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Original Article

Expression of TC1 (C8orf4), Atonal homolog 1 and β-catenin is associated with the malignant progression of ovarian carcinomas

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Abstract: Thyroid cancer 1 (TC1, C8ofr4) and Atonal homolog 1 (Atoh1) are involved in the regulation of Wnt/β-catenin signaling pathway, and participate the tumorigenesis and progression of many tumors. This study investigated the correlations among the expressions of TC1, Atoh1 and β-catenin, and their roles in the progression of ovarian carcinomas. The expressions of TC1, Atoh1 and β-catenin were examined in 112 cases of ovarian carcinomas using immunohistochemistry. The high expression rates of TC1, Atoh1 and β-catenin were 82.14% (92/112), 72.32% (81/112) and 82.14% (92/112) in ovarian carcinomas, respectively. The high expression of TC1 was correlated with the histological type (P = 0.004), differentiation (P = 0.008) and TNM stage (P = 0.028) of ovarian carcinomas. The high expression rate of TC1 in serous adenocarcinomas (89.71%, 61/68) or clear cell carcinomas (100%, 8/8) was much more than that in Mucinous adenocarcinomas (68.42%, 13/19) or Endometrioid adenocarcinomas (58.82%, 10/17). Furthermore, the expression of TC1 was positively correlated with Atoh1 expression (correlation coefficient = 0.233, P = 0.014) and β-catenin expression (correlation coefficient = 0.391, P<0.001), respectively. The expression of Atoh1 was correlated with β-catenin expression (correlation coefficient = 0.285, P = 0.002), but was not correlated with any of the clinicopathological factors of ovarian carcinomas. The high expression of β-catenin was correlated with the poor differentiation of ovarian carcinomas (P<0.001). The expressions of TC1, Atoh1 and β-catenin were increased and correlated to each other in ovarian carcinomas. TC1 and β-catenin were co-expressed and associated with the malignant progression of ovarian carcinomas.

Keywords: Thyroid cancer 1, C8orf4, atonal homolog 1, β-catenin, ovarian carcinoma

Introduction

Epithelial carcinomas are the most common ovarian cancers and the principal cause of death from gynecologic cancer worldwide. Epithelial carcinomas of ovary account for over 95% of the ovarian malignancies [1, 2]. The oncogenesis and progression of ovarian carcinoma is related to many signaling pathways and oncogenes [3, 4]. The Wnt/β-catenin signaling pathway is one of the important pathways involved in the development of ovarian carcinomas [5-8]. The activation of Wnt/β-catenin signaling pathway promotes the de-differentiation, proliferation and invasion of many tumors, such as lung cancers, liver cancers, colorectal cancers, and ovarian cancers [6, 7, 9-12]. The activation of Wnt/β-catenin pathway depends on the accumulation of β-catenin, then β-catenin forms a complex with the T-cell factor/lymphoid enhancer factor (Tcf/Lef) family of transcription factors in nucleus, leading to transcription of the target oncogenes, such as cyclin D1, c-myc and MMP7 [13, 14].

Thyroid cancer 1 (TC1, C8ofr4) and Atonal homolog 1 (Atoh1, also called Math1 or Hath1) are two proteins related to the activation of Wnt/β-catenin signaling pathway. TC1 interacts with Chibby, which inhibits the β-catenin-mediated transcriptions by binding with β-catenin. Thereby, the binding of TC1 and

Chibby relieves β-catenin from Chibby and enhances the activity of Wnt/β-catenin signaling pathway [15, 16]. The high expression of TC1 was revealed in many tumors and involved in the progression of tumors [16-19]. Atoh1 is a basic helix-loop-helix (bHLH) transcription factor and a regulating target of Notch signalling pathway. Notch pathway represses the expression of Atoh1 and control cell differentiation [20, 21]. The Wnt pathway also inhibits the expression of Atoh1 by enhancing the expressions of Hes1 and activating Notch pathway [22]. Inhibiting Wnt pathway will up-regulate Atoh1 expression [23]. But, it was also reported that β-catenin could interact with the 3' enhancer of the *ATOH1* gene and enhance the expression level of Atoh1 [24]. Atoh1 was down-regulated in many tumors and acted more likely as a tumor inhibitor [25, 26]. Interestingly, recent study showed that Atoh1 was stabilized by tumor necrosis factor α, which enriched cancer stem cells and induced high malignant potential in colon cancer cell line [27]. So, the ectopic expression of Atoh1 was found in many tumors and associated with the progression of tumors, but its function in different tumors is still need investigation [10, 27, 28].

Although, some reports indicated that the expression of TC1 and Atoh1 were involved in the development of cancers, the expressions and correlations of TC1 and Atoh1 in ovarian carcinomas were unclear. In this study, we examined the expressions of TC1, Atoh1 and β-catenin, and investigated the correlations among these three proteins and the clinicopathological factors of ovarian carcinomas.

Materials and methods

Patients and tissue specimens

All of the 112 cases of ovarian carcinomas were obtained randomly from patients who underwent surgery at the First Affiliated Hospital of China Medical University between 2010 and 2014. Patients of ovarian carcinomas included in the study were in the age range of 27-76 years, and the mean age was 49.5 years. The histological diagnosis and grade of differentiation were determined according to the classification system of the World Health Organization (2003). The histological types of ovarian carcinomas in this study including serous adenocarcinomas (*n* = 68), mucinous adenocarcinomas (*n* = 19), endometrioid adenocarcinomas (*n* = 17) and clear cell carcinoma (*n* = 8). These ovarian carcinomas showed different degrees of differentiation and were classified as well (*n* = 18), moderately (*n* = 45), or poorly (*n* = 49) differentiated tumors. Twenty-six cases showed lymphatic metastasis. The tumor stage was classified as stages I-IV (*n* = 62, 14, 31 and 5, respectively) according to the TNM classification system of the International Union Against Cancer. This study was conducted according to the regulations stipulated by the institutional review board at the China Medical University.

Immunohistochemistry

All resected specimens were fixed with 10% neutral-buffered formalin and embedded in paraffin blocks. Tissue blocks were cut into 4-μm sections. These sections were deparaffinized, rehydrated and performed by pressure cooking in a PH 6 citrate buffer for 1.5 min. Then, the sections were incubated with polyclonal rabbit anti-C8orf4 antibody (1:200; ab133885, Abcam, Cambridge, MA), monoclonal mouse anti-β-catenin antibody (610154; 1:200; BD Transduction Laboratories, KY), and polyclonal rabbit anti-Atoh1 antibody (1:200; Santa Cruz Biotechnology Inc., CA) at 4°C overnight. The detection of antibodies was accomplished using the streptavidin-peroxidase method. Some slides were also stained in the absence of primary antibodies and served as negative controls.

Evaluation of immunostaining

All the immunostained sections were evaluated by two investigators, who were blinded to the clinical data. Five views per slide were randomly examined, and 100 tumor cells were observed per view at ×400 magnification. In this study, TC1, β-catenin and Atoh1 primarily expressed in the cytoplasm in ovarian carcinomas. The intensity of TC-1, β-catenin or Atoh1 cytoplasmic staining were scored as 0, 1, 2 and 3 if negative, weak, moderate, or marked, respectively. The positive rate of each case was obtained by calculating the percentage of positively stained cells in each slide. Percentage scores were assigned as 1 (1-25%); 2 (26-50%); 3 (51-75%); and 4 (76-100%). Scores from each tumor sample were multiplied to give a final score of 0 to 12, and the tumors were finally

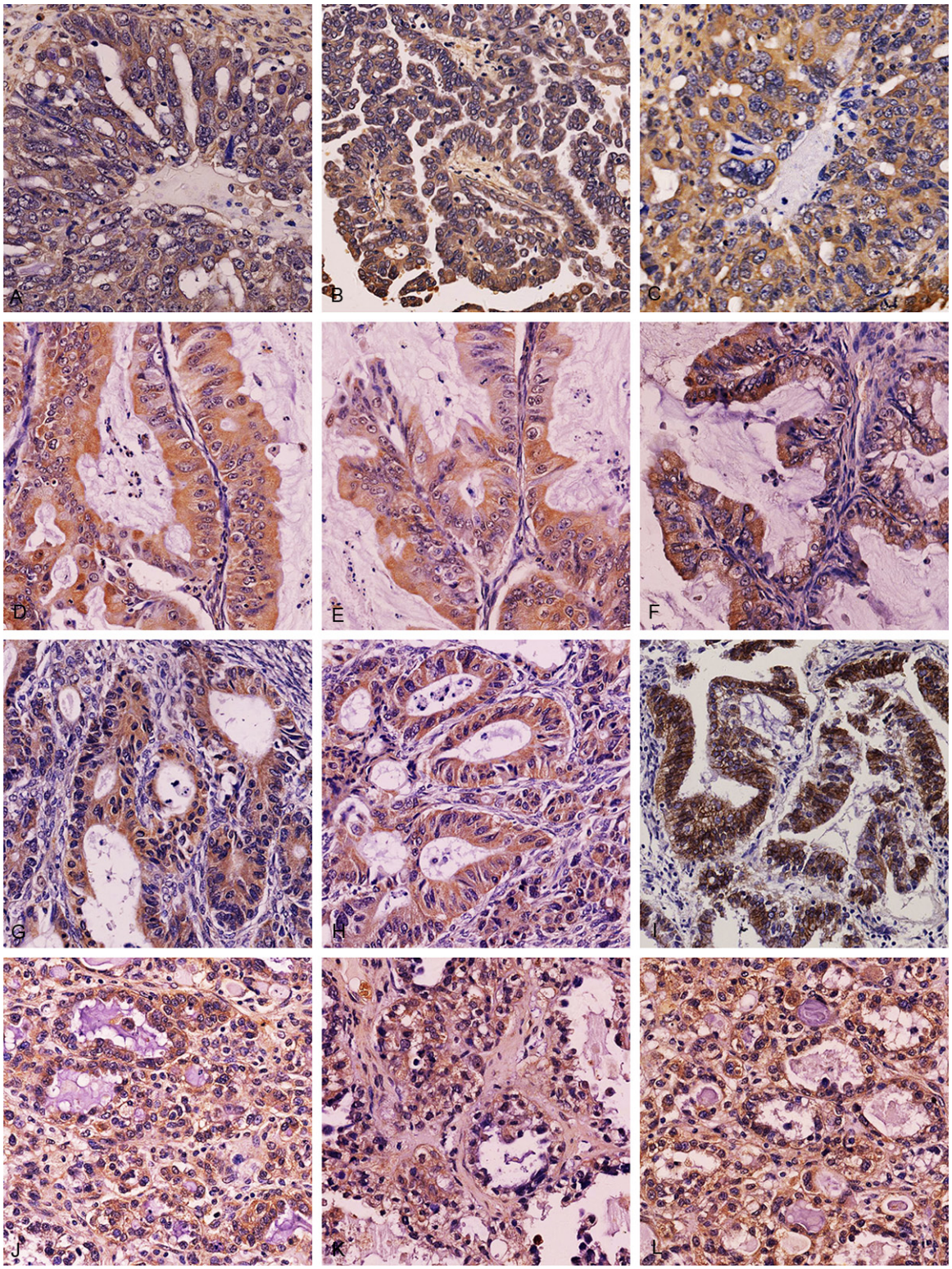

Figure 1. The expressions of TC1, Atoh1 and β-catenin in ovarian carcinomas. The high expressions of TC1 (A, D, G and J), Atoh1 (B, E, H and K) and β-catenin (C, F, I and L) were displayed in representative serous adenocarcinoma, mucinous adenocarcinoma, endometrioid adenocarcinoma and clear cell carcinoma cases, respectively. (streptavidin-peroxidase immunohistochemistry method, Original magnification, 200×).

Table 1. The correlations among the expressions of TC1, Atoh1, β-catenin and the clinicopathological parameters of ovarian carcinomas

	n	TC1 expression			Atoh1 expression			β-catenin expression		
		Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Age				0.020			0.311			0.525
<50	60	6	54		19	41		12	48	
≥50	52	14	38		12	40		8	44	
Histological type				0.004			0.350			0.960
Mucinous adenocarcinoma	19	6	13		7	12		4	15	
Serous adenocarcinoma	68	7	61		16	52		12	56	
Endometrioid adenocarcinoma	17	7	10		4	13		3	14	
Clear cell carcinoma	8	0	8		4	4		1	7	
Differentiation				0.008			0.247			<0.001
Well	18	6	12		8	10		9	9	
Moderate	45	11	34		11	34		8	37	
Poor	49	3	46		12	37		3	46	
TNM stage				0.028			0.210			0.960
I	62	15	47		19	43		12	50	
II	14	0	14		1	13		2	12	
III	31	5	26		10	21		5	26	
IV	5	0	5		1	4		1	4	
Lymphatic metastasis				0.210			0.550			0.703
No	86	18	68		25	61		16	70	
Yes	26	2	24		6	20		4	22	

determined, based on scores ≤6, and ≥8 as having low or high expression, respectively.

Statistical analysis

The Pearson's chi-square, Fisher's Exact Test, likelihood ratio test and the Spearman's correlation test were used to assay whether the expression levels of TC1, atoh1 and β-catenin were related to each other and the clinicopathologic characteristics of ovarian carcinomas. *P*-values <0.05 were considered statistically significant.

Results

TC1 was high expressed in ovarian carcinomas and correlated with the histological type, differentiation and TNM stage of ovarian carcinomas

In this study, most cases displayed high expression of TC1. The high expression rates of TC1was 82.14% (92/112). The expression of TC-1 was localized primarily in cytoplasm and also could be observed in nuclei of ovarian carcinoma cells (**Figure 1**). The high expression of TC1 was correlated with the histological type ($P = 0.004$), differentiation ($P = 0.008$) and TNM stage ($P = 0.028$) of ovarian carcinomas. The high expression rate of TC1 in serous adenocarcinomas (89.71%, 61/68) or clear cell carcinomas (100%, 8/8) was much more than that in Mucinous adenocarcinomas (68.42%, 13/19) or Endometrioid adenocarcinomas (58.82%, 10/17). The expression of TC1 was also correlated with the patients' age ($P = 0.020$), but was not correlated with the lymphatic metastasis of ovarian carcinomas ($P = 0.210$) (**Table 1**).

The expression of TC1 was positively correlated with Atoh1 and β-catenin expression, respectively

We further examined the expression of Atoh1 and β-catenin, and investigated the correlations among the expressions of TC1, Atoh1 and β-catenin in ovarian carcinomas. We found that the majority of ovarian carcinoma tissues showed high expression of Atoh1 (72.32%, 81/112). The high cytoplasmic expression of β-catenin was detected in most cases of ovarian carcinomas (82.14%, 92/112) (**Figure 1**).

Table 2. The correlations between the expressions of TC1, Atoh1 and β-catenin in ovarian carcinomas

	n	TC1 expression				Atoh1 expression			
		Low	High	Correlation coefficient	*P* value	Low	High	Correlation coefficient	*P* value
β-catenin expression				0.391	<0.001			0.285	0.002
Low	20	10	10			11	9		
High	92	10	82			20	72		
Atoh1 expression				0.233	0.014				
Low	31	10	21						
High	81	10	71						

Some cases also showed membranous expression of β-catenin along with the cytoplasmic expression. The spearman correlation test revealed that the expression of TC1 was positively correlated with Atoh1 expression (correlation coefficient = 0.233, $P = 0.014$) and β-catenin cytoplasmic expression (correlation coefficient = 0.391, $P<0.001$), respectively. Besides, the expression of Atoh1 was positively correlated with the cytoplasmic expression of β-catenin (correlation coefficient = 0.285, $P = 0.002$) (**Table 2**).

The cytoplasmic expression of β-catenin was correlated with the poor differentiation, but Atoh1 was correlated with none of the clinicopathological factors of ovarian carcinomas

The high cytoplasmic expression of β-catenin was correlated with the poor differentiation of ovarian carcinomas ($P<0.001$), but was not correlated with the patients' age ($P = 0.525$), histological type ($P = 0.960$), TNM stage ($P = 0.960$) or lymphatic metastasis ($P = 0.703$) of ovarian carcinomas (**Table 1**). As shown in **Table 1**, we did not find any correlations between Atoh1 expression and the clinicopathological factors of ovarian carcinomas.

Discussion

The Wnt/β-catenin signaling pathway regulates the de-differentiation, proliferation and invasion of cancer cells. Its activation is involved in the development of many cancers, such as lung cancers, liver cancers, colorectal cancers [9-12]. The Wnt/β-catenin signaling pathway is also play an important role in the progression of ovarian carcinomas [6, 7]. The previous studies and ours showed that β-catenin was overexpressed in ovarian carcinomas predominantly in cytoplasm [6, 29]. We found that the high expression of β-catenin was correlated with the poor differentiation of ovarian carcinomas. Other reports also showed that the ectopic expression of β-catenin and the target genes of Wnt/β-catenin pathway were correlated with the malignant phenotype and poor prognosis of ovarian carcinomas [6, 29]. Some drugs targeting the Wnt/β-catenin pathway may lead to decreased cellular proliferation and increased cell death, which indicated that Wnt/β-catenin pathway was a potential target for treatment of ovarian carcinomas [30].

TC1 and Atoh1 are both related to the expression and function of β-catenin and involved in the activation of Wnt/β-catenin signaling pathway. TC1 can upregulate the transcription activity of β-catenin by binding with Chibby and promote the activation of Wnt/β-catenin signaling pathway. Atoh1 was a regulating target of Notch signaling pathway, but its expression was also controlled by Wnt/β-catenin signaling pathway [20-24]. The expressions and functions of TC1 and Atoh1 were examined in many tumors [10, 16-19, 25-28], but were less reported in ovarian carcinomas. In this study, we investigated the expression levels of TC1 and Atoh1, and their correlations with the expression of β-catenin in ovarian carcinomas. The results showed that TC1 was high expressed in ovarian carcinomas. The high expression of TC1 was correlated with the differentiation and TNM stage of ovarian carcinomas. So, as displayed in other tumors, the high expression of TC1 contributes the progression of ovarian carcinomas. Moreover, the high expression rate of TC1 in serous adenocarcinomas (89.71%) or clear cell carcinomas (100%) was much more than that in Mucinous adenocarcinomas (68.42%) or Endometrioid adenocarcinomas (58.82%). TC1 might help to distinguish different histological type of ovarian carcinomas. Atoh1 was also high expressed in ovarian carcinomas, but

we did not find any correlations between Atoh1 and the clinicopathological factors. Further correlation analysis revealed that the expressions of TC1, Atoh1 and β-catenin were correlated with each other. It is to say that TC1 is co-expressed with β-catenin and promotes the progression of ovarian carcinomas by enhancing the activity of Wnt/β-catenin signaling pathway. On the other hand, the high level of free β-catenin could interact with the 3' enhancer of the *ATOH1* gene [24], and therefore lead to the high expression level of Atoh1in ovarian carcinomas.

To sum up, the expressions of TC1, Atoh1 and β-catenin were increased and correlated to each other in ovarian carcinomas. TC1 and β-catenin were co-expressed and associated with the malignant progression of ovarian carcinomas. TC1 and β-catenin might be potential therapeutic target for treatment of ovarian carcinomas.

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Disclosure of conflict of interest

None.

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