APOC1 is a Potential Prognostic Biomarker and Correlated With Immune Infiltration in ESCC

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Abstract

Purpose

Esophageal cancer (EC) is the sixth leading cause of cancer death worldwide. Esophageal squamous cell carcinoma (ESCC) is a predominant subtype of EC. Identifying diagnostic biomarkers for ESCC is necessary for cancer practice. Increasing evidence illustrates that apolipoprotein C-1 (APOC1) participates in the carcinogenesis. However, the biological function of APOC1 in ESCC remains unclear.

Patients and methods

We investigated the expression level of APOC1 using TIMER2.0 and GEO databases, the prognostic value of APOC1 in ESCC using Kaplan-Meier plotter and TCGA databases. We used LinkedOmics to identify co-expressed genes with APOC1 and perform GO and KEGG pathway analysis. The target networks of kinases, miRNAs and transcription factors were predicted by gene set enrichment analysis (GSEA). The correlations between APOC1 and immune infiltration were calculated using TIMER2.0 and CIBERSORT databases. We further performed the prognostic analysis based on APOC1 expression levels in related immune cells subgroups via Kaplan-Meier plotter database.

Results

APOC1 was found overexpressed in tumor tissues in multiple ESCC cohorts and high APOC1 expression was related to a dismal prognosis. Multivariate analysis confirmed that APOC1 overexpression was an independent indicator of poor OS. Functional network analysis indicated that APOC1 might regulate the natural killer cell mediated cytotoxicity, phagosome, AMPK and hippo signaling through pathways involving some cancer-related kinases, miRNA and transcription factors. Immune infiltration analysis showed that APOC1 was significantly positively correlated with M0 macrophages cells, M1 macrophages cells and activated NK cells, negatively correlated with regulatory T cells, CD8 T cells, neutrophils and monocytes. High APOC1 expression had a poor prognosis in server immune cells subgroups in ESCC, including decreased CD8+ T cells subgroups.

Conclusion

These findings suggest that increased expression of APOC1 is related to poor prognosis and immune infiltration in ESCC. APOC1 holds promise for serving as a valuable diagnostic and prognostic marker in ESCC.

Introduction

As one of the most significant diagnosed carcinomas, esophageal cancer (EC) ranks the eighth most prevalent cancer type and sixth leading cause of cancer death, with approximately 482,300 new cases and more than 400,000 deaths annually.^{1,2} Esophageal squamous cell carcinoma (ESCC) is a predominant pathological type of EC, accounting for 80% of the world's total EC cancer and up to about

95% in China.^{3,4} The warning symptoms of ESCC often become noticeable when the cancer has developed into the advanced or even metastatic stage because of the muscular and expansive nature of the esophageal.⁵ Despite the development in the diagnosis and comprehensive therapy, the prognosis of EC patients is still poor with a 5-year survival rate of approximately 20%.^{1,6,7} Therefore, it is essential to explore the molecular mechanisms and potential therapeutic targets involved in the occurrence and progression of ESCC.

Apolipoproteins are important lipoproteins that play a crucial role in maintaining the structure of lipoprotein, regulating the interaction between lipoproteins and cellular receptors and modulating the activities of enzymes associated with intravascular lipoproteins metabolism.⁸ Apolipoprotein C-1 (APOC1), located on chromosome 19, is the smallest of all apolipoproteins (only 6.6 kDa) which can be found within HDL or VLDL.⁹ Previous studies have shown that APOC1 contributes to lipid transport and metabolism in serum and plasma by binding to free lipids to form lipoprotein and transport lipids through the lymphatic and circulatory systems.¹⁰ Interestingly, recent studies have shown that the deregulation of APOC1 are detected in some malignancies including breast, gastric and prostate cancer.^{10–12} Su et al. reported that APOC1 overexpressed in prostate cancer (PCa) and APOC1 silencing inhibited cell proliferation, colony formation, cell cycle progression and promoted apoptosis.¹² Furthermore, APOC1 regulates the cell cycle and mediate the proliferation and apoptosis of the cancer cells by controlling the signal pathways for survivin, p21 and capase-3.¹³ Though these findings suggest that APOC1 may be significant in the occurrence and progression of tumor, the expression level and pattern of APOC1 in ESCC are still unknown.

In the present study, we first reviewed the APOC1 expression in different tumors in TIMER2.0 databases and observed that APOC1 overexpressed in tumor tissues in multiple ESCC datasets. We used the Kaplan-Meier plotter and TCGA databases to analyze the prognostic relationship between APOC1 and ESCC. We also explored the biological functions and signal pathways of APOC1 in ESCC and predicted the underlying targets of APOC1, including kinases, miRNAs and transcription factors. In addition, we investigated the correlation between APOC1 and immune infiltration in ESCC using TIMER2.0 and CIBERSORT.

Material And Methods

Data acquisition

The mRNA expression profiles and clinical information of ESCC patients were downloaded from TCGA database (<https://cancergenome.nih.gov/>).¹⁴ The clinical information included overall survival time, overall survival status, age, gender, TNM stage, cancer status and alcohol history. After excluding cases without data on TNM stage and overall survival time, 94 patients were enrolled in the TCGA cohort. In addition, four datasets were obtained from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>):

GSE45168(5 ESCC and 5 normal samples), GSE20347(17 ESCC and 17 normal samples), GSE70409(17 ESCC and 17 normal samples) and GSE23400(53 ESCC and 53 normal samples).¹⁵

Kaplan-Meier plotter database analysis

Kaplan-Meier plotter (<https://kmplot.com/analysis/>) is a web-based tool to assess the correlation between gene expression and prognosis. It can provide subgroups analyses based on different immune cells.¹⁶ The relationship between APOC1 expression and survival in ESCC dataset was analyzed by Kaplan-Meier plotter. In addition, we used the Kaplan-Meier plotter to perform prognostic analysis of APOC1 expression levels in ESCC based on immune cells. The log rank p-value, HR (95% CI), and survival curves were calculated and annotated for each plot.

LinkedOmics database analysis

LinkedOmics database (<http://www.linkedomics.org/admin.php>) is a new online platform that includes multi-omics data from all 32 TCGA Cancer types.¹⁷ We searched for APOC1 co-expressed genes in the ESCC dataset (n = 96) on the basis of the Pearson correlation coefficient. The analysis results were visualized by volcano plots, heat maps, or scatter plots. Gene Set Enrichment Analysis (GSEA) of the LinkInterpreter module was used to perform the analysis of biological process (BP), cell component (CC), molecular function (MF), KEGG pathways, kinase target networks, miRNA target networks and transcription factor target networks. The GSEA program was performed with 500 simulations. If p-value and false discovery rate (FDR) were both less than 0.05, the gene set was considered significantly enriched. The relationship between kinase targets and clinical information in ESCC was analyzed using the LinkFinder module. P-values less than 0.05 were considered statistically significant.

TIMER2.0 database analysis

TIMER2.0 (<http://timer.cistrome.org/>) is a comprehensive database for systematic analysis of immune infiltration in 32 different cancer types from TCGA.¹⁸ We used TIMER2.0 to analyze the expression levels of APOC1 in different cancers and the correlation between APOC1 expression and immune infiltration (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cells) in esophageal cancer.

CIBERSORT database analysis

CIBERSORT (<https://cibersort.stanford.edu/>) is a deconvolution algorithm on the basis of gene expression which infers 22 human immune cells and evaluates the relative scores for each cell type.¹⁹ In order to explore the relationship between APOC1 expression and immune infiltration, we analyzed 106 samples from the GEO cohort (GSE23400), and 53 tumors and 44 normal samples were eligible after filtering with CIBERSORT $p < 0.05$.

Statistical analysis

Student's t-test was employed to perform the different expression analysis between ESCC and normal samples. Receiver operating characteristic (ROC) curve was performed to judge the diagnostic value of APOC1 in ESCC. The ESCC patients were divided into two groups using the optimal threshold evaluated

by X-tile 3.6.1 on the basis of APOC1 expression levels.²⁰ The relationship between APOC1 expression and clinicopathological parameters was analyzed using Chi-square test, Wilcoxon signed-rank test and Kruskal-Wallis test. The overall survival (OS) between APOC1-high and APOC1-low expression groups was compared by KM analysis and log-rank test. Cox proportional hazards model was used to identify the independent indicators associated with OS. The correlation heatmap of 21 types of infiltrating immune cells was performed by “corrplot” package. The violin plot of the 22 immune cells to illustrate the difference between the ESCC and normal tissues was performed by “vioplot” package. The correlation between APOC1 and infiltrating immune cells was evaluated by Spearman correlation. The GraphPad Prism 8, SPSS 26.0 and R version 4.0 were used for statistical analyses. $P < 0.05$ was considered statistically significant.

Results

The mRNA expressions of APOC1

In order to determine the differences of APOC1 expression in tumor and normal tissues, we reviewed the expression levels of APOC1 in different tumors and normal tissues of multiple cancer types via the TIMER2.0 database. The analysis showed that APOC1 expression was significantly higher in most common tumor tissues, such as BLCA (Bladder Urothelial Carcinoma), BLCA (Breast Invasive Carcinoma), COAD (Colon Adenocarcinoma), ESCA (Esophageal Carcinoma), GBM (Glioblastoma Multiforme), HNSC (Head and Neck Squamous Cell Carcinoma), KICH (Kidney Chromophobe), KIRC (Kidney Renal Clear Cell Carcinoma), KIRP (Kidney Renal Papillary Cell Carcinoma), PRAD (Prostate Adenocarcinoma), STAD (Stomach Adenocarcinoma), THCA (Thyroid Carcinoma) and UCEC (Uterine Corpus Endometrial Carcinoma) (Fig. 1A). Then, we further studied the differences of APOC1 expression levels between esophageal squamous cell carcinoma and adjacent normal samples by analyzing four cohorts (GSE23400, GSE23407, GSE45186, GSE70409). The result demonstrated that APOC1 expression was significantly increased within paired tumor samples compared to normal esophageal samples (Fig. 1B-E). We also performed ROC curves to evaluate the diagnostic value of APOC1 upregulation for ESCC using data of GSE23400 (AUC = 0.941, $P < 0.001$) (Fig. 1F).

Association between APOC1 expression and clinicopathological characteristics

The clinicopathological data of 94 ESCC patients obtained from TCGA database were segmented into low or high APOC1 group based on the optimal threshold of the OS. According to Table 1, there was no significant difference in age, BMI, T classification, N classification, cancer status and alcohol history between two groups (all $p > 0.05$). However, high APOC1 expression was significantly associated with gender, M classification, TNM stage (all $p < 0.05$). Besides, we further studied the relationship between APOC1 expression and these different clinicopathological groups by Wilcoxon signed-rank test or

Kruskal-Wallis test. The results showed that APOC1 expression was significantly related to gender and TNM stage (all $p < 0.05$) (Fig. 2).

Table 1
Association between APOC1 expression and the clinical characteristics of ESCC patients in TCGA

Variables	APOC1 expression		χ^2	P*
	High(n = 60)	Low(n = 34)		
Age(year)			0.025	0.873
< 65	45	26		
>=65	15	8		
Gender			12.810	<0.001
Female	3	11		
Male	57	23		
BMI			6.288	0.098
< 18.5	5	1		
>=25	8	9		
18.5–24.	46	21		
unknow	1	3		
T classification			4.359	0.225
T1	2	5		
T2	21	11		
T3	33	17		
T4	4	1		
N classification			0.605	0.895
N0	31	20		
N1	20	9		
N2	3	2		
Unknow	6	3		
M classification			6.176	0.046
M0	56	27		
M1	3	2		
Unknow	1	5		

Variables	APOC1 expression		χ^2	P*
TNM stage			10.105	0.018
I	1	6		
II	37	18		
III	20	7		
IV	2	3		
Cancer status			2.685	0.261
Tumor free	48	24		
With tumor	12	9		
Unknow	0	1		
Alcohol history documented			0.631	0.729
No	14	10		
Yes	45	23		
Unknow	1	1		

Survival outcomes of ESCC patients at high and low APOC1 expression groups

We first used Kaplan-Meier plotter database to study the relationship between APOC1 and prognosis of ESCC and observed that ESCC patients with high APOC1 expression had poor overall survival (OS) (HR = 2.96 (1.27–6.88), P = 0.0089) and recurrence free survival (RFS) (HR = 3.05 (1.17–7.95), P = 0.017) (Fig. 3A-B). Then we downloaded clinical information of 94 ESCC patients from TCGA to further check the prognostic value of APOC1. The KM curve based on the optimal threshold showed that increased expressions of APOC1 were associated significantly with poor OS (p = 0.014) (Fig. 3C). In addition, we explored the independent indicators of the OS in ESCC by performing Cox regression analyses. Univariate analysis confirmed that APOC1 expression, age, gender, TNM stage, N classification and cancer status were significantly associated with the OS in ESCC (all P < 0.05). Following multivariate analysis indicated that APOC1 overexpression, age, N classification and cancer status were independent indicators of unfavourable OS in ESCC (all P < 0.05) (Table 2).

Table 2

Univariate and multivariate analysis of the correlation between clinicopathological characteristic and OS in ESCC patients

Parameters	Univariate analysis				Multivariate analysis			
	HR	95% CI (lower/upper)		P*	HR	95% CI (lower/upper)		P*
APOC1(high vs. low)	2.747	1.2	6.287	0.017	3.452	1.156	10.309	0.026
Age	1.044	1.005	1.084	0.026	1.059	1.009	1.112	0.02
Gender	0.197	0.046	0.855	0.03	0.595	0.117	3.027	0.532
BMI	0.973	0.881	1.074	0.587				
TNM stage	1.921	1.205	3.062	0.006	0.732	0.371	1.446	0.369
T classification	1.5	0.886	2.54	0.131				
N classification	2.392	1.298	4.408	0.005	2.739	1.247	6.014	0.012
M classification	2.821	0.958	8.305	0.06				
Cancer status (with tumor vs. tumor free)	4.525	2.146	9.543	< 0.001	4.422	1.864	10.492	0.001
Age began smoking	0.978	0.909	1.052	0.552				
Alcohol history	2.58	0.775	8.582	0.122				

GO and KEGG pathway analysis of co-expressed genes with APOC1 in ESCC

In order to gain the insight of APOC1 biological meaning in ESCC, correlation analyses were performed using the function module of LinkedOmics. The mRNA sequencing data of ESCC patients was chosen for analysis. According to the volcano plot (Fig. 4A), 1064 genes (dark red dots) significantly positively correlated with APOC1 whereas 185 genes (dark green dots) significantly negatively correlated with APOC1 (false discovery rate, FDR < 0.01). The top 50 significant genes positively and negatively correlated with APOC1 based on the Pearson correlation coefficient were illustrated by heat maps (Fig. 4B-C). APOC1 expression had a strong positive association with the expression of APOE ($r = 0.932$, $p = 1.17E-42$), TYROBP ($r = 0.860$, $p = 6.89E-29$) and APOC2 ($r = 0.836$, $p = 5.39E-26$), etc. The GO term annotation and KEGG pathway analysis by gene set enrichment analysis (GSEA) are presented in Fig. 5. The GO analysis results illustrated that APOC1 co-expressed genes were primarily related to adaptive immune response, cellular defense response and lymphocyte mediated immunity in the biological process(BP), MHC protein complex, immunological synapse and protein complex involved in cell adhesion in the cellular component(CC), antigen binding, immunoglobulin binding and cytokine receptor activity in the molecular function(MF). The KEGG pathway analysis showed enrichment in the natural killer cell mediated cytotoxicity, phagosome, cell adhesion molecules (CAMs), AMPK signaling pathway,

axon guidance and hippo signaling pathway, ect. These above results suggest that a widespread impact of APOC1 on the immune response.

Kinase, miRNA and transcription factor targets of APOC1 in ESCC

To further explore the underlying targets of APOC1 in ESCC, we predicted the kinase, miRNA, transcription factor targets of APOC1 co-expressed genes by GSEA. The top 5 most significant targets of kinases, miRNAs and transcription factors were shown in Table 3. The enrichment of kinases was mainly related to LCK (LCK proto-oncogene, Src family tyrosine kinase), SYK (spleen associated tyrosine kinase), LYN (LYN proto-oncogene, Src family tyrosine kinase), ITK (IL2 inducible T-cell kinase) and HCK (HCK proto-oncogene, Src family tyrosine kinase). Additionally, we found that LYN and HCK were significantly overexpressed in tumor tissues by analyzing the GSE23400 dataset (all $p < 0.05$). And increased expression of HCK was significantly associated with poor OS (Cox regression test, $p = 4.592e-02$) and pathological stage (Kruskal-Wallis test, $p = 1.242e-02$) in ESCC patients using the dataset of LinkedOmics. The enrichment of miRNAs was primarily related to MIR-26A, MIR-26B, MIR-142-3P, MIR-519E, MIR-202 and MIR-515-3P. The network of transcription factors was associated with V\$ELF1_Q6, V\$IRF_Q6, V\$STAT3_01, STTTCRNTTT_V\$IRF_Q6 and V\$PU1_Q6.

Table 3
The kinase, miRNA and transcription factor targets of APOC1 in ESCC (LinkedOmics)

Enriched Category	Gene Set	Leading Edge Number	P Value	FDR
Kinase Target	Kinase_LCK	23	0	0
	Kinase_SYK	14	0	0
	Kinase_LYN	18	0	0
	Kinase_ITK	5	0	0
	Kinase_HCK	9	0	0
miRNA Target	TACTTTGA,MIR-26A,MIR-26B	102	0	0.014
	ACACTAC,MIR-142-3P	39	0	0.017
	GGCACTT,MIR-519E	40	0	0.017
	ATAGGAA,MIR-202	41	0	0.019
	AGGCACT,MIR-515-3P	28	0	0.019
Transcription Factor Target	V\$ELF1_Q6	64	0	0.003
	V\$IRF_Q6	81	0	0.003
	V\$STAT3_01	9	0	0.005
	STTTTCRNTTT_V\$IRF_Q6	61	0	0.005
	V\$PU1_Q6	57	0	0.013
Abbreviations: Leading Edge Number: the number of leading edge genes; FDR: false discovery rate.				

Correlation between APOC1 expression and immune infiltration

Previous analyses showed that tumor infiltration is associated with the prognoses in various cancers. Therefore, we tried to explore the relationship between APOC1 expression and immune infiltration. We first accessed the correlation of APOC1 expression with immune infiltration levels in esophageal cancer by TIMER, and the results indicated that APOC1 expression was positively associated with the infiltration levels of B cells ($R = 0.3$, $P = 1.13E-4$), CD4 + T cells ($R = 0.343$, $P = 9.08E-6$), neutrophils ($R = 0.292$, $1.69E-4$), macrophages ($R = 0.576$, $P = 0$), and dendritic cells ($R = 0.331$, $P = 1.89E-5$) in esophageal cancer (Fig. 6). Then, we analyzed the fractions of 22 types of immune cells between paired tumor and adjacent non-tumorous tissues in the GEO cohort (GSE23400) by CIBERSORT. After filtering with CIBERSORT $p < 0.05$, 53 tumors and 44 normal samples were eligible (Fig. 7A). We performed the

correlation heat map of the 21 types of immune cells and eosinophils was excluded as it was not significantly expressed in ESCC and normal tissues. The correlation heatmap revealed that M0 macrophages cells negatively correlated with CD8 T cells and neutrophils. M1 Macrophages cells had a significantly positive correlation with activated NK cells (Fig. 7B). The violin plot of the 22 immune cells illustrated the difference between the normal tissues and ESCC. As shown in Fig. 7C, activated NK cells, M0 macrophages cells and M1 macrophages cells infiltrated more compared with normal control samples, while CD8 T cells, follicular helper T cells, regulatory T cells, monocytes and neutrophils infiltrated less in ESCC. Correlation analyses revealed that APOC1 had significantly positive correlation with M0 macrophages cells ($R = 0.662$), M1 macrophages cells ($R = 0.401$) and activated NK cells ($R = 0.328$), while APOC1 was negatively correlated with regulatory T cells ($R=-0.313$), CD8 T cells ($R=-0.356$), neutrophils ($R=-0.416$) and monocytes ($R=-0.455$) (Table 4).

Table 4
Significant correlation between APOC1 and infiltrating immune cells estimated by Spearman correlation coefficients

Symbol	correlation	pvalue
Macrophages M0	0.662	1.62E-13
Macrophages M1	0.401	4.59E-05
NK cells activated	0.328	1.03E-03
T cells regulatory (Tregs)	-0.313	1.81E-03
T cells CD8	-0.356	3.41E-04
Neutrophils	-0.416	2.24E-05
Monocytes	-0.455	2.80E-06

Prognostic analysis of APOC1 expressions in ESCC based on immune cells

To further investigate whether the expression of APOC1 in ESCC affected the prognosis partly on the cause of immune infiltration, we performed a prognosis analysis based on the APOC1 expression levels of ESCC in immune cells subgroups via the Kaplan-Meier plotter. The results indicated that the high expression of APOC1 of ESCC in decreased B-cells, decreased CD4 + memory T-cells, decreased CD8 + T-cells and enriched mesenchymal stem cells subgroups had poorer prognosis (Fig. 8). These above analyses suggest that high APOC1 may affect prognoses in part due to immune infiltration.

Discussion

APOC1, the smallest apolipoprotein (only 6.6 KDa), is a component of both triglyceride-rich lipoproteins and high-density lipoproteins.⁹ Previous studies have shown that APOC1 contributes to lipid transport

and metabolism in serum and plasma.^{10,13} Interesting, emerging researches highlight the relationship between APOC1 and cancers. The upregulation of APOC1 was previously reported to be associated with advanced tumor progression and dismal prognosis in patients with pancreatic cancer, colorectal cancer, lung cancer and papillary thyroid cancer.^{21–24} Nevertheless, the expression level and pattern of APOC1 in ESCC has still not been investigated. In this study, we conducted bioinformatic analysis using public data to explore the potential functions of APOC1 in ESCC.

We initially used TIMER2.0 to review the differential expression of APOC1 between tumor and normal tissues in various types of cancers and observed increased expression of APOC1 in esophageal cancer. Then, we further found that APOC1 overexpression occurred in multiple cohorts of ESCC and the mRNA expression level of APOC1 displayed favorable diagnostic value for ESCC accessed by ROC curves. In addition, we evaluated the clinical implication of APOC1 overexpression in ESCC and observed that APOC1 overexpression was correlated with gender and TNM stage. Meanwhile, Kaplan-Meier plotter analysis illustrated that ESCC patients with high APOC1 expression had unfavorable OS and RFS (all $P < 0.05$). We further confirmed the prognostic value of APOC1 by analyzing clinical information of 94 ESCC patients from TCGA. The KM analysis showed that increased expressions of APOC1 were associated significantly with poor OS ($p < 0.05$) and the multivariate analysis indicated that APOC1 overexpression was an independent prognostic factor of unfavorable OS in ESCC. These above results suggest that APOC1 may serve as a valuable diagnostic and prognostic markers of ESCC.

To probe the potential mechanisms of APOC1 upregulation in ESCC, we analyzed APOC1 co-expressed networks using GSEA. GO enrichment analysis revealed that APOC1 was mainly associated with adaptive immune response, cellular defense response and lymphocyte mediated immunity. These results suggest that APOC1 may participate in the immune response of ESCC. Moreover, the KEGG pathway analysis primarily included natural killer cell mediated cytotoxicity, phagosome, AMPK signaling pathway and hippo signaling pathway. AMPK is a crucial energy-sensing enzyme maintaining cellular energy homeostasis. It can be activated by cellular stress which increases the AMP/ATP ratio and leads to the production of metabolic poisons, the progression of hypoxia, glucose starvation, etc.²⁵ A research reported that metformin exerted its anti-proliferative effects on ESCC cells through inducing the activation of AMPK pathway, suggesting that AMPK pathway may play a crucial role in the inhibition of the growth of ESCC.²⁶ The primary functions of Hippo pathway are restricting tissue growth and modulating cell proliferation, differentiation and migration. Recent studies suggest that deregulation of hippo pathway plays an important role in cancer initiation and progression.²⁷ Gao et al reported that miR-31 could promote ESCC tumorigenesis by inhibiting LATS2 expression via the hippo pathway.²⁸ These above researches are consistent with our results, suggesting that APOC1 may participate in tumorigenesis and progression of ESCC through these signaling pathways.

To further gain insight into the regulators potentially responsible for APOC1 overexpression, we investigated networks of kinases, miRNAs and transcription factors. We observed that APOC1 in ESCC was related to the network of kinases including LCK, SYK, LYN, ITK and HCK. Additionally, LYN and HCK

showed significantly overexpressed in ESCC tissues in the GSE23400 dataset (all $p < 0.05$). And HCK overexpression was associated with the poor prognosis and pathological stage in ESCC patients (all $p < 0.05$). In fact, HCK is the important paralog of LYN. HCK plays an important role in the regulation of innate immune responses, including neutrophil, monocyte, macrophage and mast cell functions, phagocytosis, cell survival and proliferation, cell adhesion and migration. Excessive HCK activation enhances cell proliferation and survival by associating with oncogenic fusion proteins and functionally interacting with receptor tyrosine kinases.^{29,30} Consistent with previous reports, we speculate that APOC1 may regulate immune system process via HCK kinase in ESCC and further studies are needed to verify this hypothesis.

IRF family, a broad class of cytokines elicited on challenge to the host defense, are important for mobilizing immune responses to pathogens.³¹ Moreover, statistical data indicated that IRF-2 expression was tightly correlated with progression of ESCC.³² Reduction of the ratio of IRF-1/IRF-2 might lead to the enhancement of tumorigenicity of ESCC cells.³³ STAT3 plays important roles in the progression of various cancers by regulating the proliferation, invasion, angiogenesis and immune surveillance evasion.^{34,35} Previous researches have shown that the STAT3 pathway was activated in some ESCC cells and STAT3 overexpression indicated the poor prognosis of ESCC patients who had undergone curative resection.³⁶ Our study suggests that IRF and STAT3 are potential regulators of APOC1 and that APOC1 may act through these factors to regulate the immune response of ESCC.

Previous research illustrated that miRNA which normally involve in post-transcriptional regulation of gene expression can contribute to human carcinogenesis.³⁷ Thus, we tried to explore the potential regulatory miRNAs of APOC1. The miRNAs associated with APOC1 in our study participate in the process of carcinogenesis. In the current study, miR-26a and miR-26b inhibit ESCC cell proliferation through suppression of c-MYC pathway.³⁸ Previous studies showed that the upregulation of miR-519 enhanced radiosensitivity of ESCC cells and facilitated ESCC cell apoptosis via targeting PI3K/AKT/mTOR signaling pathway. The low expression level of miR-519 indicated poor prognosis of ESCC patients.³⁹ The expression of miR-202 was reported to aberrantly decreased in ESCC and the down-regulation of miR-202 was associated with the metastasis of tumor.⁴⁰ Consistent with above literature evidence, deregulation of these miRNAs may partly contribute to APOC1 overexpression in ESCC.

Tumor-infiltrating immune cells (TIICs) are crucial parts of the tumor microenvironment, which are associated with patient outcome and tumor behavior.⁴¹ We first used the TIMER2.0 database to uncover the relationship between APOC1 expression and TIICs in esophageal cancer. The results indicated that APOC1 expression had a significant positive relationship with the infiltration levels of B cells, CD4 + T cells, neutrophils, macrophages and dendritic cells in esophageal cancer. Then we further studied the roles of immune infiltration in ESCC by using CIBERSORT to analyze the GSE23400 dataset. The CIBERSORT analysis indicated that the increased infiltration of activated NK cells, M0 macrophages cells, M1 macrophages cells, and the decreased infiltration of CD8 T cells, follicular helper T cells, regulatory T cells, monocytes and neutrophils were associated with the occurrence and development of ESCC.

Analyses on the role of immune infiltration in human cancers typically focus on T cells, and the CD8 + T cells are one of the major anti-tumor immune cells in the tumor microenvironment. It has been reported that CD8 + T cells cooperate with CD4 + T cells to promote better prognosis of ESCC patients.⁴² Tregs are heterogeneous and have multiple context-dependent functions that are still not well-characterized. It may play a dual role in carcinogenesis, initially inhibiting inflammation that leads to tumorigenesis, but later suppressing anti-tumor immune responses.⁴³ Besides T cells, the innate immune system including NK cells and macrophages also role importantly in the tumor microenvironment. Several studies have shown that the infiltration of NK cells significantly increases in ESCC. And IL-6 or IL-8 secreted by primary ESCC cells impairs the function of NK cells via the STAT3 signaling pathway.⁴⁴ Tumor-associated macrophages (TAMs) have been reported to play a crucial role in inflammatory tumor microenvironment. TAM infiltration is related to poor responses to chemotherapy and unfavorable prognosis in ESCC.⁴⁵ The above research findings combined with our study indicate that the infiltrating immune cells play crucial roles in ESCC and should be the highlight of future studies. Moreover, we analyzed the correlation between the immune cells in ESCC. Our results showed that M1 macrophages cells were positively correlated with activated NK cells, and M0 macrophages cells were negatively related to CD8 T cells and neutrophils. The potential mechanisms of these correlations require further studies.

To uncover the association between APOC1 overexpression and the immune infiltration in ESCC, we preformed the correlation analysis. Our results indicated that APOC1 was significantly positively correlated with M0 macrophages cells, M1 macrophages cells and activated NK cells, and negatively correlated with regulatory T cells, CD8 T cells, neutrophils and monocytes ($|R| > 0.3$, $P < 0.05$). The correlation results are consistent with the previous studies of infiltration situation in ESCC and we speculate that APOC1 may stimulate M0 macrophages cells, M1 macrophages cells and activated NK cells or inhibit regulatory T cells, CD8 T cells, neutrophils and monocytes to participate in the tumorigenesis and progression of ESCC. Further experimental studies are needed to verify this assumption.

Furthermore, we performed the prognostic analysis of APOC1 expression levels in ESCC based on immune cells. The results showed that high APOC1 expression level had an unfavorable prognosis in the decreased B-cells, decreased CD4 + memory T-cells, decreased CD8 + T-cells, and enriched mesenchymal stem cells subgroups. These analyses suggest that the overexpression of APOC1 may partly affect the prognoses of ESCC patients due to immune infiltration, especially the decrease of CD8 + T cells.

In summary, this present study promotes our understanding of the association between APOC1 and ESCC and suggests that the upregulation of APOC1 may have an impact on the unfavorable prognosis of ESCC through the mechanism of immune infiltration. But this study also has some limitations. The study was preformed based on the data downloaded from public databases and there were few survival data of ESCC patients. It is essential to conduct additional experiments to verify our results and further clarify the role of APOC1 in ESCC.

Conclusion

In summary, APOC1 expression increased significantly in ESCC compared to normal esophageal tissues. The APOC1 overexpression was related to dismal prognosis and infiltrating immune cells. In addition, the prognostic analysis of APOC1 expression levels in ESCC based on immune cells indicated that elevated APOC1 may partly affect the prognoses of ESCC patients due to immune infiltration. Therefore, APOC1 may serve as a potential diagnostic and prognostic biomarker for esophageal squamous cell carcinoma. Further pathogenesis mechanisms and clinical studies are needed to prove the absoluteness of these findings.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during and/or analysed during the current study are available in the TCGA repository, <https://cancergenome.nih.gov/> and GEO repository, <https://www.ncbi.nlm.nih.gov/geo/>.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JX and ML designed this study. JX, ZW, ZL, XR and LX performed data collection and analysis. JX, YL, RH and AL contributed to manuscript preparation. SL, XL, ZH revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

APOC1 is significantly upregulated at the mRNA levels in ESCC. (A) Human APOC1 expression levels of different tumor types from TCGA database were detected by TIMER. (B-E) APOC1 expression between esophageal squamous cell carcinoma and adjacent normal tissues in four GEO cohorts (GSE23400, GSE20347, GSE45168, GSE70409). (F) Validation of APOC1 increase for ESCC using ROC curve. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

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Figure 2

Association between APOC1 expression and clinical parameters in ESCC. (A) Clinical stage. (B) Gender. (* $P < 0.05$, *** $P < 0.001$)

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Figure 3

Kaplan-Meier survival analysis of ESCC patients. (A) OS survival curves in Kaplan-Meier plotter databases. (B) RFS survival curves in Kaplan-Meier plotter databases. (C) Kaplan-Meier curves of OS comparing the high and low APOC1 expression grouped by X-tile plots cutoff point in TCGA cohort (n=94). OS: overall survival; RFS: relapse-free survival.

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Figure 4

Differentially expressed genes in correlation with APOC1 in ESCC (LinkedOmics). (A) Correlations between APOC1 and genes differentially expressed in ESCC were estimated by Pearson correlation coefficients. (B-C) Heat maps of positively and negatively expressed genes associated with APOC1 in ESCC (TOP 50). Red indicates positive correlation and blue indicates negative correlation. The darker the color, the stronger the correlation.

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Figure 5

Enrichment analysis of APOC1 in ESCC. The significantly enriched GO annotations and KEGG pathways of APOC1 co-expression genes in ESCC were analyzed by GSEA and the bubble diagrams display the enrichment results. (A-C) The top 10 significant terms of GO analysis (BP/CC/MF). (D) KEGG pathway analysis. NES: normalized enrichment score; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: biological process; CC: cellular component; MF: molecular function.

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Figure 6

APOC1 expressions were significantly positively correlated with B cells, CD4+ T cells, neutrophils, macrophages, and dendritic cells in esophageal cancer(ESCA) in TIMER database.

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Figure 7

Evaluation and visualization of infiltrated immune cells between tumor and adjacent normal tissues with CIBERSORT $p < 0.05$ for all eligible samples. (A) Fractions of immune cells in 44 normal and 53 tumor samples in GEO database(GSE23400). (B) Correlation heat map of 21 types of immune cells. The blue and red respectively represent negative and positive correlations. (C) Comparisons of 22 types of immune

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Figure 8

Comparison of Kaplan-Meier survival curves of ESCC patients exhibiting high and low APOC1 expression based on immune cells subgroups (Kaplan-Meier plotter). (A-H) Relationships between APOC1 of

different infiltrating immune cells subgroup and prognoses in ESCC.

Figure 8

Comparison of Kaplan-Meier survival curves of ESCC patients exhibiting high and low APOC1 expression based on immune cells subgroups (Kaplan-Meier plotter). (A-H) Relationships between APOC1 of different infiltrating immune cells subgroup and prognoses in ESCC.