The Korean Journal of Pathology
2005; 39: 313-9

Mutational and Loss of Heterozygosity Analysis of the p53 and PTEN Tumor Suppressor Genes in Breast Carcinoma

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Received : June 15, 2005
Accepted : August 10, 2005

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*This work was supported by a 2003 research grant from Chungnam National University.

Background : Although the genetic determinants of most sporadic breast cancers remain unknown, the understanding of the molecular and genetic events that contribute to breast carcinogenesis has been significantly advanced. We investigated the clinicopathologic significance of allelic imbalance or mutation of both p53 and PTEN tumor suppressor genes in sporadic breast carcinomas. **Methods :** Genomic DNA from 62 breast carcinoma cases was extracted from paraffin blocks, and PCR was performed to determine loss of heterozygosity (LOH) for DNA markers around the p53 and PTEN genes and to amplify exons 5, 6, 7, and 8 of p53 and all 9 coding axons of PTEN. **Results :** Somatic p53 mutations were detected in 6 (9.7%) of the 62 cases. LOH for DNA markers surrounding p53 was observed in 18 (29.0%) of the 62 cases. LOH for DNA markers surrounding PTEN was detected in 29 (46.8%) of the 62 cases. Only one case (1.6%) showed somatic PTEN mutations. Tumors with LOH on 17p or p53 mutation were large in size and negative for ER, had a high Ki-67 index, and exhibited p53 immunoreactivity ($p<0.05$). Tumors with LOH on 10q23 were associated with c-erbB-2 positivity ($p=0.018$). **Conclusions :** Our results indicate that LOH at 17p and/or p53 mutation is significantly associated with the aggressive pathologic parameters of breast cancer.

Key Words : Carcinoma, Breast; Genes p53; PTEN protein; Mutation; Loss of heterozygosity

Sporadic breast cancer with no previous associated family history is a major form of the disease. Although the genetic determinants of most sporadic breast cancers remain unknown, much is now known about the molecular and genetic events that contribute to breast carcinogenesis. Several genes, including BRCA1, BRCA2, and p53, confer susceptibility to familial forms of breast cancer.[1-3]

Breast cancer development is thought to be the result of recessive tumor suppressor gene (TSG) inactivation by somatic alterations, as in other types of tumors.[4] The p53 gene is located on the short arm of chromosome 17. p53 mutation is the most common genetic abnormality so far identified in human cancer and multiple loci on 17p are known to be involved in the development of breast cancer. In fact, p53 mutation/alteration is present in up to 50% of primary breast carcinomas.[5,6] Some studies have shown that alterations in p53 are associated with a poor prognosis in breast cancer, including node-negative disease.[7,8]

PTEN (phosphatase and tensin homologue deleted from chromosome 10) or MMAC1 (mutated in multiple advanced cancer) has been identified on chromosome 10q23 as a candidate TSG.[9] PTEN is somatically mutated in association with a frequent loss of heterozygosity (LOH) at 10q23.3 in a variety of tumors including advanced prostate cancers, thyroid cancer, breast cancer, and endometrial cancer.[9-12] In breast carcinomas, alterations related to the PTEN gene, including LOH in the 10q23 chromosomal region, have been reported in 30-40% of cases. However, point mutations in these gene sequences have been detected in only a small fraction (0-6%) of these primary breast cancers.[13-18] Previous studies have revealed that the result of LOH of the PTEN locus in breast carcinoma is a poor pathophenotype, although this result is controversial.[14-17]

To determine the role of both p53 and PTEN TSGs in breast carcinogenesis, we analyzed 62 primary breast carcinomas for LOH on 10q23 and 17p13, and for mutation in the exons 5-8 of p53 and all 9 coding exons of PTEN. Correlations of allelic imbalance and mutation of p53 and PTEN TSGs with clinicopathologic parameters predictive of an aggressive biologic behavior were also investigated.

MATERIALS AND METHODS

Materials

Sixty-two breast carcinomas were retrieved from the files of Chungnam National University Hospital, Daejeon, Korea. All 62 cases were invasive ductal carcinomas. Patient's ages ranged from 26 to 82 years. Histopathological slides were reviewed by two pathologists.

Methods

Immunohistochemistry

All immunohistochemical studies of ER, PR, p53, Ki-67, and c-erbB2 were performed on 4-μm sections of formalin-fixed, paraffin-embedded tissues. Prediluted antibodies against ER (Zymed Lab., San Francisco, CA, USA), Ki-67 (Zymed Lab., San Francisco, CA, USA), PR (Novocastra Lab., Newcastle, UK), p53 (Zymed Lab., San Francisco, CA, USA), and c-erbB-2 (Dako, Denmark) were obtained from commercial sources.

Immunohistochemical staining protocols for each antibody are summarized in Table 1. Immunolocalization was performed using a LSAB kit (Dako, Carpinteria, CA, USA). All assays were carried out prospectively in a automated immunostainer (Dako, Carpinteria, CA, USA). Antibody-antigen reactivity was visualized using diaminobenzidine and counterstaining with Meyer's hematoxylin.

The positive controls for ER and PR were adjacent normal breast lobules and for p53, Ki-67, and c-erbB-2 was breast carcinoma tissue with strong staining from another study. Tris-buffered saline was used instead of primary antibody in negative controls.

Positive staining was defined as nuclear staining in more than 5% of tumor cells for p53, ER, and PR, and in more than 20% for Ki-67. Scoring of c-erbB-2 membrane staining was based on a Dako Hercept test (Dako, C.A.). Positive staining was defined as intermediate or strong membrane staining in more than 10% of the tumor cell population, whereas weak staining (<10% of the tumor cell population) and cytoplasmic staining were considered to be negative. A percentage of immunopositive cells was semiquantified and calculated manually.

Table 1. Summary of immunohistochemical staining protocols

Primary antibody	Company (Country)	Dilution	Reaction time (min)	Pretreatment temp (°C), time (min)
ER	Zymed Lab. (USA)	1:70	90	Autoclave (121, 10)
PR	Novocastra (UK)	1:150	90	Autoclave (121, 10)
p53	Dako (Denmark)	l:70	60	Autoclave (121, 10)
Ki-67	Zymed Lab. (USA)	1:150	60	Autoclave (121, 10)
c-erbB-2	Dako (Denmark)	1:150	70	Autoclave (121, 10)

DNA extraction

All tumor samples were from formalin-fixed, paraffin-embedded tissue samples. Tissue blocks were required to contain at least 85% neoplastic cells. In order to meet this requirement, tumor areas were dissected from the surrounding normal tissues. DNA samples from breast carcinoma and normal breast tissue pairs were prepared. A total of 1 to 3 microdissected 5 μm sections of formalin-fixed, paraffin-embedded tumor tissue were incubated at 52°C for one or two days in 400 μL of DNA extraction buffer (0.25 μg/μL proteinase K (Roche, Germany), 20 mM Tris/HCl, pH 8.3, 5 mM $MgCl_2$, 100 mM KCl, 1% Tween-20, and 1% NP-40). The mixture was boiled for 10 min to inactivate proteinase K, followed by phenol extraction for purification, and concentration by ethanol precipitation. The isolated DNA solution was quantified spectrophotometrically.

PCR amplification of the p53 and PTEN genes

We analyzed exons 5 to 8 of the p53 gene and all nine exons of PTEN. The sequences of the required primers for the amplifications of exons 5 to 8 of the p53 gene and of exons 1-9 of PTEN have been previously described.[19,20] Exons 5 to 8 of the p53 gene and all nine exons of PTEN were amplified separately,

Table 2. Primer sets for p53 and PTEN genetic analysis

Primer	Sequence (5′→3′)
D17S654-S	FAM-GACCTAGGCCATGTTCACAGCC
D17S654-AS	GACATCCATTGGCACCACCCCAA
D17S796-S	FAM-CAATGGAACCAAATGTGGTC
D17S796-AS	AGTCCGATAATGCCAGGATG
D17S799-S	FAM-ATTGCCAGCCGTCAGTT
D17S799-AS	GACCAGCATATCATTATAGACAAGC
D10S1765-F	FAM-ACACTTACATAGTGCTTTCTGCG
D10S1765-R	CAGCCTCCCAAAGTTGC
D10S1696-F	FAM-TCCTGGGTGACAGAGTGA
D10S1696-R	GAGACAGCATTTCCATTATGA
D10S541-S	6-FAM-AAGCAAGTGAAGTCTTAGAACCACC
D10S541-AS	CCACAAGTAACAGAAAGCCTGTCTC
D10S564-S	6-FAM-TGGGAATGTGTCTTTATCCA
D10S564-AS	AGCTCTAACATAGAGGCCAGAT
D10S1739-S	6-FAM-CTGGAAAAACAACAGAGGTG
D10S1739-AS	GCTGTCTAAATCAAGGAATGTC

purified, and subjected to direct sequence analysis. PCR amplification of p53 exons 5-7 and all nine axons of PTEN were performed in a total volume of 20 μL containing 500 ng of template DNA, one unit of ExTaq polymerase (Takara, Japan), 1.25 mM dNTP, 15 pmole primers, and 2 μL of 1×reaction buffer. PCR amplification of p53 exon 8 was performed under the same conditions as exons 5-7 except that the dNTP concentration was 2.5 mM dNTP. The PCR cycles consisted of 5 min at 94℃ followed by 35 cycles for 30 sec at 94℃, 30 sec at 55℃, and 30 sec at 72℃, followed by one cycle for 7 min at 72℃.

SSCP analysis, Silver staining, and Direct DNA sequencing

Two microliters of PCR product was mixed with 6 μL of sample loading buffer containing 95% formamide (deionized), 10 mM NaOH, 0.25% Bromophenol blue, and 0.25% Xylene cyanol. The samples were denatured for 3 min at 100℃ and quickly chilled on ice. They were then loaded onto 12% polyacrylamide gel containing 1×sample buffer (33mM Tris-sulfate, 7% Glycerol, pH 8.3), and were electrophoresed at 250 V. Subsequently, gels were disassembled from the glass plate, then stained by using a Silver Stain Plus kit (BIO-RAD, USA), and air dried. Samples with abnormal bands were automatically sequenced on a Genetic analyzer (ABI, USA).

LOH analysis (Genetic analyzer method) for microsatellite polymorphisms

The method used for LOH analysis has been published previously.[20] Fluorescent microsatellite oligonucleotide primers were used. Three microsatellite markers flanking the p53 locus were used to assess LOH: D17S654, D17S796, and D17S799 (Table 2). Five microsatellite markers flanking the PTEN locus were used to assess LOH: D10S1765, D10S1696, D10S541, D10S564, and D10S1739 (Table 2). All primers used in this study were obtained from GenoTech (Daejeon, Korea).

Table 3. Mutations in the p53 and PTEN genes in breast carcinomas

Case No.	Gene-Exon	Base change	Amino acid change	Consequence
6	p53-6	AAC→AGC	Asp→Ser	Missense
11	p53-6	CTT→CGT	Leu→Arg	Missense
14	p53-6	GAG→GGA*	Glu→Gly	Frameshift
17	p53-5	CCC→TCC	Pro→Ser	Missense
40	p53-6	AGA†→AAA	Arg→Lys	Frameshift
43	p53-8	GCC→GCT	Ala→Ala	Silence mutation
56	PTEN-6	TAT→TAA	Tyr→Stop	Nonsense

*G addition, †AG deletion.

Statistical analysis

The associations between allelic imbalance and mutations of p53 and PTEN TSGs' and clinicopathologic parameters predictive of an aggressive biologic behavior were analyzed using Fisher's exact test. The associations between genetically alterated p53 and PTEN genes and histologic grade were analyzed by using Pearson's χ^2 test for trends. Results were considered to be statistically significant when the p-value was less than 0.05.

RESULTS

Somatic mutations in exons of 5 to 8 of p53 were detected in 6 (9.7%) of the 62 cases, of which 3 were missense (Fig. 1, 2), 2 were frame shift mutations, and one was a silent mutation.

Table 4. Correlations between clinicopathologic parameters and LOH at 17p or mutation of p53

Clinicopathologic parameters	LOH at 17p or mutation of p53 (%)		Total No. (N=62)	p-value
	Positive (N=23)	Negative (N=39)		
Age				0.3503
<35	3 (4.8)	2 (3.2)	5 (8.8)	
≥35	20 (32.3)	37 (59.7)	57 (91.9)	
Histologic grade				0.0244
Well	2 (3.2)	9 (14.5)	11 (17.7)	
Moderately	8 (12.9)	19 (30.6)	27 (43.5)	
Poorly	13 (22.6)	11 (16.1)	24 (38.7)	
Tumor size				0.0170
<2.0 cm	2 (3.2)	15 (24.2)	17 (27.4)	
≥2.0 cm	21 (33.8)	24 (38.7)	45 (72.6)	
Lymph node metastasis				0.0690
Positive	14 (22.6)	14 (22.6)	28 (45.2)	
Negative	9 (14.5)	25 (40.3)	34 (54.8)	
ER				0.0014
Positive	8 (12.9)	30 (48.4)	38 (61.3)	
Negative	15 (25.8)	9 (14.5)	24 (38.7)	
PR				0.1890
Positive	9 (14.5)	23 (37.1)	32 (51.6)	
Negative	14 (22.6)	16 (25.8)	30 (48.4)	
Ki-67				0.0002*
<20%	2 (3.2)	22 (35.5)	24 (38.7)	
≥20%	21 (33.9)	17 (27.4)	38 (61.3)	
p53				0.0005*
Positive	18 (29.0)	12 (19.4)	30 (48.4)	
Negative	5 (8.1)	27 (43.5)	32 (51.6)	
c-erbB-2				0.2421
Positive	8 (12.9)	8 (12.9)	16 (25.8)	
Negative	15 (24.2)	31 (50.0)	46 (73.5)	
PTEN LOH				0.4336
Positive	9 (14.5)	20 (32.3)	29 (46.8)	
Negative	14 (22.6)	19 (30.6)	33 (53.2)	

Four mutations were on exon 6, one was on exon 5, and one was on exon 8 (Table 3). LOH on 17p was observed in 18 (29.0%) of the 62 cases in at least one microsatellite marker (Table 4). p53 gene alterations, regarded as a p53 gene mutation and/or LOH for loci on 17p, were found in 23 (37.1%) of the 62 cases. The frequency of LOH detected for each of the markers was as follows: D17S654 (11 cases, 17.7%), D17S796 (7 cases, 11.3%), and D17S799 (6 cases, 9.7%) (Fig. 3).

Somatic PTEN mutations were detected in one (1.6%) of the 62 breast carcinomas, which was a nonsense mutation in exon 6 (TAT→TAA, tyrosine→stop) (Table 3, Fig. 4, 5). LOH on 10q23 was observed in 29 (46.8%) of the 62 cases in at least one microsatellite marker (Table 4). PTEN was altered in 48.4% of cases, including 29 cases with 10q23 LOH and one case of PTEN mutation. Four cases showed LOH at three markers and six cases showed LOH at two markers. The most frequent allele loss was at D10S1696 (13 cases at D10S1696 and 12 cases at D10S541) (Fig. 6).

Correlation of clinicopathological features with LOH data

The clinical and pathological parameters of 62 cases of invasive carcinomas are shown in Table 4 and 5. Tumors with LOH on 17p or a p53 mutation were histologically of high grade (p=0.0244) and large in size (p=0.070). These tumors exhibited negative ER (p=0.0014), a high Ki-67 labeling (p=0.0002), and a p53 immunoreactivity (p=0.0005) (Table 4). No correltions were found between the presence of LOH on 17p or p53 mutation and age, lymph node metastasis, PR or c-erbB-2 expression.

Tumors with LOH on 10q23 had a tendency toward c-erbB-2 positivity (p=0.0183) and p53 immunonegativity (p=0.0466) (Table 5). No correlation was found between the presence of LOH on 10q23 and tumor size, histologic grade, lymph node metastasis, ER, PR, Ki-67 expression or p53 gene alterations.

DISCUSSION

We investigated the incidences of allelic imbalances and muta-

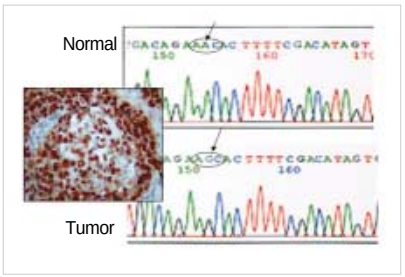

Fig. 1. Demonstration of p53 mutation (missense) in exon 6 in invasive ductal carcinoma of the breast (Case No. 6, AAC→AGC, Asparagine→Serine). The inlet shows a strong p53 immunoreactivity of the nuclei of tumor cells.

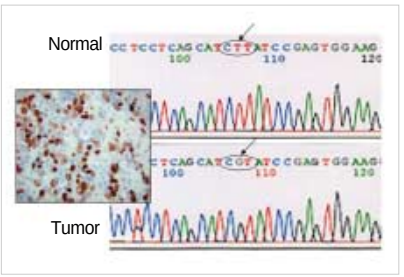

Fig. 2. Demonstration of p53 mutation (missense) in exon 6 in invasive ductal carcinoma of the breast (Case No. 11, CTT→CGT, Leucin→Arginine). The inlet shows a strong Ki-67 immunoreactivity of the nuclei of tumor cells.

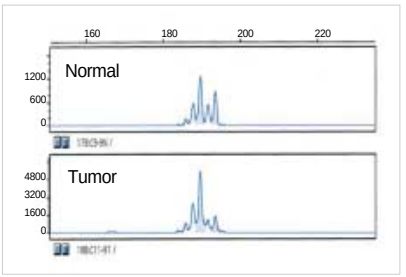

Fig. 3. Detection of LOH (D17S799) in invasive ductal carcinoma. The tumor sample shows significant loss of the shorter allele (LOH value=2.48).

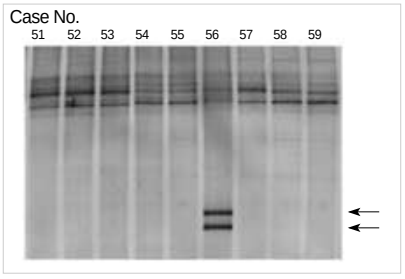

Fig. 4. Detection of PTEN mutation in exon 6 using single-strand conformational polymorphism analysis. The abnormal bands (arrows) indicate the presence of PTEN mutation.

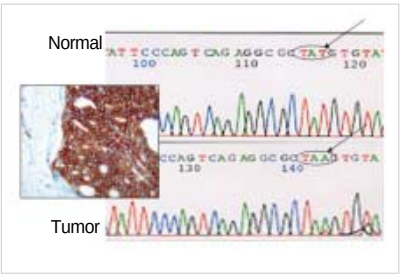

Fig. 5. Demonstration of PTEN mutation (nonsense) in exon 6 in invasive ductal carcinoma of the breast (Case No. 56, TAT→TAA, Tyrosine→Stop). The inlet shows a strong c-erbB-2 immunoreactivity of the cytoplasmic membranes of tumor cells.

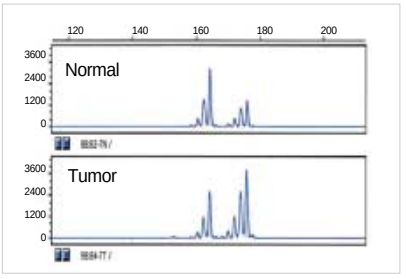

Fig. 6. Detection of LOH (D10S1696) in invasive ductal carcinoma. The tumor sample shows significant loss of the longer allele (LOH value=0.31).

Table 5. Correlations between clinicopathologic parameters and LOH at 10q23 (PTEN gene)

Clinicopathologic parameters	LOH at 10q23 (%)		Total No. (N=62)	p-value
	Positive (N=29)	Negative (N=33)		
Age				0.1762
<35	4 (6.5)	1 (1.6)	5 (8.1)	
≥35	25 (40.3)	32 (51.6)	57 (91.9)	
Histologic grade				0.9773
Well	6 (9.7)	5 (8.1)	11 (17.7)	
Moderately	11 (17.7)	16 (25.8)	27 (43.5)	
Poorly	12 (19.4)	12 (19.4)	24 (38.7)	
Tumor size				0.5809
<2.0 cm	9 (14.5)	8 (12.9)	17 (27.4)	
≥2.0 cm	20 (32.3)	25 (40.3)	45 (72.6)	
Lymph node metastasis				0.6170
Positive	12 (19.4)	16 (25.8)	28 (45.2)	
Negative	17 (27.4)	17 (27.4)	34 (54.8)	
ER				0.4365
Positive	16 (25.8)	22 (35.5)	38 (61.3)	
Negative	13 (21.0)	11 (17.7)	24 (38.7)	
PR				0.7993
Positive	14 (22.6)	18 (29.0)	32 (51.6)	
Negative	15 (24.2)	15 (24.2)	30 (48.4)	
Ki-67				0.1940
<20%	14 (22.6)	10 (16.1)	24 (38.7)	
≥20%	15 (24.2)	23 (37.1)	38 (61.3)	
p53 immunoreactivity				0.0466*
Positive	10 (16.1)	20 (32.3)	30 (48.4)	
Negative	19 (30.6)	13 (21.0)	32 (51.6)	
c-erbB-2				0.0183*
Positive	12 (19.4)	4 (6.5)	16 (25.8)	
Negative	17 (27.4)	29 (46.8)	46 (74.2)	
P53 mutation or LOH				0.4336
Positive	9 (14.5)	14 (22.6)	23 (37.1)	
Negative	20 (32.3)	19 (30.6)	39 (62.9)	

*p-value <0.05.

tions of the p53 and PTEN genes in 62 sporadic breast carcinoma cases and identified associations between clinicopathologic parameters and genetic alterations for these two genes.

The p53 gene is altered in breast carcinomas in approximately 20-40% of all cases and this is dependent on tumor size and stage of the disease.[6] Such alterations seem to be early events in breast tumorigenesis.[21] In this study, p53 gene alterations, positivity for p53 gene mutation and/or LOH for loci on 17p, were found in 23 (37.1%) of 62 cases.

p53 mutation is associated with a poor prognosis in breast cancer,[7,8,22] but mutations in different structural and functional domains of p53 have different effects on its biological activity. After adjusting for tumor stage, treatment regimen, and number of mutations, patients with p53 mutations were found to have significantly greater breast cancer specific mortality than patients without mutations. Breast cancer specific mortality and mortality due to any cause are both increased in female breast cancer patients with the following p53 mutation characteristics: silent and missense mixed mutations, transitional mutations, mutations in which guanine changed, mutations on exon 7, and multiple mutations occurring within 60 codons.[22] We showed that tumors with LOH on 17p or p53 mutation were of histologically high grade (p=0.0244) and large size (p=0.0170). These tumors were negative for ER (p=0.0014), had a high Ki-67 index (p=0.0002), and showed a p53 immunoreactivity (p=0.0005).

Recently, Bull *et al*.[6] reported that p53 mutations occurred in 24.5% of axillary node-negative breast carcinomas. Mutations were more frequently found in carcinomas with neu/erbB-2 amplification (38.9%) compared with those without neu/erbB-2 amplification (20.9%). The evaluation of tumors for p53 mutations may be beneficial to identify women at higher risk of disease recurrence or death when a tumor has neu/erbB-2 amplification, but in the absence of neu/erbB-2 amplification, the presence of p53 mutation may not provide additional independent prognostic information. We found no statistically significant correlation between p53 gene alteration and c-erbB-2 positivity.

Approximately 19% of the breast cancers examined carried a p53 mutation in exons 4-9.[23] Ethnic differences in genetic make-up, reproductive patterns, diet, socioeconomic status, and other unidentical cultural factors may be responsible for disparities in breast cancer incidence, stage at diagnosis, and survival. Compared to Caucasians (13.6%), a relatively high frequency of p53 mutation was found in Blacks (32.7%) (p=0.001). African-Brazilian women have been reported to have a larger prevalence of mutations in exons 5 and 7, whereas Caucasian women have more mutations in exon 8.[24] In the present study, four cases showed mutations of p53 in exon 6, one in exon 5, and one in exon 8.

Germline PTEN mutations have also been detected in Cowden's syndrome (CS), Lhermitte-Duclos disease, and Bannayan-Zonana syndrome.[25] PTEN involvement in human mammary carcinogenesis has been reported by studies which showed that germline PTEN mutations in CS predispose women to breast cancer and reduced PTEN protein levels in sporadic breast cancers. The PTEN gene is often mutated in primary human tumors and cell lines, but the low rate of somatic PTEN mutation found in human breast cancer has led to a debate about the role of TSG in breast cancer. However, an in vitro model indicated that PTEN has a role in breast tumorigenesis.[12]

The screening of breast cancers for LOH has resulted in a wealth of information pertaining to genomic instability and tumor development. LOH appears to occur in normal lobules adjacent to breast cancers.[26] Multiple foci of LOH are seen in in situ carci-

noma throughout the genome. By the time an invasive tumor has developed, the frequency of LOH increases at most loci.[27-29]

LOH in the 10q23 chromosomal region has been reported to occur at rates ranging between 30% and 40% in breast carcinoma cases.[13-17] Singh *et al*.[15] reported a similar frequency of LOH (41%) for one or more loci in 10q23 in sporadic breast tumors. Feilotter *et al*.[16] demonstrated LOH of PTEN in approximately 38.6% of primary tumors examined. Our results showed LOH of PTEN in 46.8% of cases in which 13 of 29 LOH-positive cases showed LOH at D10S1696 and 12 cases showed LOH at D10S541.

Rhei *et al*.[13] found only one PTEN mutation in 53 truly sporadic tumors. Feilotter *et al*.[16] reported no PTEN mutation in 70 breast tumors. In our series somatic PTEN mutations were detected in 1 (1.6%) of 62 breast carcinomas. Previously published data and our results show that LOH of the PTEN locus occurs frequently in sporadic breast carcinomas, whereas PTEN mutation is uncommon.

The biological importance of LOH and mutations of PTEN remains largely unknown. In endometrial cancers, PTEN gene alterations are associated with an endometrioid histology.[20] A strong correlation between LOH of 10q23 and poorly differentiated cancers has been suggested, however, the number of cases examined was small and the PTEN mutation status was not determined.[15] Bose *et al*.[14] reported no LOH in pure intraductal carcinoma, whereas 40% of invasive carcinomas exhibited LOH. In addition, allelic loss is significantly associated with loss of the ER, although there is no correlation between allelic loss and nuclear grade. Thus, loss of the 10q23 is strongly associated with tumor progression. Garcia *et al*.[17] also reported that significant differences in age, lymph node metastases, and higher histologic grade were found between carcinomas with and without LOH. On the other hand, lack of an association between chromosome 10 allele loss and tumor size, stage or lymph node involvement has also been reported.[16] We found no correlations between the clinicopathologic parameters of histologic grade, ER, PR and p53 expression, even though carcinomas with LOH were associated with c-erbB-2 positivity, compared with carcinomas lacking LOH (p=0.0183).

We conclude that LOH at 17p and/or the presence of p53 mutation are significantly associated with the aggressive pathologic parameters of breast cancer. LOH of the PTEN locus is frequent in sporadic breast carcinomas, whereas mutations of PTEN are uncommon. Our findings do not indicate any association between chromosome 10 allelic loss and clinicopathologic parameters, although carcinomas with LOH on 10q23 were associated with c-erbB-2 positivity (p=0.0183). Further study is required to confirm whether chromosome 10 allelic losses can be correlated with clinical parameters.

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