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ORIGINAL RESEARCH

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The expression and clinical significance of apolipoprotein C1 in cervix cancer

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Abstract

Cervical cancer (CC) is the fourth largest cancer affecting the global survival of women. Apolipoprotein C1 (APOC1) has been implicated in the pathogenesis and progression of various malignancies. Here, we investigated the expression and clinical significance of APOC1 in patients with CC. Gene expression data and the corresponding clinical features of CC were downloaded from the Gene Expression Omnibus (GEO) database. The expression levels of APOC1 were evaluated by the R software package. An enzyme-linked immunosorbent assay (ELISA) was used to detect the serum levels of APOC1 expression in 120 CC patients and 60 healthy controls. The diagnostic value of serum APOC1 level for CC was then evaluated by receiver operating characteristic curve analysis. The expression levels of APOC1 in CC tissue were significantly higher than that in adjacent tissues ($p < 0.001$). ELISA further confirmed that serum levels of APOC1 were significantly higher in CC patients than im normal controls ($p < 0.001$). With an area under the curve (AUC) of 0.826 (95% confidence interval: 0.766–0.888; $p < 0.05$), serum APOC1 can serve as a reliable molecular biomarker for differentiating CC patients from healthy controls. Moreover, we found that elevated serum APOC1 levels were significantly associated with poor clinical stage ($p = 0.025$) and positive lymph node metastasis ($p = 0.002$). In addition, serum APOC1 level was positively correlated with clinical stage ($p < 0.01$) and lymph node metastasis ($p < 0.05$) in CC patients. Thus, our results indicated that serum APOC1 was significantly higher in tissues and serum from CC patients and represents a potential novel biomarker for the diagnosis of CC.

Keywords

Cervical cancer; Apolipoprotein C1; Diagnostic markers

1. Introduction

Cervical cancer (CC) is the fourth largest cancer affecting the global survival of women after breast, colorectal and lung cancer [1]. Each year, more than half a million women are diagnosed with CC and approximately 604,127 die globally [2]. In most cases, the cause of this disease is high-risk subtypes of the human papillomavirus (HPV) [3]. Almost 90% of CC deaths occur in underdeveloped low- and middle-income areas that lack organized screening and vaccination programs for HPV [4]. Currently, the main treatment strategies for patients with CC include surgery, radiotherapy and chemotherapy [5]. While immune therapy holds broad prospects for CC, the survival rate remains disappointingly low [6]. More importantly, a major obstacle for diagnosis at present is the long preclinical stage that can span decades [3]. Therefore, identifying novel biomarkers for early diagnosis and accurate prediction to detect precancerous CC lesions before they develop into advanced cancers is of immense significance.

Apolipoprotein C1 (APOC1), located on chromosome 19, is the smallest lipoprotein (only 6.6 kDa) and participates in lipid transport and metabolism [7]. APOC1 is a secreted protein that exists in chylomicrons, interacts with APOCE, promotes the exchange of various lipoproteins in the plasma and plays an important role in plasma lipid balance [8]. In addition, APOC1 affects lipoprotein metabolism by mediating several key enzymes, including the activation of lecithin-cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL), and as a potent inhibitor of transferases and cholesteryl ester transfer protein (CETP) [9, 10]. Previous studies have shown that apolipoprotein C1 is highly expressed in the liver and macrophages, but expressed at much lower levels in the lungs, kidneys, brain, spleen, skin, adipose tissue and central nervous system [11]. APOC1 has been reported to be involved in the occurrence and development of many diseases, including tumors, atherosclerosis, diabetes, Alzheimer's disease and inflammation [12–14].

There is an increasing body of evidence supporting the fact that APOC1 is involved in the progression of cancer. In a marker phase I trial, APOC1 was found to be expressed at high levels in patients in the late stages of lung cancer; this was the case at both the mRNA and protein levels. However, the

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prognostic value of APOC1 has yet to be determined in samples of serum [15]. Previous studies have also demonstrated the pro-carcinogenic effects of APOC1; for example, APOC1 was found to promote cell proliferation during the pathogenesis of prostate cancer; other research showed that serum levels of APOC1 protein increased with disease progression [16, 17]. Furthermore, APOC1 is overexpressed in glioma cell and tissues and is known to affect cell proliferation and metastasis by regulating epithelial-mesenchymal transition and the signal transducer and activator of transcription 3 (STAT3) pathway [18]. Some researchers have reported that the upregulation of APOC1 is an effective and novel diagnostic and prognostic biomarker for breast cancer [19, 20].

However, no previous research as investigated the clinical significance of serum APOC1 in CC. In this study, we determined whether APOC1 can be used as a novel diagnostic biomarker for CC. We detected the serum levels of APOC1 and determined the expression levels of APOC1 protein in cervical tissues from patients with CC and normal controls. Then, we analyzed the association of APOC1 levels with a range of clinical characteristics in CC patients. Our study demonstrates that APOC1 represents a useful diagnostic factor for CC.

2. Materials and methods

2.1 Study participants

A total of 120 CC patients and 60 healthy controls were enrolled in this study at Changsha Hospital for Maternal and Child Health Care. CC was confirmed by pathological examination, and the clinical information of all cancer patients was registered according to medical interview records. The healthy controls were identified by physical examination.

2.2 Bioinformatics analysis

The gene expression profile datasets used in this study (GSE7803, GSE39001, GSE63514 and GSE7950) were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The R package (V3.6.3, http://r-project.org/) was used to analyze APOC1 expression in each GEO dataset. In addition, we obtained APOC1 protein expression data from the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) [21].

2.3 Serum APOC1 detection by ELISA

After 8 hours of fasting, 3 mL of venous blood were obtained from each subject. Then, the serum level of APOC1 was determined with a commercially available Human APOC1 ELISA Kit (H0465c, Elabscience Biotechnology, Wuhan, China) in a clinical laboratory under standard operating protocols.

2.4 Statistical analysis

Statistical analyses were performed in R version 3.6.2 software, SPSS (IBM SPSS Inc., Chicago, IL, USA) and GraphPad version 8.1(GraphPad Prism Software Inc., San Diego, CA, USA). The levels of APOC1 in CC and normal samples were compared by the *t*-test, and the Chi-square test was used to investigate the association between APOC1 expression and clinicopathological features. To further assess the diagnostic abilities of APOC1, we performed receiver operating characteristic (ROC) curve analysis to determine the area under the curve (AUC) and determine sensitivity and specificity. A two-tailed $p < 0.05$ was considered to be statistically significant.

3. Results

3.1 The expression of APOC1 mRNA in CC and normal tissue

First, we investigated the expression of *ApoC1* mRNA levels in normal tissue from the HPA database and found that *APOC1* mRNA was overexpressed in both the liver and adrenal glands but was expressed at low levels in all other tissues (Fig. 1A). To further investigate the expression levels of *ApoC1* mRNA in CC and normal samples, we downloaded the GSE7803, GSE39001, GSE63514 and GSE7950 datasets from the GEO database. Then, we analyzed the expression of *APOC1* in multiple CC datasets and found that *APOC1* mRNA was significantly overexpressed in CC samples ($p < 0.001$) (Fig. 1B–E).

3.2 The expression of APOC1 protein in CC and normal tissue

We obtained expression data of APOC1 protein from the HPA (Human Protein Atlas) database which provides immunohistochemical and protein expression data relating to the tissue and cellular distribution of 26,000 human proteins. We detected high levels of APOC1 protein in placenta, skin, duodenum, small intestine and colon tissues; in other tissues, APOC1 was expressed at low levels or not at all (Fig. 2A). Immunohistochemical staining revealed APOC1-positive signals in CC tissues (Fig. 2C) but not in normal cervical tissues (Fig. 2B).

3.3 Serum levels of APOC1 in cervical tumors were higher than in control tissues

To evaluate the differences in APOC1 serum levels between CC patients and controls, we used ELISA to determine the serum levels of APOC1 in 120 CC patients and 60 healthy controls. Analysis showed that the serum levels of APOC1 in CC patients were significantly higher than those in controls ($p < 0.0001$) (Fig. 3A). To further assess whether APOC1 could be used as a biomarker to diagnose CC, we performed receiver operating characteristic (ROC) curve analysis to determine diagnostic ability; results are given in Fig. 3B. The AUC for APOC1 was 0.826 (95% Confidence interval (CI): 0.766–0.888) and the cut off value was 155.345 ng/mL with a sensitivity of 0.675 and a specificity of 0.850.

3.4 The association of APOC serum levels with clinicopathological features

According to the serum levels of APOC1, 120 patients were divided into two groups to evaluate the relationships between serum APOC1 levels and clinicopathological characteristics. As shown in Table 1, high levels of APOC1 were positively correlated with clinical stage ($p = 0.017$) and tumor lymph node metastasis ($p = 0.035$). However, there were no significant associations between the serum levels of APOC1 and patient

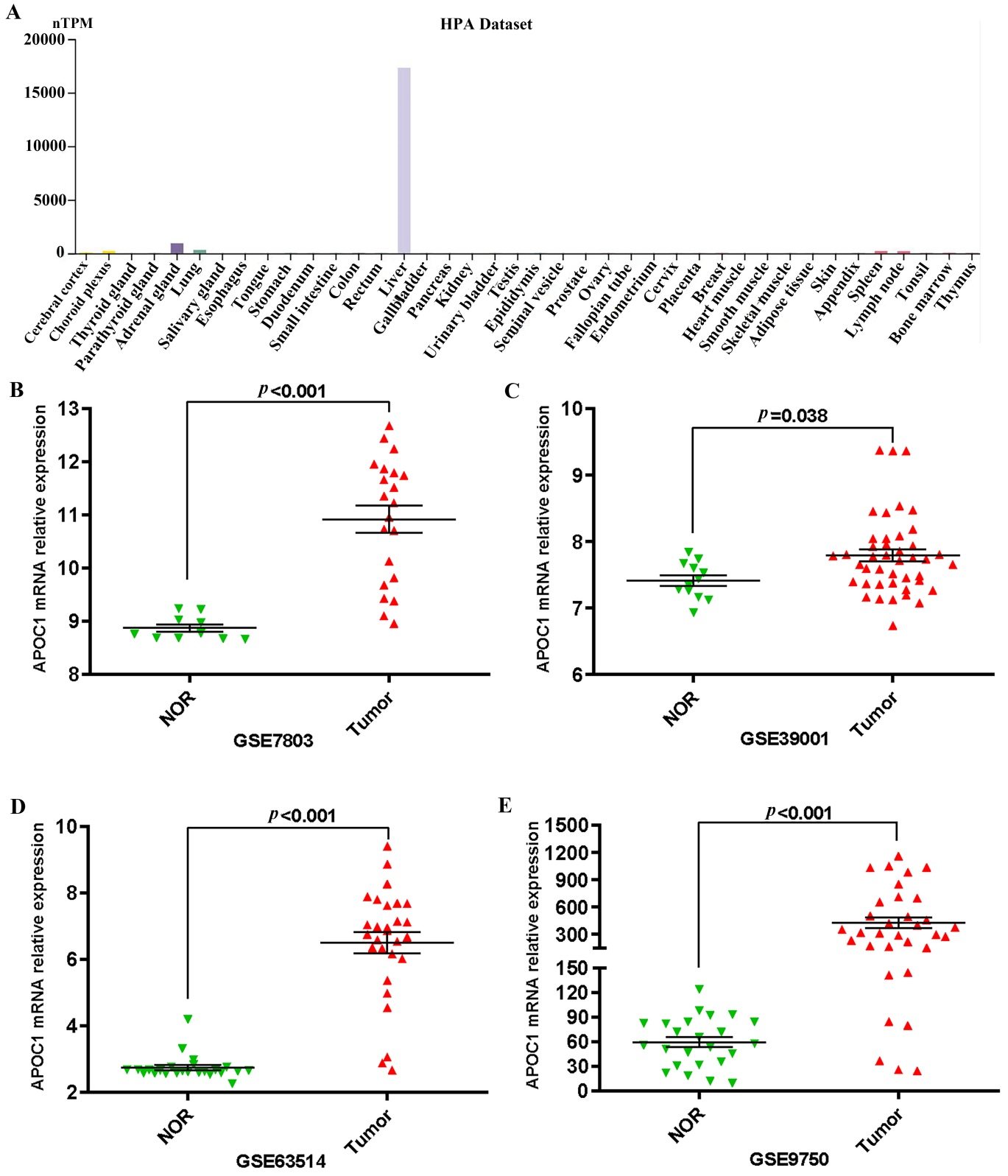

FIGURE 1. The expression of *APOC1* mRNA in CC and normal tissue (data acquired from the HPA and GEO database). (A) The expression of *ApoC1* mRNA in various tissues, (B–E) the expression of *ApoC1* mRNA in CC and normal cervical tissue (data from GSE7803, GSE39001, GSE63514 and GSE7950 datasets, respectively). HPA: Human Protein Atlas; APOC1: Apolipoprotein C1; nTPM: normalized transcript per million; NOR: normal tissue.

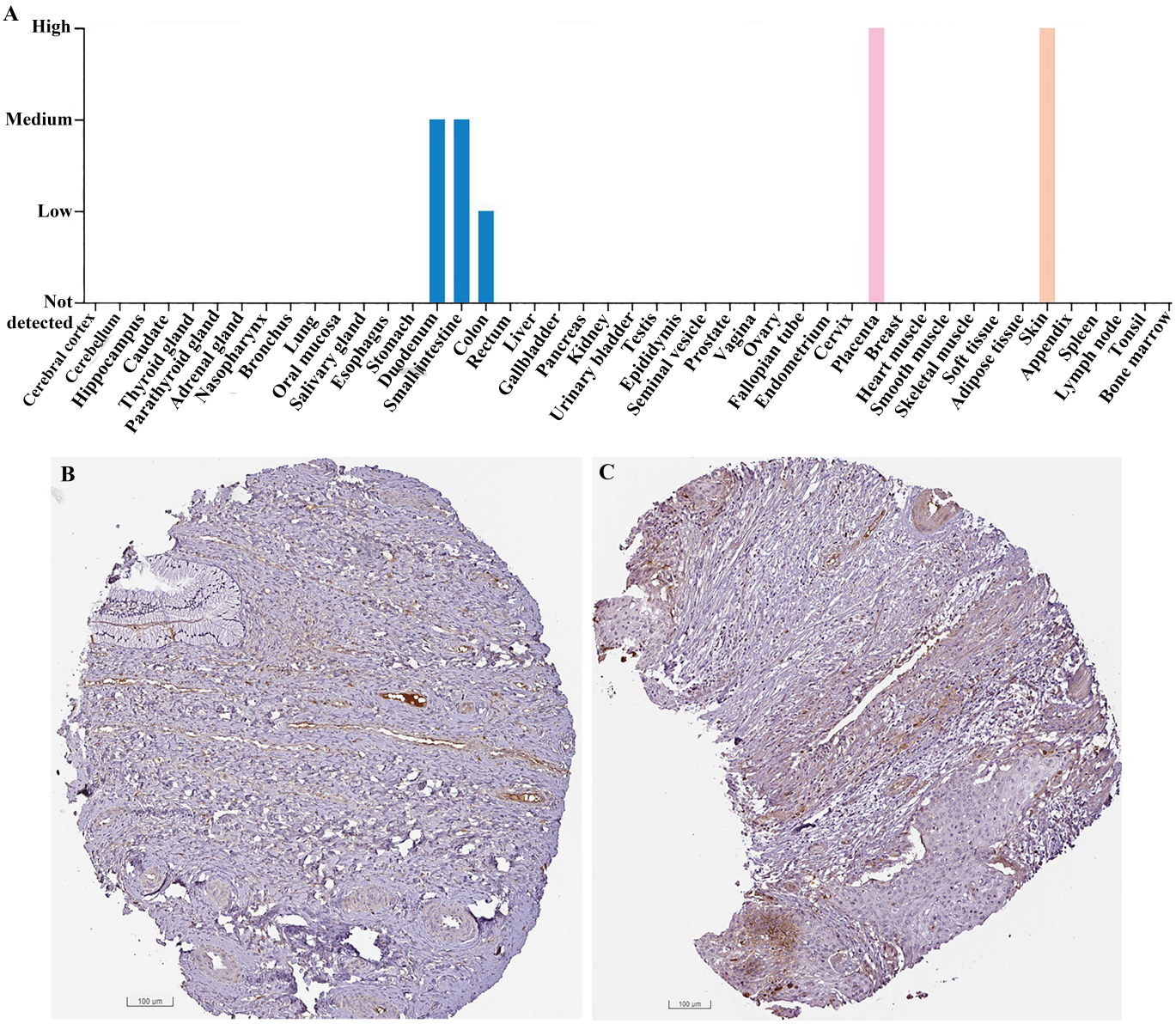

FIGURE 2. The expression of APOC1 in CC and normal tissue (data acquired from the HPA database). (A) The expression of APOC1 protein in a range of normal and healthy tissues, (B) normal cervical tissue from a 36-year-old female, and (C) CC tissue from a 39-year-old female with squamous cell carcinoma of the cervix.

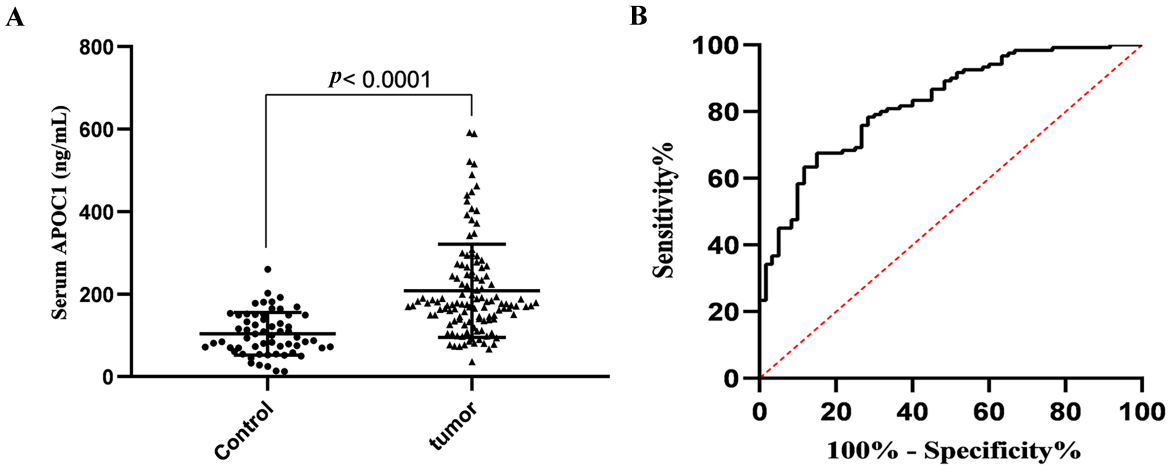

FIGURE 3. The serum expression levels and diagnostic abilities of APOC1. (A) The expression levels of APOC1 in serum and (B) the diagnostic capability of APOC1. APOC1: Apolipoprotein C1.

TABLE 1. The association between serum levels of APOC1 and clinicopathological features.

Characteristics	Total number of case (120)	APOC1 expression		*p* value
		Low (39)	High (81)	
Age (yr)				
≤50	69	22	47	0.867
>50	51	17	34	
Tumor size (cm)				
≤4 cm	57	21	36	0.334
>4 cm	63	18	45	
Clinical stages				
I–IIA	55	24	31	0.017
IIB–IV	65	15	50	
Differentiation				
Poorly	30	7	23	0.216
Moderate-well	90	32	58	
Lymph node metastasis				
No	94	35	59	0.035
Yes	26	4	22	

APOC1: Apolipoprotein C1.

age, tumor size and tumor differentiation ($p > 0.05$). Moreover, the Mann-Whitney U test indicated that serum APOC1 level was significantly increased in the poor clinical stage group ($p = 0.025$) and the positive LNM group ($p = 0.002$) when compared with those in their respective control groups. Overall, these results show that the serum levels of APOC1 are associated with the progression of CC and may represent an ideal biomarker for diagnosis.

4. Discussion

Due to the high-risk of infection by HPV sub-types, CC has gradually occurred in younger women and become a significant form of cancer affecting women's health over recent years. Despite preventive examinations of the vagina and the analysis of cervical smears represent significant diagnostic tools, the diagnosis of CC is often confirmed at an advanced stage in a highly heterogeneous manner [22]. Therefore, novel serum biomarkers for the diagnosis and treatment of CC are urgently needed. Previous studies showed that APOC1 represents an important factor for maintaining the normal physiological activities of the human body [23]. APOC1 plays a key role in the assembly and metabolism of lipoproteins, including chylomicrons, very-low-density lipoproteins (VLDL), and high-density lipoproteins (HDL), and represents an exchangeable apolipoprotein between different classes of lipoprotein [7]. Several lines of evidence have highlighted the effect of APOC1 in carcinogenesis and tumor development; however, little is known of its potential association with CC.

In this study, we found that APOC1 was highly expressed in CC patients at both the mRNA and protein level, as determined by bioinformatics data mining and comprehensive tissue analysis; these findings concur with previous literature [24–28]. Furthermore, we determined the diagnostic ability of APOC1 as a biomarker by plotting an ROC curve after the detection of serum APOC1 levels in clinical patients (AUC: 0.826); this results indicated that APOC1 possesses high diagnostic accuracy for screening CC patients from healthy controls. APOC1 is a 6.6 kDa secreted protein in the plasma and is synthesized predominantly by the liver [11]. In a previous study, Yi *et al.* [24] reported that serum APOC1 may represent a potential biomarker for the diagnosis and prognosis assessment of gastric cancer. Similarly, another study also showed that APOC1 could act as a candidate serum diagnostic marker for breast cancer [19]. The serum level of APOC1 is influenced by APOC1-producing organs, especially the liver; therefore, Ko *et al.* [15] considered that serum APOC1 may not represent a good prognosticator for lung cancer. It is undeniable that serum APOC1 has been reported to be highly expressed in several types of cancer and that APOC1 was related to a worse prognosis and more advanced tumor progression in patients [25]. Our analysis also showed that the positive correlation between high levels of APOC1 and tumor clinical stage and tumor lymph node metastasis in CC patients may suggest that APOC1 is involved in tumor progression. Given that the CC patients included in the present study formed a heterogeneous group of different histotypes, our results may be biased in terms of certain types of patients; this needs to be verified by larger studies carried out in multiple centers.

The mechanism of how APOC1 can influence the survival and malignant phenotype of cancer cells has yet to be elucidated. However, previous studies have shown that APOC1 may prevent cellular apoptosis and promote cell proliferation in pancreatic cancer [26]. Ren *et al.* [27] reported that APOC1 could promote the proliferation, migration and invasion of renal cell carcinoma *via* the Wnt3a signaling pathway.

Similarly, the siRNA-mediated knockdown of the (Reelin) RELN pathway and its downstream signals, including the VLDL receptor known to bind to APOC1, was shown to increase cell motility and aggressiveness in pancreatic cancer [29]. In terms of lung cancer, previous studies found that an increase of APOC1 may promote the proliferation of lung cancer cells by stimulating an increase of IL-6 levels; this effect was also shown to be associated with proliferation in lung cancer cell lines [15]. Another study indicated that APOC1 can regulate the ferroptosis pathway and promote the M2 to M1 transformation of macrophages in hepatocellular carcinoma [12]. Zheng *et al.* [30] also showed that APOC1 reduced ferroptosis by inhibiting the Kelch-like ECH-associated protein 1 (KEAP1)/ NF-E2-related factor 2 (NRF2) pathway and induced ferroptosis resistance by increasing the expression of cystathionine beta-synthase (CBS), a protein known to play a key role in the occurrence and development of glioblastoma. Furthermore, APOC1 has been shown to promote tumor metastasis by regulating the EMT signaling pathway [18, 20]. Collectively, these data suggested that APOC1 is associated with tumorigenesis and progression, although this needs further validation in more extensive research.

5. Conclusions

In summary, our study showed that APOC1 is overexpressed in CC and closely related to clinical characteristics. Consequently, the serum levels of APOC1 can be used as a potential novel biomarker for CC.

AVAILABILITY OF DATA AND MATERIALS

All data used and analyzed in the study are available from the corresponding author Sen Mao on reasonable request.

AUTHOR CONTRIBUTIONS

SM—conceived the study and reviewed manuscript. TF—collected specimens, carried out the ELISA and wrote the article. YZ—conducted data analysis. All authors approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All participants provided informed consent forms, and the ethics committee of the Changsha Hospital for Maternal & Child Health Care approved our study protocol (Reference number: EC-20230721-01).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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