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Expression and clinical value of activating transcription factor-3 in endometrial serous carcinoma

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Summary

Objects: To investigate the expression of activating transcription factor-3 (ATF3), its correlation with mutant-type p53 (MTP53) in endometrial serous carcinoma (ESC), and discuss the possible mechanism of its significance in this neoplasm. *Materials and Methods*: The clinical information of 33 patients aged 44-73 years, admitted to Yantai Yuhuangding hospital for surgical treatment of ESC between January 2003 and December 2013 were retrospectively reviewed. Immunohistochemistry analysis of ATF3 and MTP53 were performed on ESC and surrounding tissues. Correlation between ATF3 expression and the clinicopathological features of patients, MTP53 expression, and disease-free survival of patients were analyzed. *Results*: The expression ratio for ATF3 in ESC tissue (27.27%) was significantly lower than that in adjacent tissues (87.88%) ($p < 0.01$). There was no significant difference in positive ratio of ATF3 among each clinicopathological groups. The authors found a negative correlation between the ATF3 and MTP53 expression in ESC tissues. The three- and five-year disease-free survival rates for ATF3+ patients were both significantly higher than for ATF3− patients. *Conclusions*: ATF3 plays an important role in the tumorigenesis of ESC and is likely to be a prognostic factor for ESC.

Key words: Endometrial cancer; Serous carcinoma; Mutant type p53, MTP53; ATF3; Prognosis.

Introduction

Endometrial cancer (also called corpus carcinoma) is a gynecological cancer that arises from the endometrium and mainly affects perimenopausal and aged women [1]. Endometrial cancer, cervical cancer, and ovarian cancer are the three major malignancies in female reproductive system. The morbidity and mortality rate of endometrial cancer have been increasing in recent years across the world and the onset age tends to be younger. Endometrial serous carcinoma (ESC) is a special pathological type of high-aggressive endometrial cancer. Although the incidence of ESC only comprises 10% of total endometrial carcinomas, its high malignant degree makes the five-year survival rate to be much lower than endometrioid adenocarcinoma [2-4].

Previous studies have identified the close involvement of p53 mutation in the occurrence of serous carcinoma [5-7]. Multiple proteins have been found to regulate the function of mutant type p53 (MTP53) by affecting its conformation or promote protein degradation, therefore suppressing tumorigenesis or metastasis. Activating transcription factor-3 (ATF3) protein is an important regulatory factor for wild-type p53 (WTP53). Binding of ATF3 to WTP53 induces the change in WTP53 conformation, making it easier to bind to DNA sequences or other transcription activating factors [8]. ATF3 also competitively binds to the promoter region of p53, enhancing its transcriptional activity [9]. *In vitro* studies revealed that ATF3 could directly bind to and repress the activity of MTP53 [10]. ATF3 has been shown to function as a tumor suppressor in bladder cancer [11] and colon cancer [12]. Other studies reported a significant reduce of ATF3 expression in many tumor tissues, including lung cancer [13, 14], breast cancer [15, 16], and liver cancer [17], suggesting its role in tumorigenesis. However, the involvement of ATF3 in ESC was not clear. In this study, the authors investigated the expression profile of ATF3 in ESC and its relationship with MTP53, and discussed the possible mechanism of ATF3 in tumorigenesis of ESC. Their research might provide a novel target for treating ESC.

Materials and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

The clinical information of 33 patients admitted to Yantai Yuhuangding hospital for surgical treatment of ESC between January 2003 and December 2013 were retrospectively reviewed. Patients aged 44-73 (median age 64) years. All patients received extensive uterus and bilateral accessory resection combined pelvic lymph node dissection. None of them received chemo- or radio-

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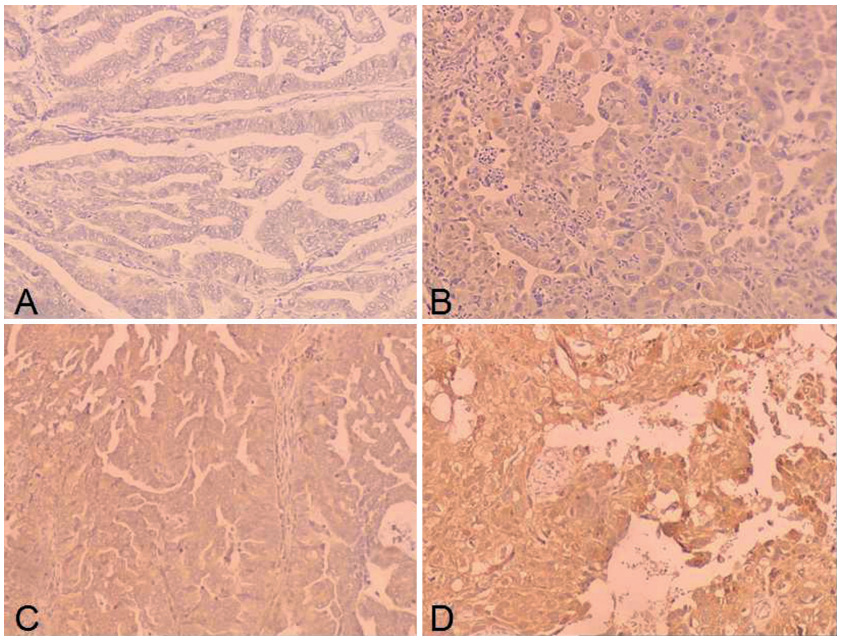

Figure 1. — Classification of the positive degree for ATF3 staining. A) Score−0. B) Score−1. C) Score−2. D) Score−3. ×100 magnification.

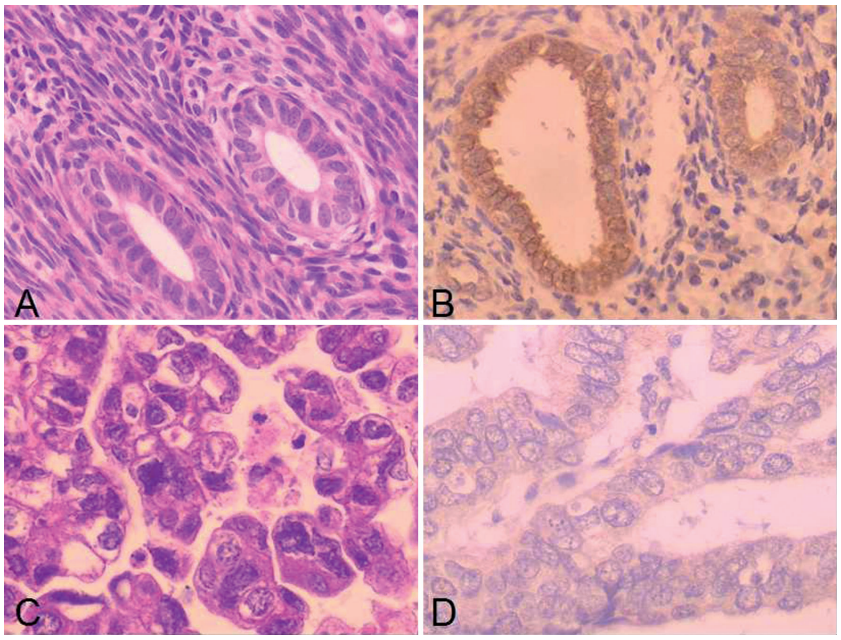

Figure 2. — Histopathologic and immune staining of ESC and surrounding tissue. A) HE stain of surrounding endometrial tissue. (×100 magnification). B) Positive ATF3 expression of surrounding endometrial tissue (×100 magnification). C) HE stain of ESC tissue section (×400 magnification). D) Positive ATF3 stain of ESC tissue section (×400 magnification).

therapy before the operation. Surgical specimens were confirmed with serous carcinoma of the endometrium. For each patient the normal endometrial tissue surrounding the tumor were used as control. Patients' age, tumor size, lymphatic metastasis, and tumor staging were analyzed. Tumor staging were determined according to the International Federation of Gynecology and Obstetrics (FIGO) staging systems for vulva, cervix, endometrium, and sarcomas [18]. Patients were followed from two to 64 (average 26.7) months which was from the day of operation until the end of study or the death of patient.

Hematoxylin-eosin stain was routinely performed on tissue sections as previously described [19]. Immunohistologic stain of MTP53 and ATP3 were performed using the EnVision two-step method [20]. Sample was classified as MTP53 positive if ≥ 75% of the nucleus of tumor cells were stained. The classification for ATF3 staining results were: (1) positive degree (PD): 0−no color; 1−light yellow, 2−brown-yellow, 3−brown. (Figure 1); (2) percentage of positive cells (PP): 0: ≤ 5%; 1: 6% to 25%; 2: 26% to 50%; 3: >50%. Samples were classified as ATF3 positive if PD×PP ≥ 3 and negative if < 3.

Statistical analysis was performed using SPSS 17.0. Significance was analyzed by χ^2 test. Correlation was analyzed by Spearman correlation test. Patients' disease-free survival was analyzed using Kaplan-Meier method and a survival curve was made. $P <$

Table 1. — *The ATF3 expression in different clinicopathological groups.*

Clinicopathological parameters		No. of cases	ATF3 +	ATF3 –
Age (years)	≥64	17	3	14
	<64	16	6	10
Tumor size **(cm)**	≥ 3.87	16	4	12
	< 3.87	17	5	12
Clinical Stage	I-II	20	6	14
	III-IV	13	3	10
Infiltration depth	MI < 50%	19	5	14
	MI ≥ 50%	14	4	10
Lymphatic metastasis	Yes	14	2	12
	No	19	7	12

MI: myometrial invasion.

Table 2. — *Correlation between ATF3 and MTP53 expression in ESC.*

		No. of cases	ATF3 expression +	ATF3 expression –	*r* value	*p* value
MTP53 expression	+	24	3	21	-0.542	0.001
	–	9	6	3		

0.05 suggested statistically significant difference.

Results

Histophathologic analysis of surrounding tissue of ESC exhibited atrophic endometrial tissue with short-columned glands and fusiform mesenchymal cells (Figure 2A). HE stain of ESC tissue showed obvious atypia (Figure 2C). Immunohistochemistry analysis showed that the positive ratio of ATF3 stain in ESC and adjacent tissues among all 33 patients was 27.27% (9/33) and 87.88% (29/33), respectively, which was significantly different ($p < 0.01$) (Figure 2B, 2D). The relationship between ATF3 expression and the clinicopathological features of patients are summarized in Table 1. There was no significant difference in the positive ratio of ATF3 among each clinicopathological groups.

The present authors analyzed the correlation between ATF3 and MTP53 expression (Table 2). Results showed a negative correlation between the ATF3 and MTP53 expression in ESC tissues.

The cumulative survival curve after surgery for ATF3 positive and negative patients are shown in Figure 3. During the follow-up time, 16 out of 33 ESC patients (48.48%) were alive while 17 (51.52%) died of the disease. The three- and five-year disease-free survival rates for ATF3+ patients were 100% and 66.7%, respectively, while for ATF3– patients, the three- and five-year disease-free survival rates were only 25.9% and 0%, respectively. Statistical analysis showed that the prognosis for ATF3 + group

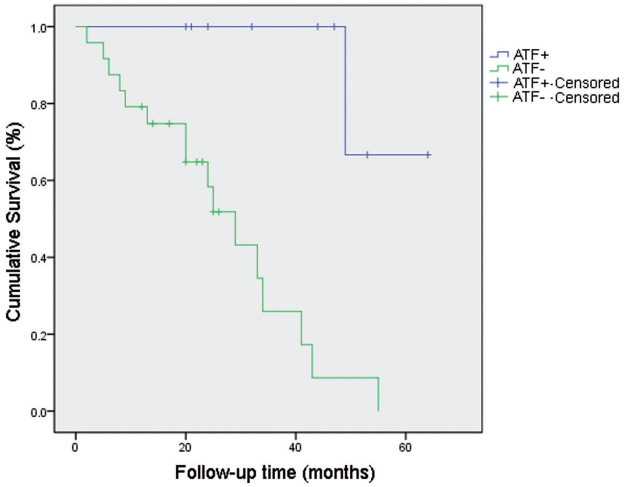

Figure 3. — Cumulative survival of ATF3+ and ATF3 ESC patients.

was significantly superior to that of the ATF3– group ($p < 0.001$).

Discussion

ATF3 has been discovered to be downregulated in many malignant tumors and participates in the tumorigenesis and tumor progression. Downregulation of inhibition of differentiation-1 (Id-1) via activation of ATF 3 and Smad induced apoptosis in prostate cancer [21]. In esophageal squamous cell carcinoma (ESCC), ATF3 was found downregulated in ESCC lesions compared with paired non-cancerous tissues. Overexpression of ATF3 led to decreased growth and invasion properties of ESCC cells *in vitro* and *in vivo*, whereas knockdown of ATF3 did the opposite [22]. ATF3 induction in colorectal cancer cells showed protumorigenic and anti-invasive properties *in vitro* [23]. Reducing the ATF3 expression in HT29 human colon cancer cell line led to increased attachment and invasiveness of the cancer cells [24]. In highly metastatic bladder cancer cells, ATF3 overexpression decreased migration *in vitro* and experimental metastasis *in vivo* by activating the actin filament severing protein gelsolin (GSN) and degradation of actin [11]. In human cervical cancer Siha cells, ATF3 played a role in blocking the cell cycle and inducing apoptosis through enhancing the activity of target gene *p53* [25]. In this study, the authors observed a significant downregulation of ATF3 in ESC than normal surrounding tissues, suggesting that ATF3 might play an important role in the tumorigenesis of ESC.

The cancer suppressor gene p53 is one of the most closely related genes with human carcinomas. For MTP53, the conformational change of p53 loses its tumor-suppressing ability and enables the malignant transformation of

normal cells. The present authors' previous work has shown that MTP53 was highly expressed in ESC tissues (unpublished data) and was an early event of ESC, indicating its involvement in tumorigenesis. The present results showed a significant negative correlation between ATF3 and MTP53 expression in ESC. This further supported that ATF3 downregulation was likely involved in the formation of ESC.

The mechanism of this involvement still remains unclear. One possible mechanism is that reduced expression of ATF3 relives its inhibition to Erythroid-E2-related factor 2 (Nrf2) expression. Nrf2 is an important nuclear transcription factor responsible for maintaining the redox homeostasis of the body. Nrf2 overexpression was suggested to be closely associated with the development of ESC and chemotherapy drug resistance [26]. Brown *et al.* found that ATF3 can repress Nrf2-mediated signaling through direct ATF3-Nrf2 protein-protein interactions [27]. Therefore ATF3-mediated inhibition of Nrf2 expression is likely to be an antineoplastic factor for ESC.

Oncogenic protein IMP3 (insulin-like growth factor II mRNA-binding protein) has also been shown to over-express in several types of malignant tissues, including ESC and is closely involved in tumorigenesis [28-31]. The expression of IMP3 was reported to be correlated with p53 in ESC [31]. It is possible that ATF3 interacts with IMP3 during ESC development and their correlation in ESC still awaits further investigation.

Matrix metalloproteinase 2 (MMP-2) is another protein highly expressed in ESC [32]. It was discovered that in ESC, ATF3 upregulated the expression of MDM2 by increasing the nuclear translocation of p53 and formed an ATF3/MDM2/MMP-2 complex that facilitated MMP-2 degradation, which subsequently led to inhibition of cell invasion [22, 33]. Another study found that nitric oxide inhibited endothelial cell migration via the inhibition of MMP-2 expression by ATF3 induction [34]. In prostate cancer cells, IL-10 inhibited MMP-2 expression by upregulating ATF3 expression, which suppressed cancer cell migration [35]. Therefore, the authors proposed that MMP-2 is likely to be a downstream protein of ATF3 in ESC.

Studies have shown that low ATF3 expression was an indicator for poor prognosis for esophageal squamous cancer [22], liver cancer [36], colon cancer [24], and lung cancer [14]. The present results showed a significantly higher disease-free survival rate in ATF3+ patients than in ATF3– patients, suggesting that ATF3 is likely to be an important prognostic factor for ESC. Human epidermal growth factor receptor 2 (HER-2) and Cyclin-D1 are the other two important prognostic factors for endometrial carcinoma and are both overexpressed in tumor tissues [37, 38]. HER-2 is a target protein for MTP53, which amplifies HER-2 signaling to promote mammary tumorigenesis [39]. Cyclin-D1 expression is also regulated by ATF3 [40]. Therefore, investigating the interaction of ATF3-MTP53-HER-2 and ATF3-Cyclin-D1 could provide a better understanding of the oncogenesis, progression, and prognosis of ESC.

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