreted SFN promotes the expression of matrix metalloproteinases in cancerous surrounding cells via an aminopeptidase N dependent mechanism, and SFN has a paracrine effect on educating stromal cells in the tumor-associated microenvironment. Here, SFN was co-expressed with RP11-25G10.2 and ZBED3-AS1. Therefore, we hypothesized that both RP11-210 M15.2 and ZBED3-AS1 may be involved in regulation of SFN and may underlie the tumor grade.

In summary, we identified several differentially expressed mRNAs and lncRNAs associated with different tumor grades of ESCC. Interestingly, the overlapped DEmRNAs (SFN and IL1RAPL2) and DElncRNA (RP11-25G10.2) overlapping for grade 1, grade 2, and grade 3 may play a crucial role in the regulation of tumor grade in ESCC. However, some limitations should be addressed. The number of samples used qRT-PCR confirmation was small. More studies are needed to uncover the precise mechanisms of ESCC.
CONSENT FOR PUBLICATION

The subjects gave written informed consent for the publication of any associated data and accompanying images.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Yufeng Cheng and Xin Gao contributed to the conception of the study. Qian Liu and Xue Chen contributed the materials and performed the experiment. Shaoping Chen, Jianmei Yang, and Qiang Liu performed the data analyses. Yufeng Cheng and Xin Gao contributed significantly in writing the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

This study was approved by the ethical committee of the Qilu Hospital, Cheeloo College of Medicine, Shandong University.

DATA AVAILABILITY STATEMENT

The dataset supporting the conclusions of this article is included within the article.

ORCID

Yufeng Cheng https://orcid.org/0000-0001-5283-5956

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-E386.Epub 2014/09/16.
2. Ohashi S, Miymoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent advances from basic and clinical studies of esophageal squamous cell carcinoma. Gastroenterology. 2015;149(7):1700-1715. Epub 2015/09/17.
3. Sakai NS, Samia-Aly E, Barbera M, Fitzgerald RC. A review of the current understanding and clinical utility of miRNAs in esophageal cancer. Semin Cancer Biol. 2013;23(6):512-521. Epub 2013/09/10.
4. Sugihara H, Ishimoto T, Miyake K, et al. Noncoding RNA expression aberration is associated with cancer progression and is a potential biomarker in esophageal squamous cell carcinoma. Int J Mol Sci. 2015;16(11):27824-27834. Epub 2015/11/28.
5. Zhou M, Guo M, He D, et al. A potential signature of eight long non-coding RNAs predicts survival in patients with non-small cell lung cancer. J Transl Med. 2015;13:231. Epub 2015/07/18.
6. Li Z, Yao Q, Zhao S, Wang Y, Li Y, Wang Z. Comprehensive analysis of differential co-expression patterns reveal transcriptional dysregulation mechanism and identify novel prognostic lncRNAs in esophageal squamous carcinoma. Oncotarget. 2017;10:3095-3105. Epub 2017/08/10.
7. Zhou M, Xu W, Yue X, et al. Relapse-related long non-coding RNA signature to improve prognosis prediction of lung adenocarcinoma. Oncotarget. 2016;7(20):29720-29738. Epub 2016/04/23.
8. Gupta RA, Shah N, Wang KC, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010;464(7291):1071-1076. Epub 2010/04/16.
9. Qiu JJ, Lin YY, Ye LC, et al. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. Gynecol Oncol. 2014;134(1):121-128. Epub 2014/03/26.
10. Ge X-S, Ma H-J, Zheng X-H, et al. HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits WtF-1 expression and activates Wnt pathway. Cancer Sci. 2013;104(12):1675-1682. Epub 2013/10/15.
11. Cook MB. Non-acid reflux: the missing link between gastric atrophy and esophageal squamous cell carcinoma? Am J Gastroenterol. 2011;106(11):1930-1932. Epub 2011/11/08.
12. Holmes RS, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. Semin Radiat Oncol. 2007;17(1):2-9. Epub 2006/12/23.
13. Idichi T, Seki N, Kurahara H, et al. Involvement of anti-tumor miR-124-3p and its targets in the pathogenesis of pancreatic ductal adenocarcinoma: direct regulation of ITGA3 and ITGB1 by miR-124-3p. Oncotarget. 2018;9(48):28849-28865. Epub 2018/07/11.
14. Xing J, Liu C. Identification of genes associated with histologic tumor grade of esophageal squamous cell carcinoma. FEBS Open Bio. 2017;7(9):1246-1257. Epub 2017/09/15.
15. Yang LX, Li BL, Liu XH, et al. RNA-seq reveals determinants of sensitivity to chemotherapy drugs in esophageal carcinoma cells. Int J Clin Exp Pathol. 2014;7(4):1524-1533. Epub 2014/05/13.
16. Haider AS, Peters SB, Kaporis H, et al. Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. *J Invest Dermatol*. 2006;126(4):869-881. Epub 2006/02/14.

17. Idichi T, Seki N, Kurahara H, et al. Regulation of actin-binding protein ANLN by antitumor miR-217 inhibits cancer cell aggressiveness in pancreatic ductal adenocarcinoma. *Oncotarget*. 2017;8(32):53180-53193. Epub 2017/09/09.

18. Li CF, Liu TT, Chuang IC, et al. PLCB4 copy gain and PLCss4 overexpression in primary gastrointestinal stromal tumors: Integrative characterization of a lipid-catabolizing enzyme associated with worse disease-free survival. *Oncotarget*. 2017;8(12):19997-20010. Epub 2017/02/18.

19. Johansson P, Aoude LG, Wadt K, et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. *Oncotarget*. 2016;7(4):4624-4631. Epub 2015/12/20.

20. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. *Nature*. 1999;401(6753):616-620. Epub 1999/10/19.

21. Shiba-Ishii A, Kim Y, Shiozawa T, et al. Stratifin accelerates progression of lung adenocarcinoma at an early stage. *Mol Cancer*. 2015;14:142. Epub 2015/08/01.

22. Ye M, Huang T, Ying Y, et al. Detection of 14-3-3 sigma (sigma) promoter methylation as a noninvasive biomarker using blood samples for breast cancer diagnosis. *Oncotarget*. 2017;8(6):9230-9242. Epub 2016/12/22.

23. Liu CC, Chang TC, Lin YT, et al. Paracrine regulation of matrix metalloproteinases contributes to cancer cell invasion by hepatocellular carcinoma-secreted 14-3-3sigma. *Oncotarget*. 2016;7(24):36988-36999. Epub 2016/05/14.

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