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Acetyltransferase P300 Inhibits the Proliferation, Invasion, and Migration of Esophageal Cancer via Survivin Acetylation

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Abstract

Background: Esophageal cancer is one of the primary death causes leading by cancer in the world, which is high morbidity and mortality. Epigenetic acetylation modification participates in and regulates the proliferation, invasion, and metastasis of various tumor cells, and the acetylation modification of tumor proteins involved by acetyltransferases may be one of the important mechanisms of esophageal carcinogenesis. The aim of this study was to investigate the correlation of acetyltransferase P300 and Survivin acetylation in esophageal cancer pathogenesis and its molecular mechanism. **Methods:** Fifty-five cases of esophageal cancer tissues and adjacent cancer tissues were collected, Survivin and P300 protein expression was measured by immunohistochemistry (SP) and protein blotting (Western Blot); Survivin acetylated protein levels were measured by coimmunoprecipitation (Co-IP); bioinformatics predicted the relationship between P300 and Survivin as the substrate, and fluorescence immunohistochemistry (IF) to verify the localization and expression of Survivin and P300 in esophageal cancer tissues; the correlation of Survivin acetylation, P300 and clinical cases characteristics was analyzed by statistics. P300 siRNA sequences were structured and transfected into EC109 cells. P300 protein expression and Survivin acetylated protein levels were determined by Co-IP. Cell viability was determined by the MTT assay, Scratch healing and Transwell chamber assay examined cell migration and invasion ability. **Results:** Survivin and P300 protein expression was significantly increased in human esophageal cancer tissues and EC109 cells. The Survivin protein was acetylated in esophageal cancer tissues and EC109 cells, and its protein acetylation rate was significantly increased; bioinformatics predicted that the acetyltransferase P300 could catalyze the acetylation of Survivin as a substrate, and the fluorescence immunohistochemistry confirmed that both Survivin and P300 simultaneously showed a high expression state in cancer

tissues; Survivin acetylation and P300 expression; Survivin acetylation and P300 were closely related with esophageal cancer stage, tissue differentiation and lymph node metastasis. The vitro experiments showed that P300 RNA interference in esophageal cancer cells can significantly reduce the Survivin protein acetylation level, while inhibiting the survival, migration and invasion capacity of EC109 cells. **Conclusion:** P300 has a correlation with Survivin acetylation in the lespathological process of esophageal cancer, P300 may be an important upstream molecule of Survivin acetylation and has an important potential value in the diagnosis and treatment of esophageal cancer.

Keywords

P300, Acetyltransferase, Survivin, Acetylation Modification, Esophageal Cancer

1. Introduction

Esophageal cancer is a common gastrointestinal tumor in the world, which has a high morbidity and mortality rate [1]. Although surgery, chemotherapy and radiotherapy have somewhat improved the prognosis of some patients with esophageal cancer, the overall survival rate remains unsatisfactory [2]. Modern medical research has found that the protein acetylation modification in epigenetics plays an important role in the occurrence and development of tumors [3] [4]. The acetylation modification of some key proteins in the body can change their function, leading to the occurrence and development of some tumors.

Survivin is an apoptosis inhibitory that is highly expressed in various tumors and suppresses tumor cell apoptosis and leads to abnormal cell proliferation [5] [6] [7] [8] [9]. Previous studies have shown that Survivin is acetylated in the esophageal tissues, and is closely associated with the proliferation, invasion, and metastasis of esophageal cancer [10]. However, the specific mechanisms of Survivin acetylation promote occurrence and development of esophageal cancer which remains unclear. Acetylation modification of proteins needs the catalysis of acetyltransferases, and the protein function after acetylation modification is altered and strengthened, participating in tumor genesis and development [11]. P300 is an important member of the acetyltransferase family, which can catalyze the acetylation of various histones and non-histones, and then participate in the occurrence and development of the tumor [12]. In this study, we mainly investigated the correlation and its molecular mechanism by examining the level of P300 expression and Survivin acetylation in the esophageal cancer pathogenesis.

2. Material

2.1. Tissues

55 specimens esophageal cancer tissues and adjacent cancer tissues were selected in the Department of thoracic Surgery of the Affiliated Hospital of Chengde

Medical College from January 2019 to May 2021; General information: There were 11 females, 44 males; ≥60 aged 42 examples, <60 aged 13 examples; TNM stage: I stage 7 examples, II stage 32 examples, III stage 16 examples; Tissue differentiation: well-differentiated 7 examples, moderately differentiated 40 examples and poorly differentiated 8 examples; 14 patients with lymph node metastasis and 41 examples without lymph node metastasis. Esophageal cancer EC109 cells and normal esophageal epithelial HEEC cells were provided by the Central Laboratory of the Affiliated Hospital of Chengde Medical University.

2.2. Main Reagent

Survivin monoclonal antibody, Acetylated-Lysine antibody and P300 antibody were purchased from Abcam company (USA); A/G agarose beads from CST company (USA); immunohistochemical kit from Hebei Beibo Experimental Products Co., Ltd. (Hebei, China). Alexa Fluor 488-conjugated sheep anti-rabbit fluorescent antibody and ATTO 594-conjugated sheep anti-mouse fluorescent antibody were purchased from Hebei Beibo Experimental Products Co., LTD. (Hebei, China).

3. Methods

3.1. Immunohistochemical Method (SP)

Tissues were sliced, xylene waxing, soaked in gradient alcohol, driplet with 3% H_2O_2 and placed at room temperature for 10 min. Citrate buffer high temperature antigen repair after membrane breaking; Slice cooling then closed with goat serum. After washing, primary antibodies were placed in a wet box and incubated for 4˚C overnight. Secondary antibodies were incubated at room temperature for 30 min. DBA coloration, then counterstained with hematoxylin.

3.2. Western Blot

Protein was extracted and concentration determined; total protein was separated by gel electrophoresis and transferred to PVDF membrane by semi-dry transfer. The med milk powder was closed and incubated with primary antibody for 40 min. After rinsing, the secondary antibodies were incubated for 30 min. After rinsing, the luminescence reaction was performed in the dark chamber and exposed into slices.

3.3. Co-Immunoprecipitation (Co-IP)

Proteins were extracted, and A/G agarose beads 5 μl and Survivin monoclonal pure antibody 5 μl were mixed, supplemented with 2× lysis buffer to a total volume of 450 μl, 400 μl of the supernatant was taken into a centrifuge tube, and a 4˚C fixed mixer was coprecipitated for 12 h. It was centrifuged at 4˚C and the supernatant was discarded. The A/G agarose beads were washed in 500 μl with 1× lysis buffer. The supernatant was discarded at 3000 r/min and centrifuged for 3 min. The supernatant was discarded at 3000 r/min and centrifuged for 3 min.

After the last wash, discard the supernatant. 1× Lysis buffer of 35 μl and equal volumes of 2× SDS loading buffer were mixed and boiled for 8 min. 3000 r/min, centrifuged; SDS-PAGE electrophoresis, electrotransfer, blocking, added primary antibody (Acetylated-Lysine antibody 1:2000, GAPGH antibody 1:1000) was incubated in 4˚C in 3% BSA overnight. It was developed after adding the secondary antibodies.

3.4. Bioinformatic Analysis and Prediction

The bioinformatics software Acetylation Set Enrichment Based (ASEB) predicts that the Survivin protein is catalyzed as a substrate [13]. ASEB can not only predict the acetylation status of the target protein, but also predict the acetyltransferase that catalyzes the acetylation modification and the possible lysine sites.

3.5. Fluorescent Immunohistochemistry (IF Method)

Tissue sections were baked in a 60˚C incubator for 20 min, section were dewaxed by xylene and ethanol, and antigen repair occurred after PBS cleaning; 5% goat serum blocked at room temperature for 30 min, shake off the excess liquid; Primary antibody was added, 4˚C overnight, 37˚C for 45 min, and washed 3 times in PBS. Fluorescence-labeled secondary antibody was added, incubated at room temperature for 1 h, and washed three times in PBS; The DAPI was stained for 10 min, washed 3 times in PBS; Anti-fluorescence quencher capping sheet and photographed.

3.6. Cells Were Cultured and Transfected

EC109 cells and esophageal epithelial HEEC cells were cultured in conventional medium. The P300 siRNA and the negative control strain of EC109 cells were transfected with the respective siRNA by using a liposome-mediated assay. Experimental group: P300 siRNA group (si-P group), negative control group: P300 siRNA empty carrier group (si-N group), blank control group: blank cell group (NC group).

3.7. MTT Experiment

Cells in the log growth phase, were made into a single-cell suspension. After cell counting, cells were seeded at 8×10^3 cells per well in 96-well plates, adding 100 μl per well, and cultured until cell density up to 80% transfection. After 12, 24, 48 and 72 h, 5 mg/ml of MTT solution of 30 μl was added per well to each plate at each time point, and the incubation was continued for 4 h. Centrifugation, abandon the upper clear. Dimethylsulfoxide was added to 150 μl and shaken at 80 r/min level for 5 to 10 minutes. The absorbance value (570 nm) was measured by a microplate meter.

3.8. Scratch Healing Experiment

Cells were plated with a single-cell suspension, and 5×10^3 cells per well were seeded into 6-well plates at 100 μl per well. In conventional culture, when the

cells were covered with the bottom of the hole, the fluid transfer gun head was made with a vertical plate surface scratch, and the culture medium was washed off the falling cells. 2 ml of serum-free medium was added to each well for a further 48 h of culture and the width of the scratches was measured under an inverted microscope.

3.9. Transwell Compartment Invasion Assay

After transfection, cells were made into a single cell suspension and cells were counted. The Matrigel glue melts into a liquid state early. The 50 mg/L Matrigel 1:8 dilution solution was coated with a polycarbonate microporous filter membrane at 8 μm aperture at the bottom of the Transwell chamber and polymerized into glue at 37˚C overnight. The chamber was UV-sterilized for 2 h, and the residual Matrigel gel solution was removed and moistened with serum-free F12 medium for 1 h at 37˚C. The Transwell chamber was placed in a 24-well plate, the chamber was removed, and 600 μl of medium was added outdoors. In chambers, 200 μl cell suspension was cultured without serum medium, and six samples were repeated in each group. The 24-well plates were routinely cultured for 48 h. PBS buffer was rinsed three times and cells in the inner layer of the microporous membrane were erased. Formaldehyde was fixed and counterstained with crystal violet. Wash the PBS for 3 times, change the liquid each time; dry the liquid with a cotton swab, and count the number of penetrating membrane cells under the microscope.

3.10. Statistical Analysis

The SPSS21.0 statistical analysis software was used. Data with normal distribution and equal variance are expressed by mean ± standard deviation, Count data were used in χ^2 analysis. Paired samples were analyzed using a t-test, and means between multiple groups were compared using one-way ANOVA. Statistical significance was determined as $P < 0.05$.

4. Results

4.1. Survivin and P300 Protein Expression in Esophageal and Adjacent Tissues

Immunohistochemistry results showed that the positive expression rate of Survivin in esophageal cancer tissue was 83.64% (46/55), higher than the beside the carcinoma tissue 23.64% (13/55); The positive expression rate of P300 in esophageal cancer tissue was 78.18% (43/55), which was higher than beside the carcinoma tissue 25.45% (14/55), and the difference was statistically significant ($P < 0.05$) (**Figure 1(A)**). Western Blot results showed that the expression of Survivin in 55 esophageal cancer tissues was (71.25 ± 2.56)%, higher than adjacent tissues (16.22 ± 2.05)%; The protein expression level of P300 in cancerous tissues was (56.35 ± 1.78)%, higher than that in adjacent tissues (11.04 ± 1.52)%, and the difference was statistically significant ($P < 0.05$) (**Figure 1(B)**).

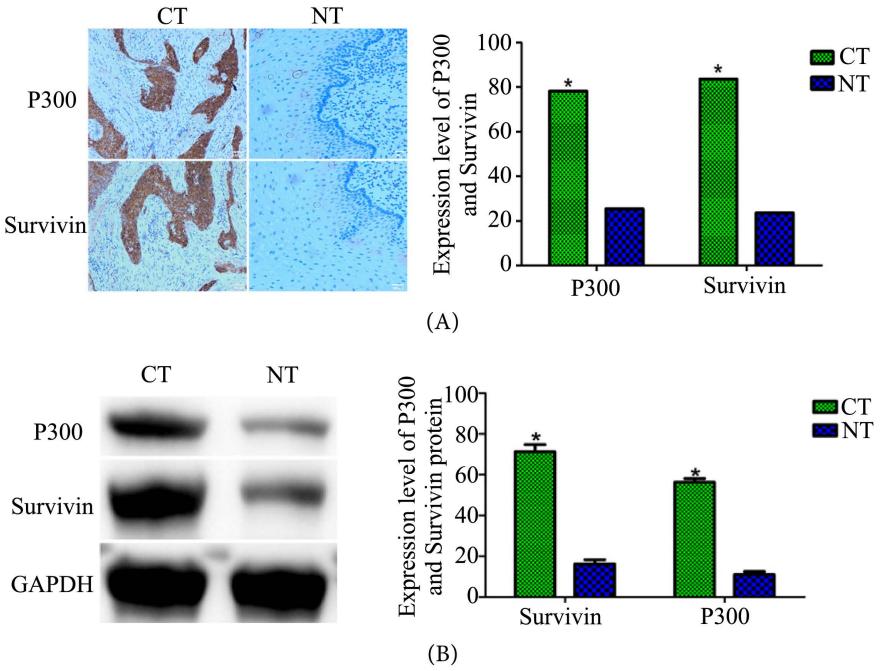

Figure 1. (A) Survivin and P300 protein expression in cancerous tissues and adjacent tissues (CT: Cancerous Tissues, NT: Adjacent tissues); (B) Survivin and P300 protein expression in esophageal cancer tissues and adjacent tissues (CT: Cancerous Tissues, NT: Adjacent tissues).

4.2. Protein Acetylation Levels of Survivin and P300 in Esophageal and Adjacent Tissues

The immunoprecipitation experiments showed that P300 protein expression in 55 cases was (64.09 ± 4.94)% of esophageal cancer tissues, higher than in adjacent tissues (12.26 ± 1.77)%, with a statistically significant difference ($P < 0.05$). Survivin protein was acetylated, and its acetylation rate was (61.59 ± 2.15)% in cancer tissue, which was significantly higher than that in adjacent tissue (10.42 ± 1.26)%, with a significant difference ($P < 0.05$) (**Figure 2**).

4.3. P300 Catalyzes Its Acetylation Prediction by Using Survivin as a Substrate

The bioinformatics software Acetylation Set Enrichment Based (ASEB) predicted that the acetyltransferase P300 can catalyze the Survivin protein as a substrate and predict the five lysine (K) sites where the Survivin may be acetylated (**Table 1**).

4.4. Survivin and P300 Protein Expression in Esophageal Cancer Tissue (IF)

Fluorescent immunohistochemistry showed that the Survivin and P300 protein simultaneously showed a high expression status in esophageal cancer tissues, and both were expressed mainly in the cytoplasm and partly in the nucleus (**Figure 3**).

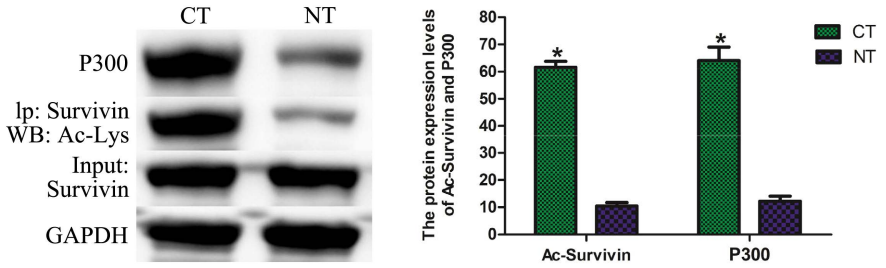

Figure 2. Survivin acetylation and P300 protein levels in cancerous and normal tissues (CT: NT: adjacent tissue).

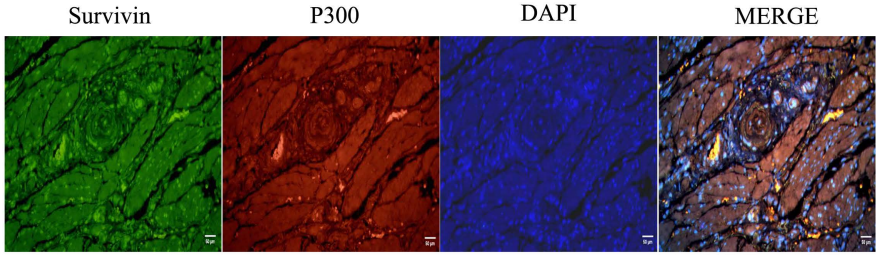

Figure 3. Immunofluorescence histochemical expression of Survivin and P300 proteins in esophageal cancer tissue (400, ruler 50 μm) Note: Survivin green fluorescence, P300 red fluorescence, DAPI cell nucleus staining, MERGE is the triplot fusion image.

Table 1. Prediction of acetylation by the acetyltransferase P300 using Survivin as a substrate.

KAT	Site	Sequence	*P*-value
P300	67	GRSLALGRKPSELGRPV	0.0247
P300	274	LGNDKCPLKRSRELKP	0.0316
P300	32	RLVYSKCLKYCPLSTVP	0.1047
P300	32	PSAYCNHDKCYPSKCS	0.2341

Notes: The smaller the *P*-value, the greater the chance of acetylation at the site.

4.5. Correlation between P300 and Survivin Protein Acetylation and Clinical Case Characteristics in Esophageal Cancer Tissue

The protein acetylation level of Survivin protein was associated with TNM stage, tissue differentiation, and lymph node metastasis of esophageal cancer ($P < 0.05$), regardless of sex and age. Positive P300 expression was closely associated with TNM stage, differentiation degree, and lymph node metastasis ($P < 0.05$), and it had no correlation with sex or age (Table 2). It is correlated between the Survivin protein acetylation and P300 positive expression in esophageal cancer tissues, its correlation coefficient is $r = 0.527$, and they showed a positive correlation (Table 3).

4.6. Expression of P300 and Survivin Acetylated Proteins in Esophageal Cancer Cell Lines

The Western blot results showed that P300 was highly expressed in esophageal

Table 2. Relationship between survivin protein acetylation and P300 and clinical case characteristics in esophageal cancer tissues (*n*, %).

Item	Number	Survivin Acetylation	*P*	P300 (positive)	*P*
Sex					
Male	44	51.35 ± 1.27		36	
Female	11	49.79 ± 2.04	0.251	5	0.155
Age					
≥60	42	63.42 ± 2.45		34	
<60	13	60.76 ± 1.56	0.138	7	0.434
TNM					
I Stage	7	32.14 ± 1.54*		2*	
II Stage	32	50.75 ± 2.04**		25**	
III Stage	16	70.58 ± 3.34**	0.012	14**	0.021
Histodifferentiation					
well-D	7	33.18 ± 1.52*		3*	
Moderately-D	40	50.64 ± 2.78**		31**	
Poorly-D	8	73.15 ± 3.46**	0.004	7**	0.018
Lymphatic metastasis					
Have	14	66.76 ± 4.57*		3*	
None	41	44.31 ± 2.18**	0.027	38**	0.021

Notes: Compared with *, ** $P < 0.05$.

Table 3. Correlation between Survivin protein acetylation and P300 expression in esophageal cancer tissues (*n*).

P300	Ac-Survivin		χ^2	*P*	*r*
	Positive	Negative			
Positive	28	13	4.615	0.032	0.527
Negative	5	9			

cancer EC109 cells, but it showed low expression in normal esophageal epithelial HEEC cells. The Survivin protein acetylation level in EC109 cells of esophageal cancer was significantly higher than the normal esophageal epithelial HEEC cells (Figure 4).

4.7. Verification of P300 Downregulation by Si-RNA Interference

The resultant si-RNA-P300 contained a green fluorescently labeled tag, and the green fluorescence expression was indicated after successful transfection in EC109 cells (Figure 5). The P300 mRNA and protein expression was decreased significantly in the si-P group via Rt-qPCR and Western Blot assays (Figure 6).

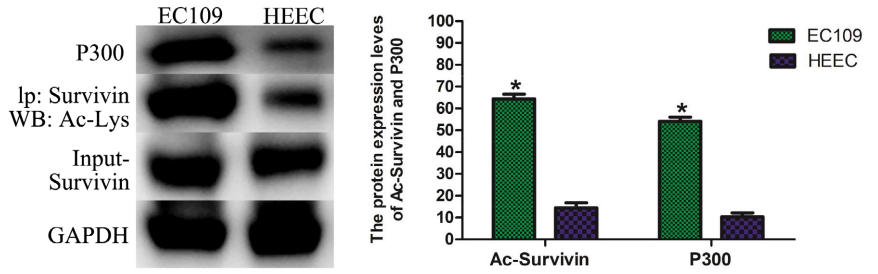

Figure 4. P300 protein expression and Survivin protein acetylation levels in human esophageal cancer cell lines.

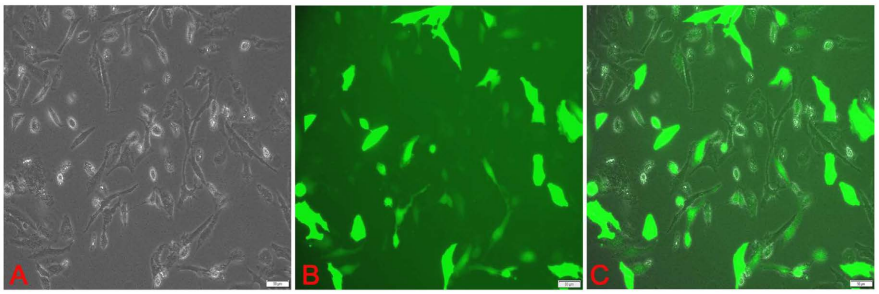

Figure 5. Fluorescence microscopy results of RNA interfering P300 transfected EC109 cells ((A): Bright field; (B): Fluorescence microscope; (C): Superposition of (A) and (B)).

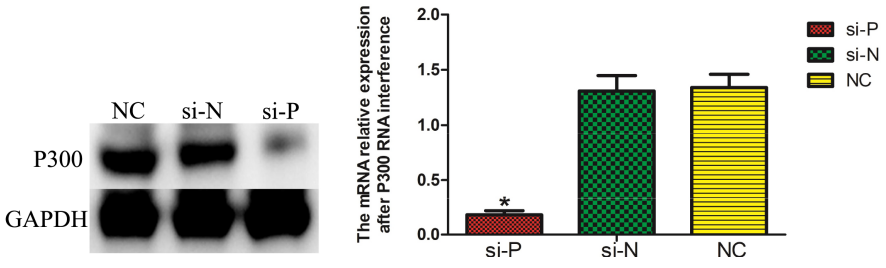

Figure 6. The mRNA and protein expression after P300 RNA interference (Group si-P: P300 siRNA group si-N: negative control group, NC: blank control group).

4.8. Effect of Survivin Acetylation after RNA Interference with P300 in EC109 Cells

Co-IP showed that Survivin acetylation decreased significantly after P300 downregulation by RNA interference in si-P group ($P > 0.05$), but no difference in si-N and NC groups ($P < 0.05$). The results suggest that inhibition of P 300 expression could significantly reduce Survivin acetylation levels in EC109 cells (**Figure 7**).

4.9. Effect of P300 Downregulation on the Proliferation, Invasion, and Migration Ability of EC109 Cells

The results of the MTT experiments showed that the absorbance values of EC109 cells were significantly decreased in the si-P group when compared to the si-N and NC groups (**Figure 8(A)**). The results of Transwell chamber experiment showed that the number of perforated cells was significantly decreased in the

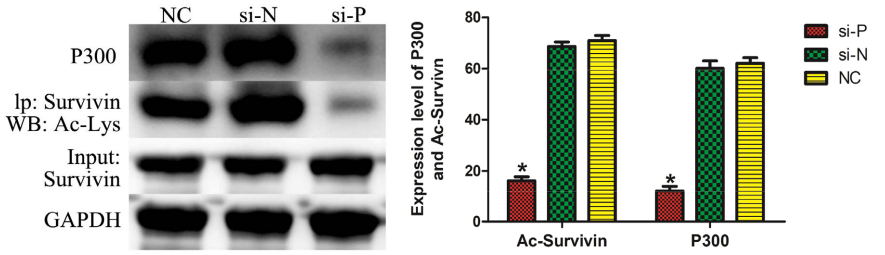

Figure 7. The Survivin acetylation level after RNA interference P300 (si-P: P300 siRNA group si-N: negative control group, NC: blank control).

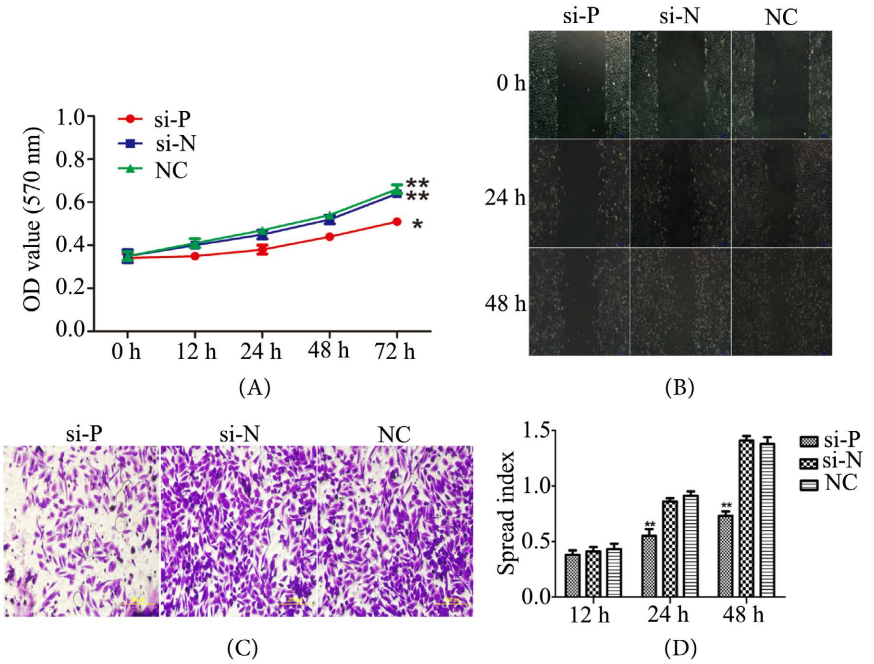

Figure 8. Effects of P300 downregulation on the proliferation, invasion and migration of EC109 cells (si-P: P300 siRNA group si-N: negative control group NC: blank control group) Note: (A): EC109 cells proliferation decreased (B), (D): EC109 cells migration decreased; (C): EC109 cells invasion decreased.

si-P group, which inhibited the invasion capacity of EC109 cells. The difference between the si-N and NC groups was significant ($P < 0.05$) (**Figure 8(B)** and **Figure 8(D)**). The results of the cell scratch healing assay showed that EC109 cells had scratch healing very slowly and decreased migration ability in the si-P group (**Figure 8(C)**).

5. Discussion

Esophageal cancer is a common gastrointestinal malignancy in esophageal epithelial tissues [14]. The early symptoms of esophageal cancer is not obvious, the discovery is mostly late manifestations, poor prognosis [15]. Survivin is the strongest member of the anti-apoptotic proteins of the family of apoptosis inhibitors, which is widely expressed in a variety of malignancies [16]. Survivin can regulate the tumor cell cycle and apoptotic pathways through complex mechan-

isms, thus promote tumor cell proliferation, accelerate tumor progression, and ultimately lead to poor prognosis in tumor patients [17] [18]. Survivin is highly expressed in both gastric and colon cancer, and is closely associated with tissue differentiation, lymph node metastasis, and prognosis in gastric and colon cancer, including [19]. Foreign studies have found that Survivin, as a cancer-promoting factor, is closely related to glioma, head and neck squamous cell carcinoma and breast cancer, and is an important factor to promote the occurrence and development of these tumors [20] [21] [22] [23]. These results suggest that Survivin is closely related to the occurrence, development and metastasis of various cancers in humans. In this study, the results of immunohistochemistry detection suggested that Survivin showed high expression in esophageal cancer tissues, but also low expression in adjacent tissues. Further quantitative detection results of immunoprotein imprinting experiments confirmed the high expression state of Survivin in esophageal cancer tissues, which is consistent with the findings of [24] [25] *et al.*, indicating that Survivin may be involved in the occurrence and development of esophageal cancer in the pathogenesis of esophageal cancer.

Protein acetylation, an important form of protein modification in epigenetics, is involved in the regulation of various biological processes such as gene transcription, proliferation, modululation, and invasion, and plays an important role in tumorigenesis [26]. The protein function can change after acetylation, induce tumor and promote tumor development. At present, there are no relevant reports on whether Survivin protein is acetylated in esophageal cancer tissues and what effect on esophageal cancer after acetylation. This experimental study confirmed by immunoprecipitation experiments that the Survivin protein can acetylate modification in esophageal cancer tissues, and its acetylation level was significantly higher than that of adjacent tissues. And the acetylation level of Survivin has correlation with TNM stage, tissue differentiation and lymph node metastasis, which indicates that acetylation of Survivin protein plays an important role in the occurrence and development of esophageal cancer. The author speculated that it may be strengthen the cancer function of Survivin protein because of acetylation of Survivin protein in esophageal cancer tissue, and thus promotes the development of esophageal cancer.

The acetylation modification of the proteins requires the catalysis of the acetyltransferase, and the protein function after the acetylation modification is changed and strengthened, participating in the tumor initiation and development [11]. P300 is an important member of the acetyltransferase family, which can catalyze the acetylation of various histones and non-histones, and participate in the occurrence and development of various tumors. Via qualitative and quantitative protein experiments, this study confirmed that P300 is highly expressed in esophageal cancer tissues, and its positive expression was associated with tumor stage, differentiation, and lymph node metastasis. This is in agreement with the experimental study by Wang [27] *et al.*, indicating that P300 and Survivin are

also involved in the development and development of esophageal cancer. However, whether P300 acts as an acetyltransferase can catalyze the acetylation modification of Survivin and regulate the occurrence and development of esophageal cancer in esophageal cancer needs further verification. In this study, bioinformatics predicted results showed that the acetyltransferase P300 can catalyze Survivin protein to acetylation as a substrate, and its multiple lysine sites can undergo acetylation. Further research found that Survivin and P300 protein are co-expressed in the cytoplasm in esophageal cancer tissues, and both showed high expression status, indicating that P300 can interact with Survivin in esophageal cancer. P300 as an acetyltransferase may be involved in the acetylation modification process of Survivin protein, which then affects the biological behavior of esophageal cancer. Further statistical analysis of the correlation between P300 and Survivin acetylation levels showed that positive P300 expression was positively associated with Survivin hyperacetylation in esophageal cancer tissues, which also suggested that P300 as an acetyltransferase may be involved in Survivin acetylation modification, promoting the proliferation, invasion, and metastasis of esophageal cancer.

Studies confirm that inhibition of acetyltransferase expression can alter the acetylation state of intracellular proteins [28]. Silencing of P300 has the potential to deacetylation and inhibit tumor cells in the mechanism of inhibiting protein acetyltransferase. Studies have confirmed that the effect of inhibiting tumor cells through inhibiting the acetyltransferase pathway in different tumor cells is also different [29] [30] [31]. In this study, the relationship of acetyltransferase P300 and Survivin acetylation modification was verified by in vitro cell experiments, and the results showed that the Survivin acetylation level in EC109 cells was significantly reduced after P300 silencing by RNA interference. This suggests that P300, acting as an acetyltransferase, can catalyze the acetylation of non-histone Survivin, which can further regulate the occurrence and development of oesophageal cancer. It was further confirmed that the proliferation, invasion and migration capacity of esophageal cancer EC109 cell were inhibited after knockdown of P300. This suggests that P300 acting as an acetyltransferase may be an important upstream molecular event to regulate the acetylation of Survivin and then affect the biological function during the occurrence and development of esophageal cancer.

6. Conclusions

In conclusion, the acetyltransferase P300 is closely related to Survivin acetylation in oesophageal cancer, and the high expression of P300 may promote the hyperacetylation state of Survivin, and both may be important biological features that promote the development and development of oesophageal cancer.

Limitations and shortcomings of this study: This study confirmed the effect of P300 on esophageal cancer cells only through Survivin acetylation at the tumor tissue and cell level, but its function *in vivo* and the validation of other specific

regulatory mechanisms need further investigation. It is of great significance for the early diagnosis and prognosis judgment of esophageal cancer to deeply explore the relationship between Survivin acetylation and acetyltransferase P300 and esophageal cancer occurrence, development, invasion, metastasis and prognosis.

Author Contributions

JX Zheng and ZY Liang conceived the hypothesis and designed the experiments. JX Zheng, JT Huang, ZY Liang performed the experiments. JT Huang and ZY Liang analyzed the data, visualized the figures and drafted the manuscript. JX Zheng revised the article.

All authors read and approved the final manuscript.

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Availability of Data and Materials

The data included in this investigation are available from the corresponding author.

Ethics Approval and Consent to Participate

All experimental protocols were implemented following the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The materials and data are carried out in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Affiliated Hospital of Chengde Medical University (NO.: 20200616).

Conflicts of Interest

The authors declare that they have no competing interests.

References

[1] Shi, J.-L., Li, B.-Q., Wang, L., *et al.* (2018) Expression and Significance of Vasohibin-1 in Oesophageal Cancer. *PLA Journal of Preventive Medicine*, **36**, 1178-1180.

[2] Dudash, M.J., Slipak, S., Dove, J., *et al.* (2019) Lymph Node Harvest as a Measure of Quality and Effect on Overall Survival in Esophageal Cancer: A National Cancer Database Assessment. *The American Surgeon*, **85**, 201-205. https://doi.org/10.1177/000313481908500229

[3] Narita, T., Weinert, B.T. and Choudhary, C. (2019) Functions and Mechanisms of Non Histone Protein Acetylation. *Nature Reviews Molecular Cell Biology*, **20**, 156-174. https://doi.org/10.1038/s41580-018-0081-3

[4] Wang, W.-X. and Wang, S.-J. (2020) Progress in the Regulation of Medullary Inhi-

bitory Cells by Acetylation Modification. *Journal of Jiangsu University*, **30**, 475-479.

[5] Lin, J., Huang, Y., Tang, F.-H., *et al.* (2020) Melatonin Induces Apoptosis in Human Osteosarcoma Cells by Regulating Survivin and Caspase-3 Expression. *Chinese Journal of Histochemistry and Cytochemistry*, **29**, 28-33.

[6] Huang, M.-L., Cai, X.-D., Huang, Z.-H., *et al.* (2018) Expression and Significance of VEGF and Survivin in Lung Adenocarcinoma. *Guangdong Medicine*, **39**, 1800-1805.

[7] Jiang, W., Dong, T., Lu, Y., *et al.* (2020) Survivin and FHIT Expression in Meningioma Patients. *Journal of Clinical Military Medicine*, **48**, 152-154.

[8] Liu, M.-H. (2019) Expression and Clinical Significance of Livin and Survivin in Gastric Adenocarcinoma Tissues. *Chinese Journal of Gerontology*, **39**, 2648-2650.

[9] Touloumis, Z., Lazaris, A. and Griniatsos, J. (2020) The Prognostic Significance of Caspase-3 and Survivin Expression in Colorectal Cancer Patients. *Journal BUON*, **25**, 2160-2170.

[10] Liang, Z.-Y., Hou, J.-S., Zhang, L., *et al.* (2019) Correlation between Survivin Acetylation Level and PCAF Expression in Oesophageal Cancer and Its Clinical Significance. *Medical Clinical Research*, **36**, 1475-1477.

[11] Ding, J.-L., Zhang, Z.-P., Liu, J., *et al.* (2021) Advances in Multi-Target Antitumor Drugs Based on Histone Deacetylase Design. *The Chinese Journal of New Drugs*, **30**, 236-245.

[12] Yang, Y. and Chen, G.-Y. (2014) P300/CBP in Malignant Ancies. *Journal of Practical Oncology*, No. 2, 188-192.

[13] Wang, L., Du, Y., Lu, M., *et al.* (2012) ASEB: A Web Server for KAT-Specific Acetylation Site Prediction. *Nucleic Acids Research*, **40**, 376-379. https://doi.org/10.1093/nar/gks437

[14] Cao, Q.-Q., Lu, Y.-Y. and Ma, Y.-C. (2021) Early Diagnosis and Early Treatment of Oesophageal Cancer. *Journal of Clinical Digestive Diseases*, **33**, 141-143.

[15] Huang, F.L. and Yu, S.J. (2018) Esophageal Cancer: Risk Factors, Genetic Association, and Treatment. *Asian Journal of Surgery*, **41**, 210-215. https://doi.org/10.1016/j.asjsur.2016.10.005

[16] Yang, Y.-J. (2020) Construction of Molybdenum Disulfide Sensing Array and Its Application in the Detection of Bladder Cancer Markers. Wuhan University, Wuhan.

[17] Zheng, Y.-W., Liu, H.-Y. and Jin, X.-M. (2019) Prospect of the Inhibitor of Apoptosis Protein Survivin in Tumor Immunotherapy. *International Journal of Immunology*, **42**, 418-421.

[18] Wu, C.-L., Yi, P., Huang, X., *et al.* (2021) Downregulation of Survivin Protein by the MDM2 Gene Inhibited Melanoma Growth. *Journal of Shanxi Medical University*, **52**, 403-408.

[19] Liang, Y., Liu, Y., Chen, J.-F., *et al.* (2018) Progress in Studying Survivin and Colorectal Cancer. *Chinese Journal of Gerontology*, **38**, 5620-5623.

[20] Tang, J.-H., Yang, L., *et al.* (2019) Bortezomib Inhibits Growth and Sensitizes Glioma to Temozolomide (TMZ) via Down-Regulating the FOXM1-Survivin Axis. *Cancer Communications* (*London*), **39**, 81. https://doi.org/10.1186/s40880-019-0424-2

[21] Krafft, U., Tschirdewahn, S., Hess, J., Harke, N.N., *et al.* (2019) Validation of Survivin and HMGA2 as Biomarkers for Cisplatin Resistance in Bladder Cancer. *Urologic Oncology*, **37**, 810.e7-810.e15. https://doi.org/10.1016/j.urolonc.2019.04.015

[22] Khan, S.-A., Burke, M., Zhu, F., *et al.* (2021) Survivin Expression and Impact on

Head and Neck Cancer Outcomes. *Oral Oncology*, **112**, Article ID: 105049. https://doi.org/10.1016/j.oraloncology.2020.105049

[23] Wu, W., Warner, M., Wang, L., He, W., *et al.* (2021) Drivers and Suppressors of Triple-Negative Breast Cancer. *Proceedings of the National Academy of Sciences of the United States of America*, **118**, e2104162118. https://doi.org/10.1073/pnas.2104162118

[24] Zeng, Y.-Z., Wei, X.-L. and Zhu, J.-L. (2018) Scientific Significance of the Combined Expression Levels of Survivin, VEGF-C and VEGFR-3 Protein in the Diagnosis and Treatment of Esophageal Cancer. *Journal of Diagnostic Pathology*, **25**, 118-121.

[25] Feng, M., Abby, M.-T., Shi, J.-Y., *et al.* (2020) Expression of miR-143, miR-145, and Survivin mRNA in Kazakh Oesophageal Cancer Tissues and Its Clinicopathological Significance. *Carcinogenic Aberration Mutation*, **32**, 29-32.

[26] Chen, H.-Y. (2017) Progress in Histone Deacetylase Inhibitors and Tumor Therapy. *Chongqing Studies*, **46**, 4280-4282.

[27] Wang, J., Wu, M., Zheng, D., *et al.* (2020) Garcinol Inhibits Esophageal Cancer Metastasis by Suppressing the p300 and TGF-β1 Signaling Pathways. *Acta Pharmacologica Sinica*, **41**, 82-92. https://doi.org/10.1038/s41401-019-0271-3

[28] Fang, C., Wang, Y. and Li, Y. (2021) Progress in Tumor Treatment with Histone Deacetylase Inhibitors and Immune Checkpoint Inhibitors. *The Chinese Journal of Lung Cancer*, **24**, 204-211.

[29] Liu, J.-N., Tang, F.-L., Ning, N., *et al.* (2018) Advances in Studying the Mechanism of Histone Deacetylase Inhibitors against Ovarian Cancer. *Medical Review*, **24**, 4842-4846.

[30] Shen, Y.-J., Yang, R., Chen, M., *et al.* (2020) HDAC Inhibitors Regulate Immune Cell Function and Their Application in Autoimmune Diseases. *China Pharmacology Bulletin*, **36**, 1481-1486.

[31] Yuan, D.-Y., Gao, X., Yang, R.-H., *et al.* (2018) The Killing Mechanism and Current Clinical Application of Histone Deacetylase Inhibitors in Multiple Myeloma Cells. *Clinical Oncology in China*, **45**, 691-694.