[CANCER RESEARCH 52, 3914–3917, July 15, 1992]

Correlation of Loss of Alleles on the Short Arms of Chromosomes 11 and 17 with Metastasis of Primary Breast Cancer to Lymph Nodes[1]

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ABSTRACT

To examine the role of loss of heterozygosity (LOH) during tumor development and/or progression, we looked for correlations between metastasis of breast cancer to a regional lymph node(s) and LOH of chromosomal arms 11p, 13q, 16q, 17p, and 17q, where frequent losses in primary tumors have been detected. No correlation between lymph node metastasis and LOH of chromosomes 13q, 16q, or 17q was observed. However, tumors showing LOH of chromosomes 11p ($\chi^2 = 10.82$, $P < 0.01$) and 17p ($\chi^2 = 6.78$, $P < 0.01$) revealed a significantly higher incidence of metastasis to a regional lymph node(s) than tumors without LOH on these chromosomal arms. Furthermore, only four of 30 (13%) patients with tumors that retained both 11p and 17p had metastasis to a regional lymph node(s), compared with 24 of 32 (75%) patients with tumors that had lost both 11p and 17p. Analysis of LOH with markers on chromosomes 11p and 17p in a large number of tumors indicated that the peritelomeric region of each of these chromosomal arms contains a tumor suppressor gene that may be associated with tumor progression, particularly metastasis to a regional lymph node(s).

INTRODUCTION

Breast cancer is the most common malignancy in females, and one of nine Caucasian women and one of 60 Japanese women are likely to develop breast cancer in their lifetimes. A number of genetic alterations associated with tumor development and progression have been reported (1, 2), and their participation in the cascade of events leading to colon cancer has been documented (3). However, the roles of distinct genetic alterations are not known during tumor development and/or progression for breast cancer. Several oncogenes (c-*myc*, c-*erbB*, and c-*erbB2*) may be involved in breast cancer (4, 5), and some reports have indicated that the amplification of the *erbB2* gene and its overexpression in tumors make the prognosis poor (6–10). However, other groups have failed to find a direct relationship between *erbB2* amplification and prognosis (11, 12).

Frequent LOH[3] in tumor DNA, which implies the presence of a tumor suppressor gene, has been detected on chromosomes 1q, 3p, 7q, 11p, 13q, 16q, 17p (two regions, the *p53* locus and the region distal to *p53*), 17q, and 18q (13–22). In breast tumors, somatic mutations detected in the *p53* gene (23), the *RB* gene (24), and the prohibitin gene (25) made each of these three genes a candidate for a tumor suppressor role. The finding of a germ line mutation in the *p53* gene in patients with Li-Fraumeni syndrome, an autosomal dominant disease associated with a high incidence of epithelial and interstitial tumors including breast cancer, further supported an important role of the *p53* gene during development of breast cancer (26, 27). Moreover, mapping of an early onset type of familial breast cancer to chromosome 17q21–23 by linkage analysis provided further evidence for the presence of a susceptibility gene for breast cancer on the long arm of chromosome 17 (28). Since the common region of LOH in sporadic breast cancer was mapped to the same region as familial breast cancer (21), hereditary and sporadic breast cancers might involve malfunction and/or removal of the same tumor suppressor gene(s).

To investigate which of the tumor suppressor gene(s) mentioned above is associated with metastasis of breast tumor to a regional lymph node(s), we looked for the correlations between histopathological lymph node status and LOH on chromosomal arms where we could detect frequent loss in a large number of primary breast cancers (11p, 13q, 16q, 17p, and 17q).

MATERIALS AND METHODS

Materials. Tumors and corresponding normal tissues of 312 patients with primary breast cancer for the allelotype study were provided from the specimens of mastectomy. Of the 312 tumors, 265 were invasive ductal carcinomas (57 papillotubular carcinomas, 76 solid tubular carcinomas, and 132 scirrhous carcinomas), and 47 were special types including 14 invasive lobular carcinomas. The histological classification of the tumors was done in accordance with the typing scheme of the Japanese Breast Cancer Society (29), which is basically the same as the WHO typing scheme for breast tumors.

DNA Extraction from Tissues. The extraction of genomic DNA from the tissues was carried out according to the method described in our previous report (19).

Probes. All probes used in this study were reported previously (21) except for probe cCI11p15-10 at chromosome 11p15 that is a variable number of tandem repeat marker isolated from a somatic hybrid.[4] Characteristics of all others were described.

Hybridization Condition. DNA samples were transferred to nylon membranes (Pall Biodyne) in the solution of 0.1 N NaOH/0.1 M NaCl. The conditions for neutralization, fixation, hybridization, and washing were according to the methods described previously (19).

Statistical Analysis. The χ^2 test and Fisher's exact test were used for statistical analysis of the results.

RESULTS

Table 1 summarizes the results of experiments to detect LOH in nearly 200 informative cases, using 21 markers on chromosomes 11p, 13q, 16q, 17p, and 17q. In this group of tumors, 41.1% showed LOH on chromosome 11p, 24.6% on 13q, 54.6% on 16q, 57.3% on 17p, and 36.1% on 17q. On the basis of this information, we looked for correlations between LOH

Received 3/27/92; accepted 5/7/92.

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[1] The work was supported in part by a grant-in-aid for cancer research from the Ministry of Education, Science, and Culture of Japan.

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[3] The abbreviation used is: LOH, loss of heterozygosity.

[4] K. Takita, A. Tanigami, T. Tokino, C. Jones, and Y. Nakamura. Identification of 57 conventional RFLP and six VNTR systems with 32 DNA clones on chromosome 11p15, submitted for publication.

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on chromosome 11p, 13q, 16q, 17p, or 17q and a histopathological *n*-factor (presence or absence of metastasis to the regional lymph node). As summarized in Table 2, 50 (66%) of 76 patients with tumors showing LOH on chromosome 11p had metastasis to a regional lymph node(s), although 46 (42%) of 109 patients with tumors, which retained chromosome 11p, had metastasis ($\chi^2 = 10.82$, $P < 0.01$). Similarly, metastases were found in 74 (65%) of 114 patients whose tumors had lost alleles on 17p; metastases were observed in 46% of patients with tumor that retained 17p ($\chi^2 = 6.78$, $P < 0.01$). However, no significant correlation was observed between regional lymph node metastasis and LOH for markers on chromosome 13q ($\chi^2 = 0.61$), 16q ($\chi^2 = 1.53$), or 17q ($\chi^2 = 0.83$).

To know whether metastasis was more frequent following loss of both 11p and 17p, we performed the analysis summarized in Table 3. In 24 (75%) of 32 patients with tumors that had lost both 11p and 17p, regional lymph node metastasis was present. On the other hand, only four (13%) of 30 patients with tumors retaining both 11p and 17p had suffered metastasis. A significant association of lymph node metastasis with LOH of both chromosomal arms was indicated ($P = 0.00000086$, Fisher's exact test). Furthermore, the chromosomal losses of 11p and 17p appeared to occur concordantly (Table 4). Although only 3 of 33 (9%) tumors retaining 17p lost 11p, 32 of 53 (60%) tumors that lost 17p also lost 11p.

Since the evidence (20, 21) has suggested that two tumor suppressor genes for breast cancer may lie on chromosome 17p (the *p53* locus and a region distal to it), we examined each region separately for correlation of LOH to lymph node metastasis (Table 5). A high incidence of lymph node metastasis was observed only among the patients with tumors missing one allele at the *YNZ22* locus ($\chi^2 = 4.26$, $P < 0.05$).

To identify the region of chromosome 11p containing a tumor suppressor gene, we examined two restriction enzyme fragment length polymorphism markers (cCI11-237 and cCI11p15-10) that are located at p15 (30). By means of a hybrid cell panel of chromosome 11, cCI11p15-10 was mapped distal (11p15.5-ter) to cCI11-237 (11p15.4-15.5).[5] The result of testing these markers in 193 breast cancers suggested that a tumor suppressor gene is located the peritelomeric region of chromosome 11p distal to cCI11-237, since 28 tumors lost the distal locus (cCI11p15-10) but retained the proximal locus (cCI11-237).

Table 1 *Loss of heterozygosity in primary breast cancer*

Chromosome[a]	No. of patients tested	Allelic losses/ informative cases
11p	193	76/185 (41.1)[b]
13q	197	35/142 (24.6)
16q	213	95/174 (54.6)
17p	222	114/199 (57.3)
17q	226	69/191 (36.1)

[a] The probes used to detect each chromosomal loss were described in our previous paper (21) except for one marker on chromosome 11p (cCI11p15-10).[4]
[b] Numbers in parentheses, percentage.

Table 2 *Correlation between LOH and lymph node metastasis in breast cancer*

Chromosome	*n*(+)[a]	*n*(−)	Total
11p[b]			
Loss	50	26	76
Retained	46	63	109
13q[c]			
Loss	21	14	35
Retained	56	51	107
16q[d]			
Loss	60	35	95
Retained	42	37	79
17p[e]			
Loss	74	40	114
Retained	39	46	85
17q[f]			
Loss	42	27	69
Retained	67	55	122

[a] *n*(+), cases with lymph node metastasis; *n*(−), cases without lymph node metastasis.
[b] $\chi^2 = 10.82$, $P < 0.01$.
[c] $\chi^2 = 0.61$.
[d] $\chi^2 = 1.53$.
[e] $\chi^2 = 6.78$, $P < 0.01$.
[f] $\chi^2 = 0.83$.

Table 3 *Correlation between lymph node metastasis and loss or retention of both 11p and 17p in tumors*

	n(+)[a]	*n*(−)	Total
Loss	24	8	32
Retained	4	26	30

[a] *n*(+), cases with lymph node metastasis; *n*(−), cases without lymph node metastasis.
[b] $P = 0.00000086$, Fisher's exact test.

Table 4 *Concordant loss of chromosomes 11p and 17p*

	Chromosome 11p		
Chromosome 17p	LOH	Retain	Total
LOH	32	21	53
Retained	3	30	33

[a] $P = 0.0000012$ by Fisher's exact test.

Table 5 *Correlation between lymph node metastasis and allelic loss of the p53 and YNZ22 loci*

	n(+)[a]	*n*(−)	Total
p53 locus[b]			
Loss	18	15	33
Retained	27	24	51
YNZ22 locus[c]			
Loss	47	22	69
Retained	36	35	71

[a] *n*(+), cases with lymph node metastasis; *n*(−), cases without lymph node metastasis.
[b] $\chi^2 = 0$.
[c] $\chi^2 = 4.26$, $P < 0.05$.

DISCUSSION

Frequent observations of LOH on chromosomes 1q, 3p, 7q, 11p, 13q, 16q, 17p, 17q, and 18q in primary breast cancers have indicated the possible presence of tumor suppressor genes on these chromosomal arms. To examine which of the putative tumor suppressor genes may be associated with lymph node metastasis of breast cancer, we have examined LOH of chromosomes 11p, 13q, 16q, 17p, and 17q in a large number of tumors. Chromosomes 1q, 7q, and 18q were not analyzed in the present study because our earlier experiments had shown that LOH on these chromosomes is not frequent (19).

The results reported here indicate a significant association between loss of tumor suppressor genes on chromosomes 11p and 17p and metastasis of breast cancer to a regional lymph node(s). Chromosomes 13q, 16q, and 17q showed no association with metastasis, although we reported a weak correlation

[5] K. Takita and Y. Nakamura, unpublished data.

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between LOH of chromosome 16q and lymph node metastasis previously (19). Patients with tumors that retained both 11p and 17p had a very low incidence (13%) of lymph node metastasis, in comparison with patients whose tumors lost both chromosomal arms (75%). Furthermore, chromosomes 11p and 17p were lost concordantly in many tumors. Loss of the short arm of chromosome 17 might trigger the loss of the short arm of chromosome 11. Moreover, three of four patients, whose tumors retained both 11p and 17p but had metastasis, had only two or three positive lymph nodes among a large number of regional lymph nodes examined: 2 positive of 27 lymph nodes; 3 of 31; and 2 of 47, respectively. As patients who have metastasis involving three or less lymph nodes can be expected to have a better prognosis than those with metastasis involving four or more lymph nodes, the present results suggest that LOH on chromosomes 11p and/or 17p may be a good marker for estimation of the risk for lymph node metastasis and prognosis.

Further analysis of chromosome 17p has indicated that the region associated with metastasis is distal to the *p53* locus. Loss of this peritelomeric region has been reported in astrocytoma, lung cancer, hepatocellular carcinoma, gastric cancer, bladder cancer, and ovarian cancer, as well as breast cancer (31–35). The unknown gene on chromosome 11p associated with lymph node metastasis is also near the telomere; this region is known to contain the gene responsible for Beckwith-Wiedemann syndrome, and frequent LOHs have been reported in hepatocellular carcinoma, rhabdomyosarcoma, lung cancer, ovarian cancer, and bladder carcinoma (36–41). A common tumor suppressor gene on 11p might account for these findings, or a cluster of genes associated with negative cell growth might be located there.

ACKNOWLEDGMENTS

We thank Akira Tanigami, Takashi Sakamoto, Hiroko Saito, and Michael Jones for helpful advice and discussion and Kazuyo Oda for secretarial assistance.

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