MICROBIOLOGY AND IMMUNOLOGY

Immunoreactivity of p53 Nuclear Protein in Differentiated Thyroid Cancer

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 122, No. 12, pp. 645-647, December, 1996
Original article submitted August 10, 1995

The study is an attempt to assess the relationship between the immunoreactivity of nuclear p53 and the classic factors predicting an unfavorable outcome of papillary thyroid cancer. Thyroid tissue from Russian and American patients with highly differentiated papillary cancer is examined. A positive immunoreactivity of p53 is detected more frequently in patients with an unfavorable prognosis (age >50 years, the presence of nondiploid DNA).

Key Words: *tumor gene suppressor; tumor differentiation; papillary thyroid cancer; p53*

Early diagnostics of thyroid cancer became particularly important after the Chernobyl disaster, when the concentration of radioactive iodine isotopes in the environment increased considerably. The thyroid gland is the major target organ for these isotopes [11]. It has been generally recognized that thyroid cancer is provoked by changes induced by exogenous factors in thyroid epithelium at the molecular level [6]. In order to assess the relationship between some clinical factors and the immunoreactivity of p53 and evaluate the role of a geographical factor, we compared the expression of p53 in patients from Russia and North America. The tumor suppressor gene p53 plays a key role in the regulation of normal cell proliferation, preventing the accumulation of mutations in somatic cells [8]. If DNA is damaged by ionizing radiation, the concentration of p53 increases, and cells remain in the G-0 phase [4,8]. This allows for DNA regeneration by blocking the transfer of a mutant gene to the daughter cells [5]. The loss of normally functioning p53 is linked with transformation of normal cells into tumor cells [6,10]. Mutations of the p53 gene often occur in patients with thyroid cancer, including the nondifferentiated variant [3,9]. Cancer cells are characterized by a high level of p53, which can be measured by immunohistochemical methods [2,7,12].

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Accumulation of p53 is associated with the dedifferentiation of papillary thyroid tumors [1,3,4]. Bearing in mind the relationship between the accumulation of p53 and the degree of differentiation of papillary cancer, we decided to assess the relationship between the expression of immunoreactive p53 and classic risk factors of unfavorable tumorigenesis (metastases, relapses, etc.): age more than 50 years and decreased ploidy of DNA.

MATERIALS AND METHODS

Tissue specimens from 28 patients aging 24-70 years (mean age 47±2 years) with papillary thyroid cancer from Russia and of 27 patients aging 17-80 years (mean age 48±0.5 years) from the USA were studied. The specimens were collected during surgery. They were fixed with formalin for 24 h and embedded in paraffin. The expression of p53 was evaluated by a modified avidin-biotin peroxidase technique [7]. Tissue samples were incubated for 12 h at 40°C with primary antibodies (DO-1 murine monoclonal antibody against the denaturation-resistant sites located near the N-terminals of normal and mutant human p53). The antibodies were omitted from the negative control preparations. Three patterns of staining were observed: diffuse, regional, and focal. A tumor was re-

0007-4888/96/0012-1208$15.00

TABLE 1. Comparative Characteristics of Thyroid Cancer in Russian and American Patients

Case characteristics	USA (Albany)	Russia (Moscow)
Age, years	48±0.5	47±2
Sex:		
male	3	1
female	24	27
Histology:		
papillary cancer	10	8
papillary follicular cancer	10	12
follicular variant	6	7
poorly differentiated cancer	1	1
Remote metastases	None	None
Positive immunoreactivity of p53	2 out of 27	8 out of 28
DNA index (ploidy):		
>1.08	9	6
<1.08	18	22

garded as p53-hyperexpressive only when the staining was sufficiently intense in two tissue samples and moderate in the third one. All other cases were regarded as p53-negative. DNA ploidy was studied on 5-μ thick sections [8]. At least 100 cells were analyzed to obtain a histogram. The DNA index of tumor cells was compared with that of the control diploid cells; the index >1.08 indicated that the cell was nondiploid. DNA was isolated from thyroid tissue, and 5-9 p53 exons were amplified in polymerase chain reaction (PCR) [12]. The results were analyzed using Fisher's ($p<0.025$) and Student's tests ($p<0.05$).

RESULTS

Immunoreactive p53 was detected in 8 (30%) out of 28 Russian patients and in 2 (7%) out of 27 American patients ($p<0.03$, Student's test, Table 1). Regional staining for p53 was observed in 1 sample of highly differentiated papillary cancer from Russian patients, while in 7 other specimens the staining was focal. In none of these samples were interstitial cells adjacent to the malignant focus stained positively for p53. In one specimen from an American patient, the lymph node containing a metastatic focus of poorly differentiated papillary cancer showed diffuse staining for p53, in all other samples the staining was focal. Analysis of DNA ploidy showed 6 (21%) nondiploid cases in the Russian group and 9 (33%) in the American group. Patients older than 50 years with nondiploid set of DNA in tumor cells ($n=24$) were assigned into the group with a high risk of unfavorable prognosis. The low-risk group included patients under 50 with diploid set of DNA. In 33% of the high-risk cases, immunoreactive p53 was located in cell nuclei, while in the low-risk group it was observed only in 6% of cases ($p<0.015$). Six percent of p53-positive cases were not associated with any risk factor, 31% were associated with one risk factor, and 38% with two risk factors ($p<0.021$). Investigation of DNA sites with high frequency of point mutations of p53 exons 5-9 by PCR revealed no mutations, which agrees with the literature data [6], confirming the low occurrence of such mutations in differentiated thyroid cancer.

A statistically significant difference was revealed in the expression of p53 in Russian and American patients with differentiated papillary cancer. In the American group, the occurrence of immunoreactive p53 was 7%, while in the Russian group it was 30%. This difference may be due to different ecological situations in Russia and USA. "Geographic" variations of the expression of immunoreactive p53 have been documented by other researchers. Presumably, the expression of p53 is associated with radioactive contamination and iodine deficiency [6,7,11]. Thus, the development of papillary thyroid cancer should be regarded in connection with exogenous and endogenous factors.

From our results it can be concluded that: 1) the expression of p53 tumor suppressor protein is increased in papillary thyroid cancer, which may be due to accumulation of mutant forms of the protein; 2) the age above 50, expression of immunoreactive p53, and the presence of nondiploid DNA are linked with unfavorable prognosis; 3) the expression of p53 in papillary thyroid cancer is different in patients living in different geographical regions.

The study was supported by grants from Lucille P. Markey Charitable Trust and Boots company (USA).

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