Role of SLC44A3-AS1 Enhancer RNA in Esophageal Cancer Prognosis

Kai Kang[1], Jingyi Wu[2], Yajun Mao[3], Jindan Kai[1], Si Chen[1] and Fei Xiong[1]

[1]*Department of Thoracic Surgery, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China*

[2]*Department of Thoracic Oncology, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China*

[3]*Operating Room, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China*

ABSTRACT

Objective: To identify key enhancer RNAs (eRNA) in esophageal cancer through a comprehensive analysis and explore its importance in esophageal cancer.

Study Design: An observational study.

Place and Duration of the Study: Department of Thoracic Surgery, Hubei Cancer Hospital affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China, from September to October 2022.

Methodology: RNA-sequencing data, survival data, and clinical data for a total of 33 tumours were gathered from TCGA (The Cancer Genome Atlas) datasets. The survival-associated eRNAs were detected by means of Spearman's correlation and Kaplan-Meier survival analyses. Enhancer RNAs linked to survival rate and their target genes in esophageal cancer were screened, and a clinical correlation analysis of key eRNAs was carried out. A functional enrichment analysis was performed and the selected key eRNAs were confirmed in pan-cancer.

Results: The key eRNA was identified as *SLC44A3-AS1*, and patients with higher expression of *SLC44A3-AS1* had worse prognosis than those with low expression. *SLC44A3-AS1* expression was significantly associated with many clinical traits, namely tumour status, grade, pathological tumour, node, metastasis (TNM) stage, tumour type, etc. According to KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment results, *SLC44A3* may affect the prognosis of esophageal cancer patients through the herpes simplex virus 1 (*HSV1*) infection pathway. According to pan-cancer validation results, *SLC44A3-AS1* was related to the survival of eight tumours. Correlations were observed between *SLC44A3-AS1* and *SLC44A3* in 32 types of tumours.

Conclusion: *SLC44A3-AS1* plays a key role in esophageal cancer related to prognosis, which may be a new therapeutic target for clinical exploration.

Key Words: *SLC44A3-AS1, Enhancer RNA, Esophageal cancer, Prognosis.*

How to cite this article: Kang K, Wu J, Mao Y, Kai J, Chen S, Xiong F. Role of SLC44A3-AS1 Enhancer RNA in Esophageal Cancer Prognosis. *J Coll Physicians Surg Pak* 2023; **33(09)**:964-971.

INTRODUCTION

Esophageal cancer ranks 8th in terms of cancer incidence and 6th in overall death rate.[1] Surgery can be performed to treat esophageal cancer, and about 1/4 of newly diagnosed patients can be treated by surgery.[2] Chemotherapy, radiotherapy, targeted therapy and combinations, thereof, are considered treatment options for patients whose tumours are too advanced to undergo surgery.[3] However, the five-year survival rate of patients with esophageal cancer is still very low, ranging from about 10% to 30% in most countries.[4,5]

Correspondence to: Dr. Jindan Kai, Department of Thoracic Surgery, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China
E-mail: casine1018@163.com

Received: October 12, 2022; Revised: July 13, 2023; Accepted: July 24, 2023
DOI: https://doi.org/10.29271/jcpsp.2023.09.964

Long non-coding RNAs (lncRNAs) are related to many diseases in humans, such as cancers.[6-8] Enhancer RNAs (eRNAs), a lncRNA subclass, play important roles in many biological processes.[9,10] There are thousands of eRNAs that have been identified in the human cells, and many of them mediate target gene activation.[9] eRNAs can regulate the expression of oncogene and tumour suppressors and participate in the regulation of cancer signalling. They also play key roles in tumour progression and tumourigenesis,[9] such as lung cancer,[11] breast cancer,[12] indicating that eRNAs have potential values in cancer diagnosis and prognosis.

SLC44A3-AS1 may be a potential biomarker and therapeutic target for esophageal cancer. This study aimed to identify the prognostic eRNAs involved in esophageal cancer progression and seek their therapeutic targets.

METHODOLOGY

The study was approved by the Institutional Ethics Committee of Hubei Cancer Hospital affiliated to Tongji Medical College,

Huazhong University of Science and Technology (Ethical Approval Number: LLHBCH2022YN-045). It analysed data on 33 tumour types obtained from the TCGA (The Cancer Genome Atlas) database, from September to October 2022.

The RNA-seq gene expression profiles, clinical data, and survival datasets in this study were from TCGA (The Cancer Genome Atlas) database using the UCSC Xena website.[13] The gene expression RNAseq (Workflow Type: HTSeq-FPKM), clinical and survival data from GDC TCGA esophageal cancer were all downloaded from TCGA database, and the same approach was applied to the gene expression RNAseq, clinical and survival data for the other 32 types of cancer. All data can be freely downloaded online.

Ensemble IDs in the RNA-seq gene expression profiles were converted to their corresponding gene symbols using gene transfer format (GTF) files for humans. The eRNA IDs were transported into gene symbols using human GTF files. The eRNA expression profiles of esophageal cancer were obtained in the TCGA database. The eRNA expression matrixes were merged with the esophageal cancer survival data by means of the limma package in R software. The clinical data for esophageal cancer were screened, and 9 types of clinical information for analysis were retained, including age, gender, cancer status, grade, smoker/non-smoker, race, stage, tumour centre and type. Age was divided into two groups of <60 and ≥60 years. Tumour stage was classified into four stages as I, II, III, and IV, while those without data information were marked as unknown.

The Kaplan-Meier method was used for evaluating the prognostic eRNAs in esophageal cancer, and survival-associated eRNAs were selected with $p<0.05$ as the standard cut-off values. The patients with esophageal cancer were categorised into high-expression and low-expression groups following the median expression of each eRNA. The survival differences between the two groups were evaluated and the survival curve was drawn, with $p<0.05$ used as the cut-off value.

The correlation between eRNAs and their target genes was assessed through correlation analysis. eRNAs with $r>0.4$ and $p<0.01$ were screened for further analyses, and correlation analysis plots were therefore generated. The x- and y-axis respectively represent the expression levels of eRNAs and eRNAs-targeted genes. The main eRNAs linked to survival and target genes, which play important roles in esophageal cancer, were obtained through Spearman's correlation analysis. The statistical significance was determined at $p<0.001$ and $r>0.4$.

SLC44A3-AS1, as an eRNA with a significant p-value, was not reported in esophageal cancer. Hence, the correlation between *SLC44A3-AS1* and clinical traits of esophageal cancer was further analysed.

For the identification of more targeted genes of *SLC44A3-AS1* in esophageal cancer, co-expression analysis was performed. The co-expressed genes of *SLC44A3-AS1* in esophageal cancer were screened through Spearman's correlation analysis. Statistical significance was considered at $p<0.001$ and rank correlation coefficient $r>0$. The correlation between *SLC44A3-AS1* and *SLC44A3* in 33 types of tumours was examined.

The org. Hs.eg.db package of R software was used to analyse gene IDs corresponding to the target genes related to the main eRNA. Then, GO [Cellular Component (CC), Biological Process (BP), and Molecular Function (MF)] and KEGG pathway enrichment analyses were made. KEGG pathways and GO terms were considered significantly enriched when the p-value <0.05.

The *SLC44A3-AS1* expression data and its target gene *SLC44A3* were obtained, while the expression matrix was combined with the survival data of the pan-cancer. According to each eRNA's median expression value, patients were divided into low-expression and high-expression groups. The Kaplan-Meier method was used to assess the prognostic eRNAs in pan-cancer. The association between eRNAs and their targeted genes was determined by correlation analysis.

All of the statistical analyses were performed using R 3.6.1. The Kaplan-Meier method was employed to analyse the differences between the two groups with high and low expression of *SLC44A3-AS1*, and spearman correlation analysis was applied to verify the correlation between *SLC44A3-AS1* and their target genes. The difference in *SLC44A3-AS1* expression was analysed in different clinical traits by Wilcoxon signed-rank test. Pearson correlation analysis was used to find out the genes that were co-expressed with *SLC44A3-AS1*. Functional enrichment analysis was performed using the clusterprofiler R package. The Kaplan-Meier method was used to assess the prognostic eRNAs in pan-cancer. The association between eRNAs and their targeted genes in pan-cancer was determined by Pearson correlation analysis.

RESULTS

A total of 34 eRNAs were significantly linked to the survival of esophageal cancer patients according to the esophageal cancer-associated RNA-seq expression profiles. The link between eRNAs and their target genes was determined by the means of correlation analysis. The results showed that 11 highly-expressed eRNAs, including *AC007255.1*, *SLC44A3-AS1*, *FOXP4-AS1*, *AC025871.2*, *AL021391.1*, *AP000696.1*, *LINC01006*, *LINC01271*, *CCDC18-AS1*, *SPAAR*, *WDFY3-AS2*, were significantly associated with poor survival of patients with esophageal cancer. In order to obtain new prognostic-related genes, eRNA *SLC44A3-AS1* was selected for the following analyses because it had the most significant p-value but was not reported in esophageal cancer. Among esophageal cancer patients, the OS time of high expression of *SLC44A3-AS1* was worse than that of low expression ($p = 0.041$, Figure 1a), and the correlation coefficient with the target gene *SLC44A3* was 0.83 ($p<2.2e-16$; Figure 1b).

The clinical traits of these patients are demonstrated in Table I. The eRNA *SLC44A3-AS1* expression was related to the cancer status and age of the patients. In Figure 2, eRNA *SLC44A3-AS1* expression in tumour patients was higher than those without tumours, while the eRNA *SLC44A3-AS1* expression was lower in

patients younger than 60 years (Figure 2a). In different grades, the expression of *SLC44A3-AS1* in G1 patients was significantly lower compared with G2 and G3 patients (Figure 2c). As observed in Figure 2d, patients with N1 showed significantly higher expression of *SLC44A3-AS1* than those with N0. Concerning the races (Figure 2e), white patients showed highly expressed *SLC44A3-AS1*. In Figure 2f, stage III patients showed significantly higher *SLC44A3-AS1* expression than stage II patients. As observed in Figure 2g, the expression of *SLC44A3-AS1* was significantly linked to central tumour location. A higher expression of *SLC44A3-AS1* was observed in distal esophageal cancer patients than in the middle. Among different tumour types (Figure 2h), the eRNA *SLC44A3-AS1* expression in adenocarcinoma patients was significantly higher compared to the other patients' types.

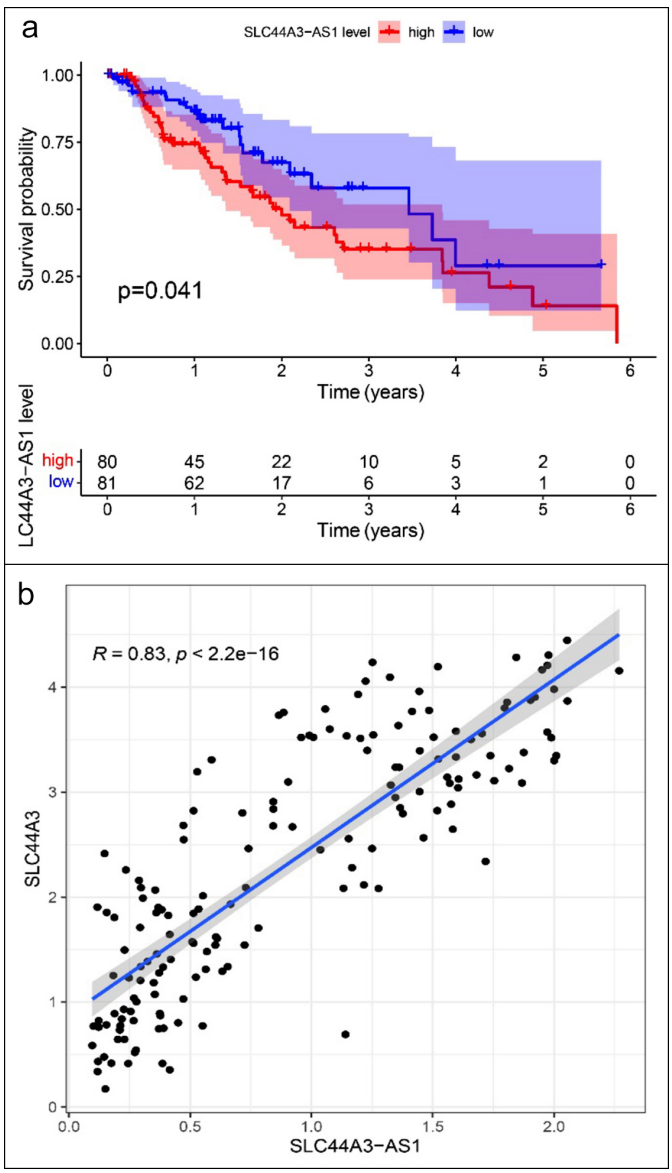

Figure 1: The risk role of *SLC44A3-AS1* for esophageal cancer; (a) Kaplan-Meier overall survival between high *SLC44A3-AS1* expression and its low expression in esophageal cancer patients; (b) Spearman correlation analysis between *SLC44A3-AS1* and *SLC44A3*.

Table I: Association between clinical traits of esophageal cancer patients and *SLC44A3-AS1* expression.

Covariates	Type	Table Stat
Age	<60	75(46.3%)
	>=60	87(53.7%)
Gender	Female	23(14.2%)
	Male	139(85.8%)
Cancer_status	Tumour free	61(37.65%)
	With tumour	35(21.6%)
	Unknown	66(40.74%)
Grade	G1	16(9.88%)
	G2	66(40.74%)
	G3	44(27.16%)
	Unknown	36(22.22%)
Smoker/ Non Smoker	<20	26(16.05%)
	>=20	60(37.04%)
	Unknown	76(46.91%)
Race	Asian	38(23.46%)
	Black or african american	6(3.7%)
	White	100(61.73%)
	Unknown	18(11.11%)
Stage	I	10(6.17%)
	II	53(32.72%)
	III	51(31.48%)
	IV	14(8.64%)
	Unknown	34(20.99%)
Tumour central	Distal	113(69.75%)
	Mid	42(25.93%)
	Proximal	6(3.7%)
	Unknown	1(0.62%)
Type	Adenocarcinoma	79(48.77%)
	Cystic, Mucinous, and Serous Neoplasms	1(0.62%)
	Squamous Cell Neoplasms	82(50.62%)

In order to find more targeted genes of eRNA *SLC44A3-AS1*, co-expression analysis in esophageal cancer was performed with r >0.4 and p<0.01. A total of 4383 targeted genes were identified ultimately. Among 4383 target genes, the top 10 genes of *SLC44A3-AS1* were ranked and finally determined according to the correlation value, which are *SLC44A3, AC006042.1, RAB17, SMPDL3B, ICA1, RAB20, CHN2, LINC01558, SMIM24, AL117382.2*.

The GO enrichment analysis displayed that eRNA-related TFs (transcription factors) were enriched in the glycoprotein metabolic and biosynthetic process in the BP group, apical part of cell and plasma membrane in the CC group, as well as anion transmembrane transporter activity in the MF group (Figure 3a). KEGG enrichment analysis showed that eRNA-related TFs were enriched in herpes simplex virus 1 (HSV1) infection, Ras-associated protein 1 (Rap1) signaling pathway, and tight junction (Figure 3b). The colour intensity implies the p-value: the stronger the colour is, the lower the p-value and the more substantial the enrichment will be. In addition, SLC44A3-AS1 was distinctly related to neutrophil-mediated immunity. Ninety-three genes were enriched in the signalling pathway (Table II).

The prognostic role of *SLC44A3-AS1* and its association with target genes in pan-cancer were determined by the means of survival and correlation analysis. The evaluation of the prognosis of pan-cancer was based on the expression level of each eRNA, using Kaplan-Meier method. Then, the median expression value of eRNAs was calculated based on which the patients were divided into low-expression and high-expression groups.

Table II: Ninety-three genes associated with *SLC44A3-AS1* are enriched in neutrophil-mediated immunity (adjusted p <0.05 and r >0.4).

Gene	Spearman Correlation Coefficient r	Gene	Spearman Correlation Coefficient r	Gene	Spearman Correlation Coefficient r
ENPP4	0.762	MLEC	0.611	PECAM1	0.479
FUCA1	0.735	S100P	0.607	CEACAM6	0.475
SERPINA1	0.732	DNAJC3	0.607	ASAH1	0.471
TMEM63A	0.723	CTSH	0.602	MVP	0.467
CYSTM1	0.715	CYBA	0.601	STBD1	0.466
AOC1	0.712	ORM2	0.601	RAB27A	0.466
PTPRJ	0.702	PRSS3	0.588	ALOX5	0.465
GCA	0.69	GHDC	0.585	ANPEP	0.458
NHLRC3	0.688	SPTAN1	0.584	TCIRG1	0.457
ADGRE5	0.683	TTR	0.583	CAT	0.454
ALDH3B1	0.681	GUSB	0.581	SLC27A2	0.45
APAF1	0.672	MGAM	0.577	FRK	0.448
ATP11A	0.672	COTL1	0.569	CST3	0.446
OLFM4	0.668	CD55	0.569	LRG1	0.445
FUCA2	0.664	VNN1	0.561	SDCBP	0.444
PIGR	0.662	LGALS3	0.554	PRDX4	0.44
LYZ	0.658	SNAP23	0.552	KCNAB2	0.439
SERPINB6	0.652	CTSA	0.545	AGPAT2	0.439
PRSS2	0.648	CANT1	0.543	ACAA1	0.436
ATP8A1	0.643	HEXB	0.538	APEH	0.428
CD63	0.642	LCN2	0.536	CEACAM8	0.421
SVIP	0.64	TRPM2	0.535	PADI2	0.418
RAB37	0.637	RHOF	0.529	DNASE1	0.415
GLB1	0.637	PLAC8	0.527	AP2A2	0.414
IQGAP2	0.635	CTSZ	0.526	CEACAM1	0.413
PTPRB	0.632	CD93	0.512	RHOA	0.412
TNFRSF1B	0.632	ANXA3	0.499	TXNDC5	0.411
CTSS	0.629	PRKCD	0.496	BRI3	0.41
PTPRN2	0.621	DGAT1	0.496	SERPINA3	0.41
RNASET2	0.619	GNS	0.495	CEACAM3	0.404
ORMDL3	0.613	DDOST	0.492	CFP	0.404

Table III: List of 33 types of tumours related to *SLC44A3-AS1* and *SLC44A3*.

eRNA	Target	Cancer Type	cor	corPval
SLC44A3-AS1	SLC44A3	Adrenocortical carcinoma	0.798953	0
SLC44A3-AS1	SLC44A3	Bladder urothelial carcinoma	0.780606	0
SLC44A3-AS1	SLC44A3	Adrenocortical carcinoma	0.627351	0
SLC44A3-AS1	SLC44A3	Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.727385	0
SLC44A3-AS1	SLC44A3	Cholangiocarcinoma	0.857915	1.55E-08
SLC44A3-AS1	SLC44A3	Colon adenocarcinoma	0.580665	0
SLC44A3-AS1	SLC44A3	Lymphoid neoplasm diffuse large B-cell lymphoma	0.801867	7.43E-12
SLC44A3-AS1	SLC44A3	Esophageal cancer	0.830174	0
SLC44A3-AS1	SLC44A3	Glioblastoma multiforme	0.703909	0
SLC44A3-AS1	SLC44A3	Head and neck squamous cell carcinoma	0.535101	1.57E-38
SLC44A3-AS1	SLC44A3	Kidney chromophobe	0.772902	0
SLC44A3-AS1	SLC44A3	Kidney renal clear cell carcinoma	0.371966	0
SLC44A3-AS1	SLC44A3	Kidney renal papillary cell carcinoma	0.677006	0
SLC44A3-AS1	SLC44A3	Acute myeloid leukemia	0.732071	1.28E-26
SLC44A3-AS1	SLC44A3	Brain lower grade glioma	0.853526	2.5E-151
SLC44A3-AS1	SLC44A3	Liver hepatocellular carcinoma	0.722753	1.17E-61
SLC44A3-AS1	SLC44A3	Lung adenocarcinoma	0.605153	0
SLC44A3-AS1	SLC44A3	Lung squamous cell carcinoma	0.708217	0
SLC44A3-AS1	SLC44A3	Mesothelioma	0.732629	0
SLC44A3-AS1	SLC44A3	Ovarian serous cystadenocarcinoma	0.665409	0
SLC44A3-AS1	SLC44A3	Pancreatic adenocarcinoma	0.604594	0
SLC44A3-AS1	SLC44A3	Pheochromocytoma and paraganglioma	0.728468	0
SLC44A3-AS1	SLC44A3	Prostate adenocarcinoma	0.568799	0
SLC44A3-AS1	SLC44A3	Rectum adenocarcinoma	0.641432	0
SLC44A3-AS1	SLC44A3	Sarcoma	0.649646	6.40E-33
SLC44A3-AS1	SLC44A3	Skin cutaneous melanoma	0.733416	0
SLC44A3-AS1	SLC44A3	Stomach adenocarcinoma	0.675076	0
SLC44A3-AS1	SLC44A3	Testicular germ cell tumours	0.916486	3.74E-63
SLC44A3-AS1	SLC44A3	Thyroid carcinoma	0.560261	0
SLC44A3-AS1	SLC44A3	Thymoma	0.86158	0
SLC44A3-AS1	SLC44A3	Uterine corpus endometrial carcinoma	0.717948	0
SLC44A3-AS1	SLC44A3	Uterine carcinoma	0.670267	4.88E-08
SLC44A3-AS1	SLC44A3	Uveal melanoma	0.907103	0

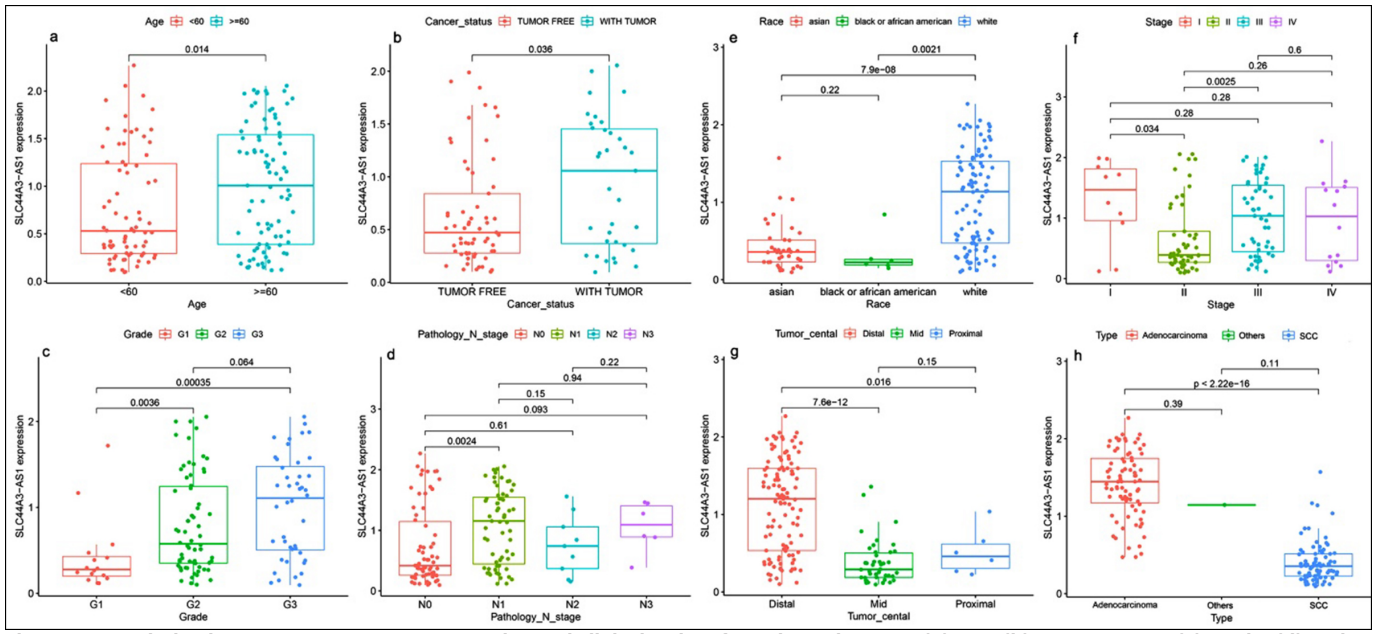

Figure 2: Correlation between *SLC44A3-AS1* expression and clinical traits of esophageal cancer; (a) age; (b) cancer status; (c) grade; (d) pathological N stage; (e) race; (f) stage; (g) tumour central; (h) type.

Finally, it was found that the eRNA *SLC44A3-AS1* also plays an important role in a variety of tumours, including bladder urothelial carcinoma, uveal melanoma, glioblastoma multiforme, brain lower grade glioma, kidney renal clear cell carcinoma, ovarian serous cystadenocarcinoma, rectum adenocarcinoma, and uterine corpus endometrial carcinoma. The survival curves for *SLC44A3-AS1* in the 8 types of tumours are presented in Figure 4. In addition, *SLC44A3-AS1* and its target genes were related to 32 kinds of tumours (Table III).

DISCUSSION

Non-coding RNA (ncRNA) plays a crucial part in the incidence and expansion of many major human diseases.[10] The role of enhancers attracted widespread attention not only because of its role in gene transcription and enhancer function mediation, but also because it overlaps with no risk loci, which are related to diseases.[14] eRNAs (500-5000 bp), which could produce through enhancer transcription,[15] are crucial cis-regulatory components promoting the eukaryotic gene expression.[16] They can stimulate the downstream gene expression by activating enhancer activity or by combining with other protein factors to promote the enhancer-promoter loop formation.[17] The role of eRNAs in tumour treatment is well understood.[9,18] In this study, the prognosis-related eRNAs in esophageal cancer was explored and examined.

It was found that eRNA *SLC44A3-AS1* was up-regulated in esophageal cancer, and patients with high eRNA *SLC44A3-AS1* expression had a weaker prognosis. These findings showed that the expression of *SLC44A3-AS1* was positively linked to esophageal cancer progression. The up-regulation had an obvious correlation with many clinical characteristics, such as tumour status, tumour centre location, grade, *etc*. *SLC44A3-AS1* may be an important factor in predicting the prognosis of esophageal cancer. According to the KEGG pathway enrichment results, *SLC44A3* affected the survival of esophageal cancer patients through the HSV1 infection pathway. The pan-cancer validation results in this study showed that *SLC44A3-AS1* was related to survival in 8 types of tumours (bladder urothelial carcinoma, uveal melanoma, Glioblastoma multiforme, renal clear cell carcinoma, low grade glioma, ovarian serous cystadenocarcinoma, rectum adenocarcinoma, and endometrial carcinoma). In this study, the expression of *SLC44A3-AS1* was associated with its target gene *SLC44A3*, which had similar results across 32 tumour types. These results show that *SLC44A3-AS1* can serve as an independent predictor of esophageal cancer.

In esophageal cancer, the *SLC44A3-AS1* expression was positively related to the *SLC44A3* expression. *SLC44A3* may be the target gene of eRNA *SLC44A3-AS1*. *SLC44A3* is a member of the *SLC44A1-5* (*SLC44* family of solute carriers), which can function as choline transporters.[19] A previous study revealed that *SLC44A3* was differently expressed between normal and uveal melanoma.[20] Hou *et al*. proved that a DNA methylation-driven signature (10MeSig) composed of 10 MDPGs containing *SLC44A3* is an independent predictive factor for the survival of patients with uveal melanoma.[21] In this study, *SLC44A3-AS1* is involved in esophageal cancer progression by the regulation of *SLC44A3*.

To clarify the mechanism by which *SLC44A3-AS1* mediates esophageal cancer occurrence, GO and KEGG enrichment analyses were performed.

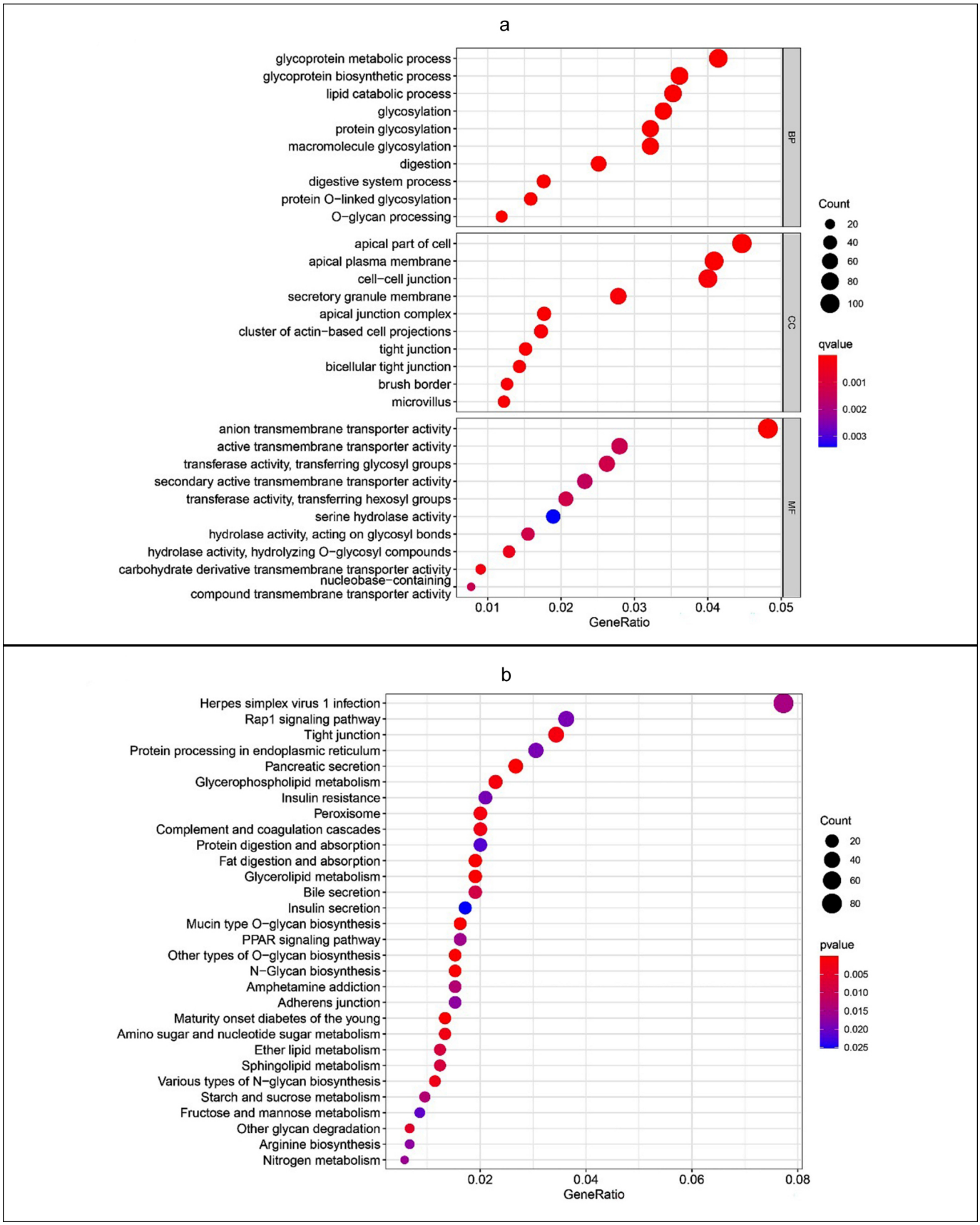

Figure 3: Biological functions of *SLC44A3-AS1*; (a) The top 10 GO terms; (b) The top 30 KEGG pathway enrichment analysis results.

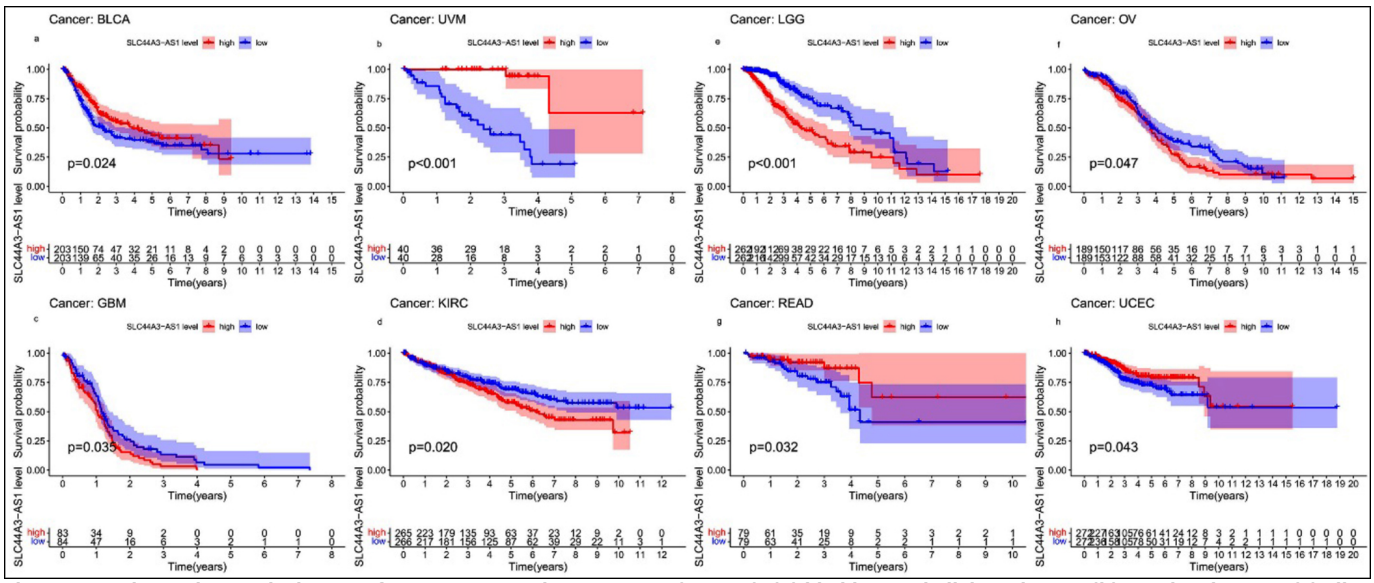

Figure 4: Kaplan-Meier survival curves for *SLC44A3-AS1* in pan-cancer (p<0.05); (a) bladder urothelial carcinoma; (b) uveal melanoma; (c) glioblastoma multiforme; (d) Kidney renal clear cell carcinoma; (e) brain lower grade glioma; (f) ovarian serous cystadenocarcinoma; (g) rectum adenocarcinoma; (h) uterine corpus endometrial carcinoma.

eRNA-associated TFs were mainly enriched in the HSV1 infection pathway, indicating that eRNAs might regulate the HSV1 infection pathway through eRNA-related TFs and suggesting that eRNAs could regulate the HSV1 infection pathway. It is a potential eRNA target for improving the oncolytic virus therapy effect, providing a potential reason for the reported mechanism of using HSV1 to treat premalignant lesions and hence, delay cancer progression.[22]

SLC44A3-AS1 is distinctly related to neutrophil-mediated immunity. lncRNAs are considered important regulators of neutrophils for cancer. lncRNA HOTTIP (homeobox A cluster transcript at the distal tip) stimulates ovarian cancer cells' immune escape by up-regulating programmed death-ligand 1 (PD-L1) in neutrophils.[23] In this study, *SLC44A3-AS1* was strongly associated with tight junction. Tight junction proteins can help maintain cellular integrity.[24] The tight junction barrier imbalance can stimulate cancer cell invasion and metastasis.[25]

CONCLUSION

The eRNA *SLC44A3-AS1* expression was up-regulated in esophageal cancer tissues. An overexpression of *SLC44A3-AS1* also indicated a poor prognosis in esophageal cancer patients. Significant correlations were observed between *SLC44A3-AS1* expression and clinical traits (e.g., central tumour location, cancer status, grade, etc.). Through pan-cancer validation, *SLC44A3-AS1* was found to be related to the survival of eight other types of tumours.

ETHICAL APPROVAL:

This study was approved by the Institutional Ethics Committee of Hubei Cancer Hospital affiliated to Tongji Medical College, Huazhong University of Science and Technology, Hubei, China (Ethical Approval Number: LLHBCH2022YN-045).

PATIENTS' CONSENT

Consent was not obtained as this study analysed data through TCGA data mining, and all patient information was sourced from publicly available databases.

COMPETING INTEREST:

The authors declared no competing interest.

AUTHORS' CONTRIBUTION:

KK, JW, YM: Collected and analysed data, and wrote the manuscript.

JK, SC: Analysed data, worked on data acquisition.

KK, FX: Worked on conception and design, interpretation, critical revision, and final approval.

All authors have approved the final version of the manuscript to be published.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; **71(3)**:209-49. doi: 10.3322/caac.21660.
2. Chen M, Liu PP, Chen YG, Chen ZW, Shen MM, Liu XH, *et al*. Long noncoding RNA FAM201A mediates the radiosensitivity of esophageal squamous cell cancer by regulating ATM and mTOR expression *via* miR-101. *Front Genet* 2018; **9**:611.doi: 10.3389/fgene.2018.00611.
3. He SM, Xu J, Liu XJ, Zhen YS. Advances and challenges in the treatment of esophageal cancer. *Acta Pharm Sin B* 2021; **11(11)**:3379-92.doi: 10.1016/j.apsb.2021.03.008.
4. Allemani C, Matsuda T, Carlo VD, Harewood R, Matz M, Nikšić M, *et al*. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): Analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* 2018; **391(10125)**:1023-75. doi: 10.1016/ S0140-6736(17)33326-3.

5. Arnold M, Rutherford MJ, Bardot A, Ferlay J, Andersson TML, Myklebust TÅ, *et al*. Progress in cancer survival, mortality, and incidence in seven high-income countries 1995-2014 (ICBP SURVMARK-2): A population-based study. *Lancet Oncol* 2019; **20(11)**: 1493-1505. doi: 10.1016/S1470-2045 (19)30456-5.
6. Li YS, Jiang TTF, Zhou WW, Li JY, Li XH, Wang Q, *et al*. Pan--cancer characterization of immune-related lncRNAs identifies potential oncogenic biomarkers. *Nat Commun* 2020; **11(1)**:1000. doi: 10.1038/s41467-020-14802-2.
7. Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. *Cancer Cell* 2016; **29(4)**:452-63. doi: 10.1016/j.ccell.2016.03.010.
8. Xiang YD, Hua QQ. The role and mechanism of long non--coding RNA HOTAIR in the oncogenesis, diagnosis, and treatment of head and neck squamous cell carcinoma. *Clin Med Insights Oncol* 2023; **17**: 11795549231169099. doi: 10.1177/ 11795549231169099.
9. Zhang Z, Lee JH, Ruan H, Ye YQ, Krakowiak J, Hu QS, *et al*. Transcriptional landscape and clinical utility of enhancer RNAs for eRNA-targeted therapy in cancer. *Nat Commun* 2019; **10(1)**: 4562. doi: 10.1038/s41467-019-12543-5.
10. Lee JH, Xiong F, Li WB. Enhancer RNAs in cancer: Regulation, mechanisms and therapeutic potential. *RNA Biol* 2020; **17(11)**:1550-19. doi: 10.1080/15476286.2020.1712895.
11. Qin N, Ma ZJ, Wang C, Zhang EB, Li YC, Huang MT, *et al*. Comprehensive characterization of functional eRNAs in lung adenocarcinoma reveals novel regulators and a prognosis-related molecular subtype. *Theranostics* 2020; **10(24)**: 11264-277. doi: 10.7150/thno.47039.
12. Zhu C, Li L, Zhang Z, Bi MJ, Wang H, Su WY, *et al*. A non--canonical role of YAP/TEAD is required for activation of estrogen-regulated enhancers in breast cancer. *Mol Cell* 2019; **75(4)**: 791-806 e8. doi: 10.1016/j.molcel.2019.06.010.
13. Gonzalez JN, Zweig AS, Speir ML, Schmelter D, Rosenbloom KR, Raney BJ, *et al*. The UCSC genome browser database: 2021 update. *Nucleic Acids Res* 2021; **49(D1)**: D1046-D57. doi: 10.1093/nar/gkaa1070.
14. Chen H, Li CY, Peng XX, Zhou ZC, Weinstein JN, Liang H. A pan-cancer analysis of enhancer expression in nearly 9000 patient samples. *Cell* 2018; **173(2)**:386-99 e12. doi: 10.1016/j.cell.2018.03.027.
15. Andersson R. Promoter or enhancer, what's the difference? Deconstruction of established distinctions and presentation of a unifying model. *Bioessays* 2015; **37(3)**: 314-23.
16. Benoist C, Chambon P. In vivo sequence requirements of the SV40 early promotor region. *Nature* 1981; **290(5804)**: 304-10. doi: 10.1038/290304a0.
17. Lam MTY, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oishi Y, *et al*. Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* 2013; **498(7455)**: 511-5. doi: 10.1038/nature12209.
18. Li P, Lin ZP, Liu QZ, Chen SY, Gao X, Guo WX, *et al*. Enhancer RNA SLIT2 inhibits bone metastasis of breast cancer through regulating P38 MAPK/c-Fos signaling pathway. *Front Oncol* 2021; **11**:743840. doi: 10.3389/fonc.2021.743840.
19. Traiffort E, O'Regan S, Ruat M. The choline transporter-like family SLC44: Properties and roles in human diseases. *Mol Aspects Med* 2013; **34(2-3)**:646-54. doi: 10.1016/j.mam.2012.10.011.
20. Xu BB, Ma RQ, Ren H, Qian Je. Genome-wide analysis of uveal melanoma metastasis-associated LncRNAs and their functional network. *DNA Cell Biol* 2018; **37(2)**:99-108. doi: 10.1089/dna.2017.4015.
21. Hou P, Bao SQ, Fan DD, Yan CC, Su JZ, Qu J, *et al*. Machine learning-based integrative analysis of methylome and transcriptome identifies novel prognostic DNA methylation signature in uveal melanoma. *Brief Bioinform* 2021; **22(4)**: bbaa371. doi: 10.1093/bib/bbaa371.
22. Woo, Y, Reid V, Kelly KJ, Carlson D, Yu Z, Fong Y. Oncolytic herpes simplex virus prevents premalignant lesions from progressing to cancer. *Mol Ther Oncolytics* 2020; **16**:1-6. doi: 10.1016/j.omto.2019.11.003.
23. Shang A, Wang WW, Gu CZ, Chen C, Zeng BJ, Yang YB, *et al*. Long non-coding RNA HOTTIP enhances IL-6 expression to potentiate immune escape of ovarian cancer cells by upregulating the expression of PD-L1 in neutrophils. *J Exp Clin Cancer Res* 2019; **38(1)**:411.doi: 10.1016/j.omto.2019.11.003.
24. Qin ZM, Fang DC, Fang Y. Low expression of occludin: A predictor of poor prognosis in esophageal squamous cell carcinoma. *Int J Clin Exp Pathol* 2017; **10(7)**:7451-9.
25. Kuo KT, Chen CL, Chou TY, Yeh CT, Lee WH, Wang LS. Nm23H1 mediates tumour invasion in esophageal squamous cell carcinoma by regulation of CLDN1 through the AKT signaling. *Oncogenesis* 2016; **5(7)**: e239. doi: 10.1038/oncsis.2016.46.