EFFECTS OF p53 OVEREXPRESSION ON NEOPLASTIC CELL PROLIFERATION AND APOPTOSIS IN THYMIC CARCINOMA

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ABSTRACT

Objective: To investigate p53 overexpression and its correlation with neoplastic cell proliferation and apoptosis in 20 thymic carcinomas. Methods: 20 surgical samples of thymic carcinoma were collected randomly during the past 15 years in the Guangzhou area. Immunohistochemical staining was performed using LSAB method with anti-p53 monoclonal antibody (DO-7) and proliferating cell nuclear antigen (clone PC 10) as primary antibodies. The p53 index was indicated by the number of p53 positive cells among 100 carcinoma cells. More than 25 percentage of p53 positive cells found in tissue sections was recognized as p53 overexpression. Carcinoma cell proliferation activity was assayed by PCNA index (PI), and apoptosis degree was evaluated by TUNEL (TdT-mediated dUTP-X nick end labeling) index (TI) using Boehringer Mannheim *In Situ* Death Detection Kit. Results: P53 positive cells could be found in vast majority of thymic carcinomas (19/20) and the overexpression rate reached 35% (7/20). The median PI (40%) of 7 cases with p53 overexpression was higher than that (31%) of 13 cases without p53 overexpression, but there was no statistical significance that existed between these two data ($P>0.05$). The median TI (0.5/HPF) of 7 p53 overexpression cases was much lower than that (4.5/HPF) of 13 non-overexpression cases, and there was a significant difference statistically ($P<0.05$). Conclusion: p53 expression was a frequent finding in thymic carcinoma cells, and the p53 overexpression which might represent p53 inactivation or gene mutation was often involved in thymic carcinogenesis. The median PCNA index of p53 overexpression group was higher than that of non-overexpression group though there existed no statistical difference. This indicates that the inhibiting function of p53 on cell proliferation seemed lost in p53 overexpressed thymic carcinomas. It is worthy to be specially mentioned that the inducing function of p53 on cell apoptosis was markedly lost in p53 overexpressed thymic carcinomas. Taken together, the overexpressed p53 that could represent aberrant p53 protein had not only lost its proliferation-inhibiting but also its apoptosis-inducing function in thymic carcinomas which might play an important role in thymic carcinogenesis.

Key words: Thymic carcinoma, Proliferation, Apoptosis, p53

Wild-type p53 protein could regulate cell proliferation and apoptosis. Loss of its function might play a crucial role in tumourigenesis of some malig nancies.[1] The inactivation of p53 protein could be resulting from p53 gene mutation or binding with other cellular or viral proteins, such as mdm-2 or ZEBRA encoded by Epstein-Barr virus.[2] Thymic carcinoma is a rare thymic epithelial malignancy. Does p53 protein overexpression develop in thymic carcinomas? If so, what role does p53 overexpression play on neoplastic cell proliferation and apoptosis in this carcinoma? The authors collected 20 thymic carcinomas during the past 15 years in the Guangzhou area and investigated the issues mentioned-above.

MATERIALS AND METHODS

Materials

Formalin-fixed paraffin-embedded specimens of 20 thymic carcinoma were randomly selected from 5

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hospitals in Guangzhou for this study during the period from 1982 to 1997.

Methods

P53 protein expression was detected by use of anti-p53 monoclonal primary antibody DO-7 immunohistochemically. Neoplastic cell proliferative activity was indicated by proliferating cell nuclear antigen (PCNA) index that was detected by primary antibody PCNA immunohistochemically. The primary antibodies and LSAB kit were manufactured by DAKO Company. The working dilution used for DO-7 and PCNA were both 1:100. Apoptotic cells were detected by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) method using "*In Situ* Cell Death Detection Kit" of Boehringer Mannheim Company (Cat. No. 1 684 809). The working procedures followed the kit instructions.

The numbers of p53 and PCNA positive cells were calculated within at least 1,000 epithelial neoplastic cells for every case and were indicated as p53 expression index and PCNA index (PI), respectively. p53 overexpression was termed when p53 expression index was >25%. The TUNEL index (TI) was valued by the number of apoptotic epithelial neoplastic cells per high-power field (10×40) on the average.

RESULTS

The patients with thymic carcinoma studied consisted of 15 men and 5 women with a median age of 41.5 years. Five of them had myasthenia gravis symptom. All of the 20 tumor masses had infiltrated and adhered to the surrounding tissues with a 9.6 cm mean maximal diameter. Metastasis to lungs, pleurae and/or diaphragm was found in six cases. According to the cytohistological standards described in "Ackerman's Surgical Pathology",[3] the histological types of these 20 thymic carcinomas were of squamous cell carcinomas (9 cases), lymphoepithelioma-like carcinomas (7 cases, Figure 1), undifferentiated carcinoma (1 case), adenocarcinoma (1 case), adenosquamous carcinoma (1 case), and mixed carcinoma (1 case, with a mixed appearance of squamous cell carcinoma, lymphoepithelioma-like carcinoma and carcinoid-like features).

The ranges of p53 expression (Figure 2) index, PCNA (Figure 3) index and TUNEL (Figure 4) index were 0–90%, 0.05–95% and 0–16/HPF, and the medians were 5.8%, 34.5% and 3.38/HPF, respectively.

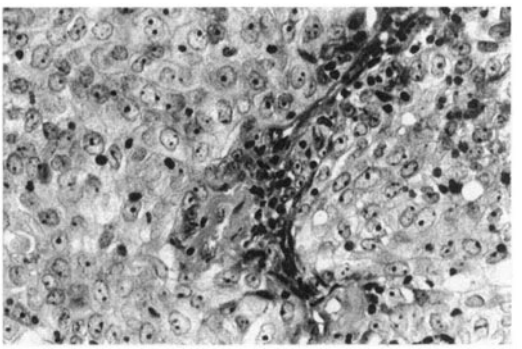

Fig. 1. Lymphoepithelioma-like carcinoma of the thymus, H & E ×100.

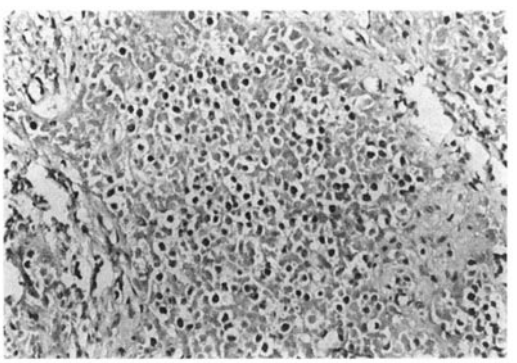

Fig. 2. Poorly differentiated squamous cell carcinoma of the thymus showing many p53 nuclear positive neoplastic cells. LSAB IHC using anti-p53 DO-7 primary antibody, ×50.

Table 1. Comparison of PCNA index and TUNEL index in p53 over- and non-over-expression groups

P53 index	n	PCNA index		TUNEL index	
		Median	Range	Median	Range
>25%	7	40.0	8.0–95.0	0.5	0.0–3.0
≤25	13	31.0	0.05–75.0	4.5	0.0–16.0
Total	20	34.5	0.05–95.0	3.4	0.0–16.0

The median PCNA index was 40% in 7 cases with p53 overexpression and 31% in 13 cases without p53 overexpression.

There was no positive or negative rank correlation

between PCNA index and p53 expression index (r_S= 0.3842, P>0.05). The median PCNA index (40%) of 7 p53 overexpression cases was higher than that (31%) of 13 p53 non-overexpression cases although there was no statistical significance (r_S= 0.3842, P>0.05). A significant negative correlation was found between p53 expression index and TUNEL index by rank correlation test (r_S= –0.5030, P<0.05). The median TUNEL index (0.5/HPF) in 7 p53 overexpression cases was significantly lower than that (4.5/HPF) in 13 p53 non-overexpression cases. The authors also studied the correlation between PCNA index and TUNEL index and found no significant difference (r_S=–0.1038, P>0.5) (Table 1).

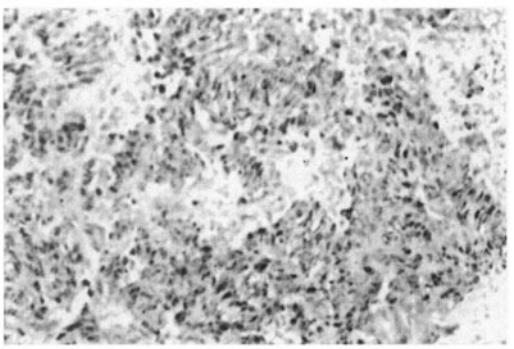

Fig. 3. Spindle cell carcinoma of the thymus showing many PCNA positive neoplastic cells. LSAB IHC using PCNA primary antibody, ×50.

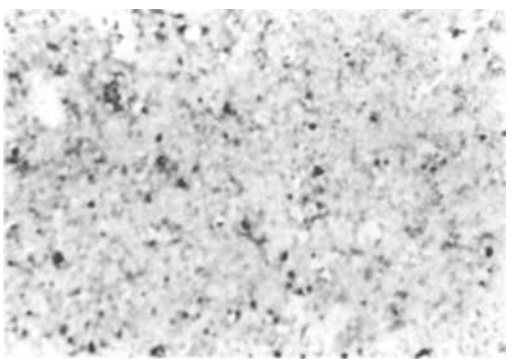

Fig. 4. Poorly differentiated squamous cell carcinoma of the thymus showing apoptotic cells, TUNEL ×50.

DISCUSSION

It is popularly recognized that there are two principal functions of wild-type p53 protein, namely, arresting the cells in G1 phase and inducing cell apoptosis in response to DNA damage.[4] By the logic of events, the cell population with functional inactivation of p53 protein would alter normal regulation of cell cycle and result in cancer formation. Indeed, a number of papers concerning the carcinogenic role of inactivated p53 protein playing in malignancies have been reported.[1] The inactivation of p53 protein may resulting from gene mutation or the forming of a protein complex with other cellular or viral proteins. Thymic carcinoma is an uncommon tumor. So far as the authors know, only a few papers about the effects of p53 overexpression in thymic carcinoma have been reported up to date.[5–11] The authors herein report that 95% of thymic carcinoma (19/20) had p53 positive neoplastic cells found in tissue sections, and the p53 overexpression rate reached 35% (7/20). These data were consistent with those reported by other researchers,[5–9] so it could be concluded that both p53 expression and overexpression are not infrequent events in thymic carcinomas. If the p53 overexpression detected by immunohistochemistry could represent the p53 gene mutation, then p53 mutation might play a crucial role in thymic carcinogenesis. This point of view coincided with the findings of Tateyama that the p53 gene in thymic carcinomas always underwent point mutation.[9] In addition, Oyama reported that detection of p53 gene mutations is useful as a diagnostic factor in thymic carcinoma.[6] Accordingly, we suggest that immunohistochemical detection of p53 protein might be also a useful mark for diagnosis of thymic carcinoma.

The results showed that the median PCNA index (40%) of the p53 overexpression group was higher than that (31%) of the non-overexpression group though there was no statistical difference. However, the enhancing effect of inactivated p53 protein seemed still present in the 20 thymic carcinomas. How to interpret the result basing upon the above statistical analysis? First of all, the number of thymic carcinomas with p53 overexpression investigated might be too small (only 7 cases) to reveal the existing correlation between p53 and cell proliferation. Furthermore, as we known, the wild-type p53 protein exerts its function on cell proliferation through a series of molecular events, such as transactivation of cyclin-dependent kinase inhibitor p21 WAFl/CIPl, phosphorylation of pRB, and others. That is to say, cell proliferation is a very complex process and is regulated by multiple related proteins. It is the overall balance between these cellular factors that determine whether a cell remains resting or dividing. Mutation or aberrant expression of such proteins is also able to influence the cell proliferation. In this study, the authors have not investigated other cell proliferation-associated proteins, therefore can not give a complete story concerning the neoplastic cell proliferation events that happened in these 20 thymic carcinomas. In addition, new opinions have been reported about the functions of wild-type p53 protein recently. For example, the p53 expression could convert p21WAF1/CIP1-mediated growth arrest into apoptosis, and p53-mediated apoptotic pathway is dominant over the growth arrest pathway in cell cycle regulation in many human cancer cell lines.[12] It was also

reported that G1 arrest was relaxed or absent in comparison with arrest in normal diploid fibroblasts, despite presenting of seemingly normal p53 and p21WAF1/CIP1 responses in most wild-type p53 cancer cell lines.[13] So, the issue concerning the effect of p53 overexpression on neoplastic cell proliferation in thymic carcinoma is worthy to be further clarified.

It is recognized that the p53 as an important upstream regulation gene can transactivate the expression of p21WAF1/CIP1 and bax, as well as downregulate bcl-2, thus induce apoptosis.[14] The data obtained in this study showed that the median TUNEL index of p53 over-expression group (0.5/HPF) was significantly lower than that (4.5/HPF) of p53 non-overexpression group. This meaningful result clearly demonstrated the effect of wild-type p53 protein on cell apoptosis itself in thymic carcinomas. The wild-type p53 protein presented in 13 thymic carcinomas without p53 overexpression and exerted its apoptosis inducing function. On the other hand, the p53 protein might be mutant type in 7 thymic carcinomas with p53 overexpression, and had lost the function to induce cell apoptosis.

In conclusion, the alteration of p53 tumor suppressor gene is a critical event in thymic carcinogenesis and the immunohistochemical detection of p53 protein could be useful in pathological diagnosis for thymic carcinoma. The overexpressed p53 presented in thymic carcinomas may have lost its functions of regulating proliferation and apoptosis.

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