OVEREXPRESSION OF P53 PROTEIN IN MALIGNANT BREAST TUMORS: AN IMMUNOHISTOCHEMICAL STUDY

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Abstract - The P53 protein is expressed in all normal cells and appears to function in cell cycle regulation. Abnormally high levels of the protein are found in many different types of cancer. In breast cancer, overexpression of P53 is associated with point mutations within highly conserved regions of the P53 gene. These altered genes encode stable P53 protein that can be detected by standard immunohistochemical techniques.

In this study, we examined 47 cases of primary breast carcinoma for the presence of P53 protein using immunohistochemistry methods employing monoclonal antibody against the clone, DO-7. Of these specimens, 25.5% had widespread overexpression of P53. A significant positive correlation waz found between P53 overexpression and younger age ($P<0.05$). There was a tendency for P53 overexpression in premenopausal women and the higher tumor grades, althought these did not acheive significance. The P53 overexpressoin was not correlated with tumor size, tumor type, nodal status and side of involved breast. The overexpressoin of P53 may itself be a prognostic factor in human breast cancer.

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INTRODUCTION

Breast cancer is the most common cancer among women in developed countries (1). The tumor suppressor gene P53 is one of the most commonly mutated genes in human cancer of all types (2). Mutations in the P53 gene have been proposed to occur in 16-46% of breast cancer and appear to be a strong indicator of poor prognosis, independent of other risk factors (3, 4, 5). The P53 protein is a nuclear phosphoprotein normally expressed at very low levels in all human cells and serves to regulate cell growth and division (6). This protein is a transcriptional activator that binds to specific DNA sequences in the control regions of genes, influencing their expression. This leads to expressoin of specific genes necessary for inhibition of cell growth or, alternatively, apoptosis (7). Recent studies indicate that the P53 protein is involved in gene transcription, DNA synthesis and repair, senescence, genomic plasticity, and in programmed cell death (8-13). In humans, the P53 gene is localized on chromosome 17 p 13.1 with 16-20 kilobase (kb) length. The presence of P53 gene mutations has been observed with varying degrees of incidence in a broad range of primary tumors, including carcinomas of lung (15), pancreas carcinomas (16), carcinomas of the colon (17, 18), squamous cell carcinomas of the larynx (19), malignant gliomas (20) and above all, breast carcinomas. In addition, P53 mutations have been found to occur in germ cell lines; these have been identified in female patients with the hereditary Li-Fraumeni syndrome, the principal feature of which is the occurrence of breast carcinoma (21). The wild-type P53 has an extremely short half-life (15-20 min) but the mutant P53 protein is much more stable, with a half-life of 4-12h, and it accumulates in the nucleus. It is therefore possible to perform an immunocytochemical diagnosis in tumor tissue to visualize this nuclear accumulation directly. The immunohistochemical procedure for determining the presence of P53 in paraffin-embedded tissue sections requires the application of monoclonal

antibodies that are of high specificity and affinity, and thus exhibit no cross-reactivities (14). The aim of the present study was to assess the relationship between the overexpression of P53 and breast carcinoma in Iranian patients compared with those reported from other countries and to investigate its association with other clinicopathologic parameters in patients with primary breast cancer.

MATERIALS AND METHODS

The study is based on forty-seven females with primary breast cancer and specimens were from biopsies or mastectomies performed at the Cancer Institute of Imam Khomeini Hospital between April 1996 and April 1998. The patients included both premenopausal and postmenopausal females. In addition to menopausal status, clinical information was collected regarding age, tumor grade, tumor size, histological type and nodal status. Patients ranged form 25 to 76 years (mean 51 years). At the time of diagnosis, 22 patients were premenopausal, and 25 were postmenopausal. Samples embedded in paraffin were routinely cut and placed on microscope slides. A section from each block was stained with hematoxylin and eosin and analyzed independently to confirm the nature of each specimen prior to immunohistochemical staining. After drying, the sections were deparaffinized, washed, and then incubated with 3% H_2O_2 to block endogenous peroxidase. After washing in phosphate-buffered saline (PBS, PH=7.4 ± 0.05), the paraffin sections were put into 10 mM citrate buffer (PH=6.0) and heated in an Autoclave at 120°C and 20 bar for 5 min for heat-mediated antigen retrieval. Cooling and washing were followed by incubation with protein blocking agent (Merck, Germany) to inhibit nonspecific binding. For the demonstration of P53 protein, we used a monoclonal antibody (Isotype: Ig G2b, kapp) against the clone DO-7 (Denmark) diluted 1:50 with PBS. The sections were incubated overnight, rinsed whith PBS and then incubated with a biotinylated secondary antibody for 30 min (Merck, Germany), followed by further incubation with horseradish peroxidase-conjugated streptavidin (Merck, Germany; 30 min). After washing with PBS, the site of antigen-antibody reaction was revealed by means of the strept AB complex/HRP method with diaminobenzidine (Abbott, Chicago, USA) for 10 min. After washing, counterstaining of tumor cell nuclei was performed using Harris's hematoxylin for 1 min. The section was washed with distilled water. Positive and negative controls were performed for both immunohistochemical staining procedures. All sections were analyzed independently by two observers at the light-microscope level, before comparing the results. Any specimen for which uncertainty or disagreement existed was restained. All specimens were divided as follows:

- Negative sections exhibited either the complete absence of any immunohistochemical staining reaction or a weakly positive staining reaction in which less than 10% of the tumor cell nuclei showed staining.
- Positive sections exhibited nuclear staining in ≤10% of the tumor cell nuclei (14).

Comparisons of the distributions of categorical variables among patients with wild-type and overexpression of P53 with respect to mean age of patients were performed. A P-value of less than 0.05 was considered to be significant.

RESULTS

In the present study, specimens from 47 patients were confirmed to be malignant by surgical pathologic examination. Of these, 40 tumors were infiltrating ductal carcinoma (IDC), 4 were infiltrating lobulary carcinoma (ILC), one was invasive breast carcinoma (IBC), 1 was medullary carcinoma (MC), and 1 was comedo carcinoma (CC). Thirteen cases were lymph node negative (25.3 percent), and in 33 cases (71.7

percent) nodes were involved. In 1 case, axillary sampling had not been performed. Also, 2 tumors (4.3 percent) were grade I, 25 tumors (53.2 percent) were grade II, and 20 (42.5 percent) were grade III carcinoma.

In this study, of the total of 47 primary breast tumors investigated, 35 (74.5 %) exhibited negative immunohistochemical staining of P53 (i.e., staining of<10% of tumor cell nuclei). The other 12 tumors (25.5%) demonstrated clearly positive staining (i.e., 10-100% of tumor cell nuclei; Table 1).

Table 1. Retationship between P53 overexpression and histologic type of breast cancer

Histologic type	P53 No.	Negative %	P53 No.	Positive %
Infiltrating ductal carcinoma (n=40)	30	75	10	25
Infiltrating lobular carcinoma (n=4)	3	75	1	25
Invasive breast carcinoma (n=1)	1	100	0	0
Medullary carcinoma (n=1)	1	100	0	0
Comedo carcinoma(n=1)	0	0	1	100
Total(n=47)	35	74.5	12	25.5

Evaluation of the results was not complicated by the presence of nonspecific staining. There was no statistically significant relationship between P53 mutations and the various histologic types of breast cancer in this study (Table 1). Similarly, no correlation, was found with tumor size, nodal status and side of involved breast (Table 2). There was a tendency for P53 positive tumors in the premenopausal women, although this did not achieve significance (34.8% $P53^+$ in premenopause vs. 16.7% $P53^+$ postmenopause, $X^2=2.03$, $P=0.154$). The higher tumor grades contained a larger proportion of P53 positive tumors (grade I, 0/2; grade II, 8/25 and grade III, 4/20; $X^2=1.56$, $P=0.46$). Overexpression of P53 showed a significant correlation with younger age (t-Value = 2.07, DF=45, P=0.044, Table 2).

Table 2. Relationship between P53 overexpression and clinical parameters in breast cancer

	Total	P53 No.	Negative	P53 No.	Positive %	P-value
Nodal status						
Negative	13	10	76.6	3	23.1	
Positive	33	25	75.8	8	24.2	0.61
Tumor type						
Ductal	40	30	75	10	25	
Lobular	4	3	75	1	25	
Other	3	2	66.7	1	33.3	0.53
Tumor size (cm)						
<1	4	4	100	0	0	
1-3	26	17	65.4	9	34.6	
3-5	11	10	90.9	1	9.1	
>5	3	2	66.7	1	33.3	0.47
Histologic grade						
1	2	2	100	0	0	
2	25	17	68	8	32	
3	20	16	80	4	20	0.46
Menopausal status						
Pre (≤50 yr.)	23	15	65.2	8	34.8	
Post (>50 yr.)	24	20	83.3	4	16.7	0.154
Side of involved breast Left	28	21	75	7	25	
Right	18	13	72.2	5	27.8	0.49
Average age at diagnosis (yr) (Mean ± SD)	47	35	53.14±12.11	12	44.75±12.66	0.044

DISCUSSION

The nuclear phosphoprotein P53 appears to play a role in the regulation of cell proliferation. It is expressed in all cells, late in the G_1 phase of the cell cycle and may regulate the entry of cells into the S phase (DNA synthesis) (22). Failure to regulate P53 expression may lead to uncontrolled cell growth. Direct evidence for the oncogenic capacity of P53 has come from the demonstration that P53 can be immortalized and, in cooperation with an activated ras oncogene, transform rodent cells invitro (23). However, only mutant forms of the P53 protein are capable of cell transformation (24). Furthermore, normally expressed wild-type P53 may actualy have a tumor suppressor effect (25).

Overexpression of P53 appear to be among the most common molecular alterations in human cancer. High levels of P53 expression have been

detected in many different types of primary adult carcinomas, including breast, lung and colorectal cancers (26-28).

Immunohistochemical analysis of primary breast tumors appears to be an accurate method of screening for the presence of P53 protein overexpression.

We have confirmed the expression of elevated of P53 in 12 (25.5%) out of 47 tumors. The finding of other authors were 16 to 46% in different countries.

Application of X^2-test revealed the absence of any correlation between the accumulation of P53 in tumor cell nuclei and the established prognostic parameters, tumor size and lymph node status. Most studies, including the present one, didn't find a correlation between P53 overexpression with tumor size (14, 26, 29 - 32). In fact, we and several other investigators have demonstrated P53 overexpression in ductal carcinoma in situ, particularly the comedo type, by immunohistochemical or genetic methods (33-35).

This study showed a tendency for P53 positive tumors to occur in the premenopausal period, although the results did not achieve significance. We found a significant correlation of mutant P53 with younger age. Similar results were obtained by other investigators in the past years (26, 29, 31, 33, 36) in their immunohistochemical analysis of P53 expressoin in breast cancer. In contrast, Thompson and coworkers (30, 37) and Isola and coworkers (38) did not find a correlation with age. In this series of tumors, poorly differentiated cancers contained P53 overexpression more frequently than well-differentiated cancers, although this did not achieve significance (Table 2). Similar results were obtained by Caleffi (31) and Mazars (39). In contrast, several investigators found significant correlations between P53 overexpressoin and poor histologic differentiation (14, 26, 40). No correlation was observed between P53 expressoin and lymph node status, suggesting that P53 does not relate to tumor progression.

The overexpression of P53 in breast cancer suggests that P53 may plays a major role in the carcinogenic activity.

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