Expression and prognostic significance of TAp73 and ΔNp73 in FIGO stage I-II cervical squamous cell carcinoma

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Abstract. The purpose of the present study was to investigate the expression of TAp73 and ΔNp73 in cervical squamous cancer cells, and to evaluate the prognostic significance of TAp73 and ΔNp73 expression in patients with International Federation of Gynecology and Obstetrics (FIGO) stage I-II cervical squamous cell carcinoma (SCC). The immunohistochemical expression of TAp73 and ΔNp73 was evaluated in 59 FIGO stage I-II cervical SCC tumor samples. Correlations with clinicopathological characteristics were determined by χ^2 test. The prognostic impact of TAp73 and ΔNp73 expression with regard to overall survival (OS) was determined by the Kaplan-Meier method. High TAp73 and ΔNp73 expression was detected in 79.7% (47/59) and 76.3% (45/59) of patients, respectively. The expression of TAp73 and ΔNp73 was not associated with age, FIGO stage, pathological differentiation or lymph node metastasis. The 3-year OS rates associated with low and high TAp73 expression were 75.0 and 83.0%, respectively (χ^2=0.33; P=0.568), whereas those associated with low and high ΔNp73 expression were 100.0 and 75.6%, respectively (χ^2=3.90; P=0.048). High expression levels of TAp73 and ΔNp73 were frequently observed in the cervical squamous cancer cells. Overall, high expression levels of ΔNp73 may indicate an unfavorable prognosis in patients with early-stage cervical SCC.

Introduction

Cervical cancer is the second most prevalent female cancer worldwide, and 80-90% of cervical cancers are classified as squamous cell carcinoma (SCC) based on pathological findings (1,2). The majority of patients with early-stage cervical cancer can be treated with radical surgery or radiotherapy (3). Komaki *et al* (4) reported 5-year overall survival (OS) rates of 89.4 and 79.3% in patients with International Federation of Gynecology and Obstetrics (FIGO) stage I and II cervical cancer, respectively. Pathological factors, including histological type, tumor diameter, lymph node metastasis, lymph vascular space invasion, depth of stromal invasion and parametrial extension, are currently considered independent prognostic factors for survival (5). However, there is no consensus regarding the role of these risk factors for the individual patient (6). The identification of novel markers to accurately predict the prognosis of patients with cervical cancer is therefore necessary.

The transcription factor p53 and its two homologs, p73 and p63, form the p53 gene family. These three genes encode proteins with high similarity at the structural and functional level (7,8). The p53 gene is the prototype tumor suppressor in human cancers due to its proapoptotic and antiproliferative functions in response to oncogenic stress. The p53 gene is mutated and its function is lost in ~50% of human cancers (9,10). Despite its significant homology to p53, p73 is not a classic Knudson-type tumor suppressor gene, and it has several complex isoforms with opposing functions, including transactivation domain p73 (TAp73) and p73 with inhibitory proteins lacking TA (ΔTAp73). ΔTAp73 is expressed in four different forms as follows: ΔNp73, ΔN'p73, ΔEx2p73 and ΔEx2/3p73, of which ΔNp73 is the predominant form. ΔNp73 is a potent transdominant inhibitor of TAp73 and wild-type p53. The p73 locus encodes a tumor suppressor (TAp73) and a putative oncogene (ΔNp73) (11,12).

Recent studies have demonstrated that TAp73 and ΔNp73 are overexpressed in a number of solid tumors, including lung, ovarian, hepatocellular, breast and colon cancers, and their expression levels are associated with prognosis in patients with these cancers (10-14). However, few studies have investigated the expression levels of TAp73 and ΔNp73 and their prognostic significance in cervical cancer, particularly in early-stage cervical SCC (15). In the current study, the expression of TAp73 and ΔNp73 was investigated in cervical squamous cancer cells, and their prognostic significance was evaluated in patients with FIGO stage I-II cervical SCC.

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Patients and methods

Patients and specimen selection. Paraffin-embedded post-operative tissue samples were obtained from the archives of the Department of Pathology, the Second Affiliated Hospital of Soochow University (Suzhou, Jiangsu, China), between January 2009 and December 2010. A total of 59 tumor samples were retrospectively retrieved from patients with FIGO stage I-II cervical SCC. Approval for the current project was obtained from the Ethics Committee of the Second Affiliated Hospital of Soochow University.

The main characteristics of the 59 patients were summarized in Table I. The median age of the patients was 42 years (range, 22-68 years). According to the FIGO stage, the cohort consisted of 44 patients with stage I and 15 patients with stage II SCC. All patients underwent radical surgery. A total of 7 patients with a post-operative pathological diagnosis of pelvic lymph node metastasis received radiotherapy. The target volume included the whole pelvis, and radiation was delivered in four or two fields (anterioposterior and postero-anterior beams). A total dose of 5,000 cGy was delivered in fractions of 200 cGy/day for 5 days per week. Systemic adjuvant treatment was administered to 8 patients; these patients received 80 mg/m^2 cisplatin and 135 mg/m^2 Taxol every 3 weeks for 2-4 cycles.

Immunohistochemistry. Three serial slides, each 3-μm thick, were cut from paraffin-embedded tissues. One slide was used for hematoxylin/eosin staining and the other two were subjected to immunohistochemical staining using the two-step procedure. Mouse monoclonal anti-human TAp73 antibody (IMG-246; dilution, 1:100; Imgenex, Novus Biologicals, Littleton, CO, USA) and mouse monoclonal anti-human ΔNp73 antibody (ab13649; dilution, 1:100; Abcam, Cambridge, MA, USA) were used. Following deparaffinization at 65˚C and hydration, the slides were subjected to antigen retrieval by pressure-cooking for 5 min at 120˚C. Endogenous peroxidase activity was neutralized using peroxide block placement on the slides for 15 min at room temperature. The slides were then incubated with anti-TAp73 and anti-ΔNp73 monoclonal antibodies for 30 min at 4˚C, followed by incubation with peroxidase-conjugated polymers (ChemMate EnVision/HRP; Gene Tech, Shanghai, China) for 30 min at room temperature. The chromogen reaction was developed in 3,3'-diaminobenzidine tetrahydrochloride (Gene Tech) for 10 min. Finally, hematoxylin was used as a light nuclear counterstain. The negative control used was an immunoglobulin G2b isotype antibody (Dako, Shanghai, China), ensuring the same concentration of immunoglobulins used in the anti-TAp73 and anti-ΔNp73 antibodies.

Assessment of TAp73 and ΔNp73 expression. All slides were evaluated independently by two experienced pathologists. The intensity of staining was recorded as negative, weak or strong. In accordance with the description in the study by Giatromanolaki *et al*, only strongly-stained cells were considered positive cells (16). The extent of staining was expressed as a percentage of positive cells to total cells and was recorded after examining all optical fields at x200 magnification. The mean value was used to score all samples. Cells were classified into four categories according to the percentage of positive-staining cells as follows: 1, 0-24%; 2, 25-49%; 3, 50-74%; and 4, 75-100%. The scoring pattern was defined as follows: 1-2, low expression (<50% positive cells); and 3-4, high expression (≥50% positive cells).

Table I. Patient characteristics.

Characteristic	Value
Patients, n (%)	59 (100.0)
Median age (range), years	42 (22-68)
Pathological diagnosis, n (%)	
Cervical squamous cell carcinoma	59 (100.0)
FIGO stage, n (%)	
I	44 (74.6)
II	15 (25.4)
Radical surgery, n (%)	59 (100.0)
Post-operative radiotherapy, n (%)	7 (11.9)
Post-operative chemotherapy, n (%)	8 (13.6)

FIGO, International Federation of Gynecology and Obstetrics.

Statistical analysis. Statistical analysis was performed using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA) The correlation between the expression of TAp73 and ΔNp73, and the clincopathological characteristics was examined using the χ^2 test. OS rates were determined using the Kaplan-Meier method and log-rank test. OS time was defined from the day of surgery to the day of mortality or last follow-up. For all tests, two-sided $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of TAp73 and ΔNp73 in cervical SCC cells. The expression of TAp73 and ΔNp73 was detected in the nucleus and cytoplasm of the cervical SCC cells. High TAp73 and ΔNp73 expression was detected in 79.7% (47/59) and 76.3% (45/59) of patients, respectively (Fig. 1A and B). No significant correlation between TAp73 and ΔNp73 expression was observed ($\chi^2=0.415$; $P=0.519$).

Association of TAp73 and ΔNp73 expression with clinicopathological characteristics. Table II shows the associations between the expression levels of TAp73 and ΔNp73 in the 59 patients with FIGO stage I-II cervical SCC and several clinicopathological characteristics. No significant association was observed between TAp73 and ΔNp73 expression and age, FIGO stage, pathological differentiation and lymph node metastasis (Table II).

Correlation between TAp73 and ΔNp73 expression and overall survival. The average duration of follow-up was 41 months (range, 2-61 months). Analysis of the Kaplan-Meier plots showed that the 3-year OS rate of all patients was 81.4%.

Table II. Association of TAp73 and ΔNp73 expression with clincopathological characteristics.

Variables	No. of patients	TAp73 expression, %		χ^2	P-value	ΔNp73 expression, %		χ^2	P-value
		Low	High			Low	High		
Age, years									
≤42	33	24.2	75.8	0.704	0.401	30.3	69.7	1.788	0.181
>42	26	15.4	84.6			15.4	84.6		
FIGO stage									
I	44	22.7	77.3	0.609	0.435	25.0	75.0	0.155	0.694
II	15	13.3	86.7			20.0	80.0		
Pathological differentiation									
Low	12	33.3	66.7	1.600	0.449	33.3	66.7	1.215	0.545
Medium	28	17.9	82.1			17.9	82.1		
High	19	15.8	84.2			26.3	73.7		
Lymph node metastasis									
No	52	21.2	78.8	0.180	0.672	26.9	73.1	2.471	0.116
Yes	7	14.3	85.7			0.0	100.0		

FIGO, International Federation of Gynecology and Obstetrics.

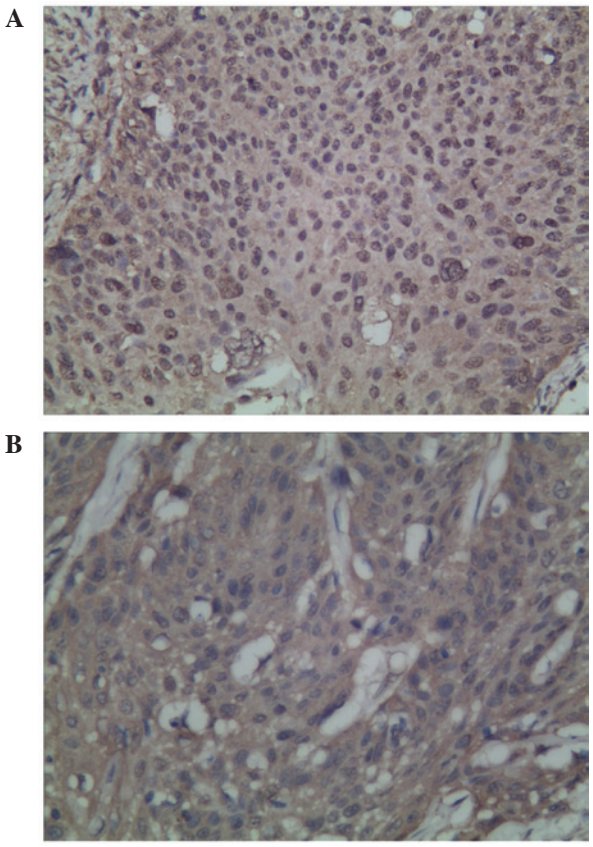

Figure 1. Expression of TAp73 and ΔNp73, as assessed by immunohistochemistry in cervical squamous cell carcinoma cells: (A) High expression of TAp73; and (B) high expression of ΔNp73.

The 3-year OS rates in patients with low and high expression levels of TAp73 were 75.0 and 83.0%, respectively (χ^2=0.33; P=0.568; Fig. 2A), whereas those in patients with low and high

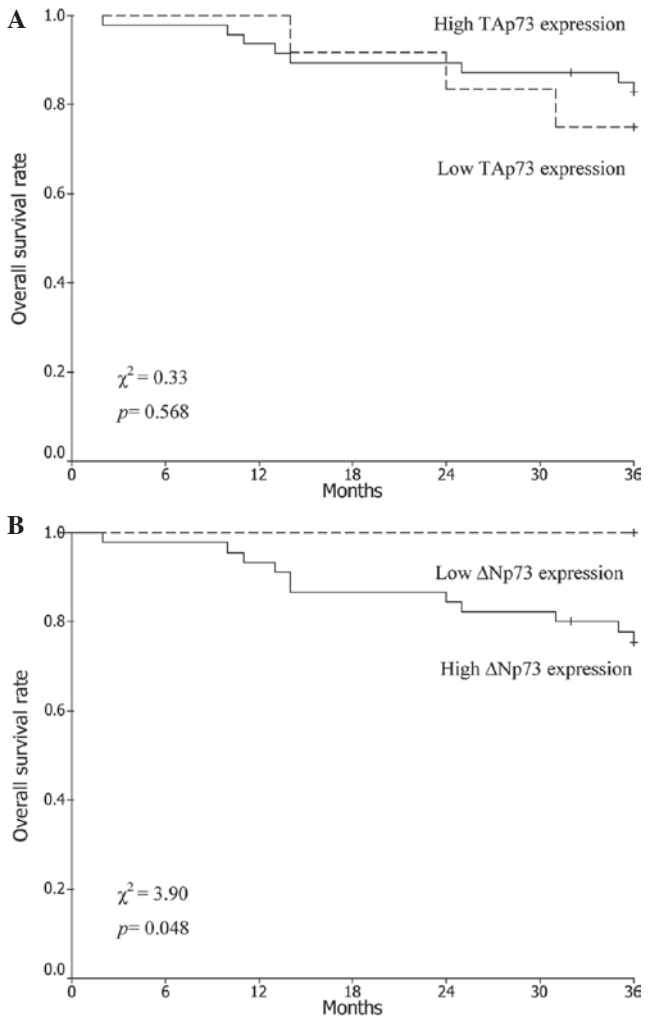

Figure 2. Kaplan-Meier curves for TAp73 and ΔNp73 expression in patients with International Federation of Gynecology and Obstetrics stage I-II cervical squamous cell carcinoma: (A) TAp73 and (B) ΔNp73 expression groups.

expression levels of ΔNp73 were 100.0 and 75.6%, respectively (χ^2=3.90; P=0.048; Fig. 2B).

Discussion

Previous studies reported that the TAp73 gene mimics p53 suppressor activities, showing proapoptotic effects. However, the ΔNp73 gene was shown to have an antiapoptotic function, in which cooperation with oncogenic RAS induces cell transformation, confers drug resistance and induces the phosphorylation of retinoblastoma protein (11,12,17). The different expression levels of the TAp73 and ΔNp73 isoforms may determine tumorigenesis and resistance to chemo(radio) therapy, as the predominance of ΔNp73 may confer pro-tumorigenic properties (18). A previous study showed that the TAp73 and ΔNp73 isoforms were significantly overexpressed in a number of solid tumors compared with the corresponding normal tissues, suggesting that the balance between these two isoforms may play a role in the regulation of cell proliferation and cell death (19). Hofstetter *et al* (20) showed that TAp73 and ΔNp73 were expressed in 88.0% (73/83) and 57.8% (48/83) of ovarian cancer samples, respectively. Liu *et al* (15) reported the positive expression of TAp73 and ΔNp73 in 41.0 and 30.8% of patients with cervical cancer, respectively. Moreover, cancers that expressed a higher level of ΔNp73 tended to express a lower level of TAp73. Müller *et al* (11) also reported that TAp73 and ΔNp73 are inversely regulated and showed that the high expression of TAp73 is correlated with the low expression of ΔNp73. These results suggest that the expression of the two isoforms is upregulated via different mechanisms in different cancers. In the current study, it was found that 79.7% (47/59) and 76.3% (45/59) of patients with cervical SCC exhibited high expression levels of TAp73 and ΔNp73, respectively. However, no significant correlation was observed between TAp73 and ΔNp73 expression (χ^2=0.415; P=0.519).

With respect to the clinical significance of TAp73 expression in cancers, Castellino *et al* (21) reported that medulloblastoma patients with high expression of TAp73 showed favorable disease-free survival and overall survival times. Similarly, Liu *et al* (15) showed that TAp73 overexpression predicted a better survival in patients with cervical SCC receiving radiotherapy. However, Hofstetter *et al* (20) reported that TAp73 expression had no prognostic significance in ovarian cancer. In the present study, the 3-year OS rates in patients with low and high expression levels of TAp73 were 75.0 and 83.0%, respectively, in patients with FIGO stage I-II cervical SCC after radical surgery (χ^2=0.33; P=0.568). Further study is required to determine the prognostic significance of TAp73 expression.

Emerging evidence suggests that ΔNp73, rather than TAp73, is the main physiologically relevant component of tumor-associated p73 overexpression and that it functionally overrides the frequent concomitant increase in TAp73. Several studies have shown that a high expression level of ΔNp73 is correlated with tumor progression and poor survival rates in a number of cancer types. Uramoto *et al* (9) showed that ΔNp73 expression in lung cancer is not correlated with clinicopathological factors, including histological type, pathological tumor stage and node stage. However, lung cancer patients with high ΔNp73 expression exhibited a lower 5-year survival rate than those with low ΔNp73 expression. Similarly, Müller *et al* (11) reported that a high expression level of ΔNp73 is correlated with reduced survival in hepatocellular carcinoma patients. Liu *et al* (15) found that ΔNp73 overexpression is significantly associated with resistance to radiotherapy, disease recurrence and poor survival in patients with cervical SCC. These findings indicate that ΔNp73 may be a potential marker for predicting the prognosis and sensitivity to radiotherapy in patients with cervical SCC. In the current study, it was shown that the 3-year OS rates of FIGO stage I-II cervical SCC patients with low and high expression levels of ΔNp73 were 100.0 and 75.6%, respectively, following radical surgery (χ^2=3.90; P=0.048). Taken together, these findings suggest that ΔNp73 is associated with an unfavorable prognosis due to its role in chemo(radio) therapy resistance and tumor aggressiveness.

In conclusion, TAp73 and ΔNp73 were frequently overexpressed in the cervical SCC cells in the present study. High expression of ΔNp73 may indicate an unfavorable prognosis in early-stage cervical SCC. However, this retrospective study was potentially limited by the relatively small number of patients. Additional larger studies are required to reach a definitive conclusion.

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