Analysis of Genetic Alterations Related to the Development and Progression of Breast Carcinoma

Koichi Nagayama and Masahiro Watatani¹

First Department of Surgery, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589

To study genetic alterations related to the development and/or progression of breast carcinoma, we examined amplification of the ERBB2, INT2, and MYC genes, as well as loss of heterozygosity (LOH) at loci on 11p, 16q, 17p (*D17S5* and *TP53*), 17q (*D17S74* and *NME1*), and 18q by restriction fragment length polymorphism analysis. The subjects were 26 patients with small breast carcinomas (≤ 2 cm) and 88 patients with larger breast carcinomas (2 to < 5 cm). All patients were free of distant metastasis. As tumor diameter increased, the frequency of oncogene amplification and LOH at all loci except *D17S5* increased. However, there was no relationship between tumor diameter and amplification of specific oncogenes or allelic loss at specific loci. LOH at *D17S5* was detected in 40% of small breast carcinomas (≤ 2 cm) and 43% of larger breast carcinomas (2 to < 5 cm). There was a significant correlation of LOH at *D17S5* with INT2 amplification or with LOH on 11p, 16q, and 18q. These findings suggest that LOH at *D17S5* may be involved in the early stage of breast carcinoma development, while INT2 amplification and LOH at 11p, 16q, and 18q appear to be genetic alterations that occur with tumor progression. In addition, as lymph node metastases were significantly related to amplification of the ERBB2 and MYC genes, and LOH of the NME1 gene, these genetic alterations may play a role in the mechanism of lymph node metastases.

Key words: Breast carcinoma — Oncogene amplification — Restriction fragment length polymorphism analysis — Loss of heterozygosity

Recent progress in molecular biology has led to the identification of various oncogenes and tumor suppressor genes. The accumulation of more than one abnormal oncogene and tumor suppressor gene (multistage alterations) may be involved in the development and progression of breast carcinoma. Important genetic alterations of proto-oncogenes that have been detected in breast carcinoma include amplification of the ERBB2, INT2, and MYC genes.¹⁻³ Frequent loss of heterozygosity (LOH) on 1p, 1q, 3p, 7q, 11p, 13q, 16q, 17p, 17q, and 18q have been observed by Southern blot analysis using restriction fragment length polymorphism (RFLP), indicating that putative tumor suppressor genes located on these chromosomal arms may be related to the development and/or progression of breast carcinoma.⁴

Conceivably, carcinoma progression is associated with further modification of tumor cell DNA, giving rise to clones with new genetic alterations. This process eventually generates a clone with metastatic potential. Most solid tumors are detected only after reaching a size large enough to cause symptoms, and thus are likely to have accumulated various genetic alterations. Studies of colon carcinoma have clarified the correspondence between morphologic and genetic alterations in the adenoma-carcinoma sequence.⁵ However, corresponding altera-

tions in breast carcinoma still remain obscure, and how such genetic alterations and their accumulation affect the development and/or progression of breast carcinoma has not been clarified.

We classified primary breast carcinomas into two groups on the basis of tumor size (≤ 2 cm and 2 to < 5 cm). We then analyzed ERBB2, INT2, and MYC gene amplification, as well as LOH on 11p, 16q, 17p (*D17S5* and *TP53*), 17q (*D17S74* and *NME1*), and 18q by RFLP analysis. We also studied genetic alterations related to lymph node metastases in these tumors.

MATERIALS AND METHODS

Materials From 114 patients with untreated breast carcinoma who underwent mastectomy at Kinki University Hospital beginning in January 1990 through October 1992, tumor tissue and corresponding normal tissue were collected. The tissue samples were immediately frozen and stored in liquid nitrogen. The samples included 26 with diameter ≤ 2 cm and 88 with diameter of 2 to < 5 cm. Lymph node metastases were absent in 75 patients and present in 39 patients. None of the patients showed distant metastasis. The histological diagnosis was infiltrating ductal carcinoma in 108 patients (95%), including 24 (22%) with papillotubular carcinoma, 66 (61%) with solid-tubular carcinoma, and 18 (17%) with

¹ To whom correspondence should be addressed.

scirrhous carcinoma.⁶⁾ In addition, there were 2 patients with mucinous carcinoma, 3 with medullary carcinoma, and 1 with infiltrating lobular carcinoma.

DNA extraction and Southern blot hybridization High-molecular-weight DNA was extracted as described previously.⁷⁾ In brief, frozen tissues were incubated in SDS-Proteinase K (10%, 500 μ g/ml), then DNA was extracted with phenol:chloroform:isoamyl alcohol. Ten μ g of genomic DNA was digested with an appropriate restriction enzyme and subjected to electrophoresis on 0.8% or 1.0% agarose gels. Following DNA transfer to nylon filters (Hybond N⁺, Amersham), DNA was fixed to the filters with UV Crosslinker (Nippon Genetics).⁸⁾ DNA was then hybridized for 15 h at 42°C with ³²P-labeled probes. The filters were washed and autoradiography was performed at -70°C. The degree of amplification of the ERBB2, INT2, and MYC genes was then quantitated by densitometry (PDA-65, Konika) to determine the density ratios of the signal from tumor to that from normal tissue.

Probes The probes and restriction enzymes used in this study are listed in Table I. The ERBB2, INT2, and MYC gene probes were supplied by the Japanese Cancer Research Resources Bank. The NM23-H1 probe was obtained from Dr. Patricia S. Steeg of the National Cancer Institute (NCI, USA), National Institute of Health (NIH). The other RFLP markers were kindly provided by Dr. Yusuke Nakamura, Department of Biochemistry, Cancer Institute, Tokyo.

Statistical methods Relationships between tumor diameter, lymph node metastases, and genetic alterations were determined by the chi-squared test or Fisher's exact test.

RESULTS

Oncogene amplification and LOH of several chromosomal arms Oncogene amplification and LOH on 11p, 16q, 17p (*D17S5* and *TP53*), 17q (*D17S74* and *NME1*),

and 18q were analyzed in the breast carcinomas < 5 cm in diameter without distant metastasis. As shown in Table I, ERBB2, INT2, and MYC gene amplifications were found in 25% (28/114), 17% (19/114), and 7% (8/114) of the tumors, respectively. The informative patients for 11p, 16q, and 18q in this study were 29, 83 and 70, respectively (Fig. 1). In the case of 17p (*D17S5* and *TP53*), 62 and 65 samples were respectively informative, while 57 and 48 samples were respectively informative for 17q (*D17S74* and *NME1*). LOH at *D17S5* was observed in 26 of 62 informative samples (42%). However, the frequency of LOH at the *HRAS*, *D17S74*, *NME1*, and *D18S5* loci was 20% or less.

Genetic alterations and tumor diameter Tumor diameters were classified into two groups (≤ 2 cm and 2 to < 5 cm). Amplification of the ERBB2 and INT2 genes was observed in 15% (4/26) and 12% (3/26) of the samples in the ≤ 2 cm group and in 27% (24/88) and 18% (16/88) of samples in the 2 to < 5 cm group, respectively (Table II). MYC amplification was observed in 9% (8/88) of samples in the 2 to < 5 cm group, but was not observed in the ≤ 2 cm group. The frequency of ERBB2, INT2, and MYC amplification increased as tumor diameter increased, but amplification of specific oncogenes was not related to tumor diameter.

LOH at *D17S5* was observed in 6 of 15 samples (40%) in the ≤ 2 cm group and in 43% of samples in the 2 to < 5 cm group. Using markers for 11p, 17q, (*D17S74* and

Fig. 1. Southern blot analysis with the probe YNZ22 (*D17S5*) showing LOH of DNA from tumor (T) and normal (N) tissue of breast cancer patients. Each DNA sample was digested with *TagI*. Case 1 is non-informative and case 2 retains constitutional heterozygosity. Case 3 shows LOH.

Table I. Genetic Alterations in 114 Primary Breast Carcinomas

Probe	Locus	Restriction enzyme	No. of informative patients with amplification or LOH/no. of informative patients (%)
pKX044	<i>ERBB2</i>	<i>EcoRI</i>	28/114 (25)
SS6	<i>INT2</i>	<i>BamHI</i>	19/114 (17)
pSPT-MYC	<i>MYC</i>	<i>EcoRI</i>	8/114 (7)
c-H-ras	<i>HRAS</i>	<i>BamHI</i>	5/29 (17)
p79-2-3	<i>D16S7</i>	<i>TagI</i>	23/83 (28)
YNZ22	<i>D17S5</i>	<i>TagI</i>	26/62 (42)
BHP53	<i>TP53</i>	<i>BamHI</i>	13/65 (20)
CMM86	<i>D17S74</i>	<i>TagI</i>	10/57 (18)
NM23-H1	<i>NME1</i>	<i>BglII</i>	7/48 (15)
OS-4	<i>D18S5</i>	<i>TagI</i>	11/70 (16)

Table II. Relationship between Tumor Size and Genetic Alterations in Primary Breast Carcinomas

Genetic alterations	Tumor size (%)		<i>P</i>
	(0-2 cm)	(2.1-5 cm)	
Amplification			
<i>ERBB2</i>	4/26 (15)	24/88 (27)	NS
<i>INT2</i>	3/26 (12)	16/88 (18)	NS
<i>MYC</i>	0/26 (0)	8/88 (9)	NS
LOH			
<i>HRAS</i>	0/5 (0)	5/24 (21)	NS
<i>D16S7</i>	2/14 (14)	21/69 (30)	NS
<i>D17S5</i>	6/15 (40)	20/47 (43)	NS
<i>TP53</i>	1/16 (6)	12/49 (24)	NS
<i>D17S74</i>	0/12 (0)	10/45 (22)	NS
<i>NME1</i>	0/11 (0)	7/37 (19)	NS
<i>D18S5</i>	0/15 (0)	11/55 (20)	NS

No. of positive tumors/no. of informative tumors. *P* values were calculated by χ^2 test or Fisher's exact test. NS: not significant.

Table III. Relationship between Lymph Node Metastases and Genetic Alterations in Primary Breast Carcinomas

Genetic alterations	Lymph node metastases (%)		<i>P</i>
	n (-)	n (+)	
Amplification			
<i>ERBB2</i>	12/75 (16)	16/39 (41)	0.01
<i>INT2</i>	10/75 (13)	9/39 (23)	NS
<i>MYC</i>	2/75 (3)	6/39 (15)	0.01
LOH			
<i>HRAS</i>	2/18 (11)	3/11 (27)	NS
<i>D16S7</i>	16/56 (29)	7/27 (26)	NS
<i>D17S5</i>	17/39 (44)	9/23 (39)	NS
<i>TP53</i>	10/44 (23)	3/21 (14)	NS
<i>D17S74</i>	4/34 (12)	6/23 (26)	NS
<i>NME1</i>	2/34 (6)	5/14 (36)	0.01
<i>D18S5</i>	7/47 (15)	4/23 (17)	NS

No. of positive tumors/no. of informative tumors. *P* values were calculated by χ^2 test or Fisher's exact test. NS: not significant.

Table IV. Associations among Various Genetic Alterations in Primary Breast Carcinomas

Genetic alterations	Genetic alterations									
	<i>ERBB2</i> Amp.	<i>INT2</i> Amp.	<i>MYC</i> Amp.	<i>HRAS</i> LOH	<i>D16S7</i> LOH	<i>D17S5</i> LOH	<i>TP53</i> LOH	<i>D17S74</i> LOH	<i>NME1</i> LOH	<i>D18S5</i> LOH
<i>ERBB2</i> Amp.	—									
<i>INT2</i> Amp.	NS	—								
<i>MYC</i> Amp.	NS	NS	—							
<i>HRAS</i> LOH	NS	NS	0.002	—						
<i>D16S7</i> LOH	NS	NS	NS	NS	—					
<i>D17S5</i> LOH	NS	0.02	NS	0.04	0.01	—				
<i>TP53</i> LOH	NS	0.05	NS	NS	NS	0.01	—			
<i>D17S74</i> LOH	NS	NS	0.03	NS	0.03	NS	0.04	—		
<i>NME1</i> LOH	0.02	NS	0.01	NS	0.04	NS	NS	NS	—	
<i>D18S5</i> LOH	NS	NS	NS	NS	0.03	0.005	0.01	NS	NS	—

P values were calculated by χ^2 or Fisher's exact test. NS: not significant.

NME1), and 18q, LOH was detected in 21%, 22%, 19% and 20% of samples in the 2 to < 5 cm group, while no LOH was detected in the ≤ 2 cm group. With respect to LOH on the 16q or p53 gene, the frequency of LOH also increased as tumor diameter increased. However, the relationship between LOH on a specific locus and tumor size was not statistically significant.

Genetic alterations and lymph node metastases Seventy-five of the 114 tumors were histologically negative for lymph node metastases. *ERBB2* amplification was found in 16% of these 75 tumors. On the other hand, 41% of the tumors with lymph node metastases showed *ERBB2* amplification. The *ERBB2* amplification was significantly related to lymph node metastases ($P=0.01$) (Table III). The frequency of *MYC* amplification was 3% in tumors

without lymph node metastases and 15% in tumors with lymph node metastases, so that a statistically significant association was observed. Although *INT2* amplification was more frequently detected in breast carcinomas with lymph node metastases than in those without nodal metastases, there was no significant relationship between amplification of this gene and nodal metastases.

LOH at the *D17S5* locus was detected in approximately 40% of the tumors with and without lymph node metastases. LOH at the *NME1* locus was found in 2 of 34 informative patients (6%) without lymph node metastases, whereas it was detected in 5 of 14 informative patients (36%) with lymph node metastases, a statistically significant relationship ($P=0.01$). Using the CMM86 probe (another marker for 17q), LOH was

detected more frequently in tumors with lymph node metastases than in those without nodal metastases, but the difference was not statistically significant.

Relationship between alterations of the individual genes
Table IV shows the associations between alterations of individual genes. Since MYC amplification and LOH on 11p, 17q (*D17S74* and *NME1*), and 18q were not detected in breast carcinomas with a diameter ≤ 2 cm, we excluded these genetic alterations for analysis of association among individual genes. The ERBB2 amplification was not related to any other genetic alterations, but INT2 amplification was related to LOH on 17p (*D17S5* and *TP53*). LOH at *D17S5* was related to LOH on 16q. Moreover, as shown in Table V, 7 of 37 tumor samples which were informative for *D17S5* and *TP53* loci showed LOH at these two loci, but 19 retained two alleles at both loci. Although we observed a significant association between LOH at *TP53* and *D17S5* ($P=0.01$), 10 of these 37 tumors showed LOH at *D17S5* but had no allelic loss at the *TP53* locus.

DISCUSSION

In this study, we investigated amplification of the ERBB2, INT2, and MYC genes in primary breast carcinomas and assessed LOH at multiple loci by RFLP analysis. Although the frequencies of ERBB2 and INT2 amplification were identical to those reported previously, our study showed that the frequency of MYC amplification was only 7%.^{9,10} Garcia *et al.* reported that MYC amplification was frequently observed in inflammatory breast carcinoma and suggested a correlation with the aggressiveness of breast carcinoma.¹¹ Although our study excluded inflammatory or locally progressive breast carcinoma, MYC amplification was detected in relatively large breast carcinomas with extensive lymph node metastases, suggesting biological aggressiveness.

We have previously studied overexpression of the ERBB2 oncoprotein in primary tumors, as well as in metastatic lymph nodes, by immunostaining.¹² When ERBB2 oncoprotein is overexpressed in the primary lesion, it is also overexpressed in the metastatic lymph nodes, and the overexpression of ERBB2 oncoprotein is more prominent in the metastatic lymph nodes, compared to the primary tumor. Although the present study did not prove a relationship between ERBB2 amplification and tumor size, it confirmed the relationship between ERBB2 amplification and lymph node metastases. Borg *et al.*, as well as ourselves, have confirmed that ERBB2 amplification is frequently observed in breast carcinoma with lymph node metastases.¹³ Since abnormalities of the ERBB2 gene are observed in 10%–40% of all breast carcinomas, this gene is likely to play a role in the development of a subpopulation of breast carci-

Table V. Concordant LOH at the *TP53* and *D17S5* Loci in Breast Carcinomas

	<i>D17S5</i> LOH		<i>P</i>
	+	–	
<i>TP53</i> LOH +	7	1	0.01
–	10	19	

P values were calculated by Fisher's exact test.

noma. In addition, ERBB2 gene abnormalities seem to be strongly related to the process of lymph node metastases.

Abnormalities of p53 genes have been detected in various tumors.¹⁴ Abnormalities including the expression and LOH of p53 gene have been reported in 10–60% of non-hereditary breast cancers. Li-Fraumeni syndrome, which includes juvenile bilateral breast cancer, is attributable to a germ-line mutation of the p53 gene.¹⁵ LOH of p53 gene was detected in 20% of our 114 tumors by RFLP analysis. Coles *et al.* and Sato *et al.*, respectively, reported LOH of p53 in 27% and 37% of the tumors they examined, using a combination of probe and restriction enzymes identical to those used in this study.^{16,17} In contrast, Davidoff *et al.* observed LOH of p53 (using the combination of a p53 cDNA probe and the *Bgl*II enzyme) in 5 of 9 breast cancers.¹⁸ They also observed p53 gene expression in 2 of 15 intraductal carcinomas by immunostaining and considered that abnormal p53 gene expression occurs in the early stage of breast carcinoma development.¹⁹ However, the low frequency of allelic loss of p53 gene may suggest that it is less related to the development and/or progression of breast carcinoma than to the development of colon or lung carcinoma.

Using the YNZ22 probe, we detected allelic loss at *D17S5* in 42% of breast carcinomas. Sato *et al.* also found LOH at *D17S5* in 47% of breast carcinomas.²⁰ When a tumor suppressor gene is lost through a chromosomal aberration, some polymorphic loci close to the tumor suppressor gene may be lost simultaneously. In the present series, 7 of 37 tumor samples informative for *D17S5* and *TP53* showed allelic loss at both loci. However, 10 of the 37 samples showed LOH at *D17S5* but not at *TP53*, suggesting the existence of a new tumor suppressor gene near or at 17p13.3, as Coles *et al.* and Sato *et al.* previously documented.^{16,17} This putative tumor suppressor gene may play an important role in the early stage of breast carcinoma development. A detailed deletion-mapping study around this locus using a panel of polymorphic markers may be worthwhile.

The NME1 gene identified by Steeg *et al.*, recently assigned to 17q22, is a candidate for a metastatic suppressor gene.^{21,22} We detected LOH of NME1 in 36% of carcinomas with lymph node metastases and confirmed a relationship between lymph node metastases and LOH at

this locus. Hall *et al.* reported linkage of early onset familial breast cancer to D17S74.²³⁾ In addition, the prohibitin gene, another possible tumor suppressor gene in breast carcinoma, has recently been localized to 17q21.²⁴⁾ Thus, analysis of the long arm of chromosome 17 may be essential in identifying genes related to the development and/or progression of breast carcinoma.

Accumulation of multiple genetic alterations is presumed to be necessary for the transformation of a normal mammary cell to a malignant cell. Sato *et al.* have suggested that the first event in breast carcinoma development is LOH on 17p followed by the ERBB2 amplification.²⁰⁾ We propose that allelic loss of specific loci on 17p, especially near and at 17p13.3, is the first important event in breast carcinoma development. We consider that allelic loss of 11p, 16q, and 18q also occurs subsequent to the allelic loss of 17p and that this whole sequence of

genetic alterations may be involved in the progression of breast carcinoma. The analysis of cancer-related genetic alterations in breast carcinoma has just started, and extensive further studies in this field are required.

ACKNOWLEDGMENTS

We would like to express our gratitude to Professor Masayuki Yasutomi at the First Department of Surgery, Kinki University School of Medicine, for his valuable advice and encouragement. We also wish to thank Dr. Yusuke Nakamura for his very generous gifts of RFLP probes, Dr. Patricia S. Steeg for generously providing the NM23-H1 probe, and Dr. Isamu Nishisho for the OS-4 probe. This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

(Received June 10, 1993/Accepted August 10, 1993)

REFERENCES

- Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science*, **235**, 177-182 (1987).
- Lidereau, R., Callahan, R., Dickson, C., Peters, G., Escot, C. and Ali, I. U. Amplification of the *int-2* gene in primary human breast tumors. *Oncogene Res.*, **2**, 285-291 (1988).
- Escot, C., Theillet, C., Lidereau, R., Spyrtatos, F., Champeme, M-H., Gest, J. and Callahan, R. Genetic alteration of the *c-myc* proto-oncogene (*MYC*) in human primary breast carcinomas. *Proc. Natl. Acad. Sci. USA*, **83**, 4834-4838 (1986).
- Callahan, R., Cropp, C. S., Merlo, G. R., Liscia, D. S., Cappa, A. P. M. and Lidereau, R. Somatic mutations and human breast cancer. *Cancer*, **69**, 1582-1588 (1992).
- Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, **319**, 525-532 (1988).
- Japanese Breast Cancer Society. The general rules for clinical and pathological recording of breast cancer. *Jpn. J. Surg.*, **19**, 612-632 (1989) (in Japanese).
- Watatani, M., Perantoni, A. O., Reed, C. D., Enomoto, T., Wenk, M. L. and Rice, J. M. Infrequent activation of *K-ras*, *H-ras*, and other oncogenes in hepatocellular neoplasms initiated by methyl(acetoxymethyl)nitrosamine, a methylating agent, and promoted by phenobarbital in F344 rats. *Cancer Res.*, **49**, 1103-1109 (1989).
- Southern, E. M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, **98**, 503-517 (1975).
- Clark, G. M. and McGuire, W. L. Follow-up study of HER-2/*neu* amplification in primary breast cancer. *Cancer Res.*, **51**, 944-948 (1991).
- Callahan, R. and Campbell, G. Mutations in human breast cancer: an overview. *J. Natl. Cancer Inst.*, **81**, 1780-1786 (1989).
- Garcia, I., Dietrich, P.-Y., Aapro, M., Vauthier, G., Vadas, L. and Engel, E. Genetic alterations of *c-myc*, *c-erbB-2*, and *c-Ha-ras* protooncogenes and clinical associations in human breast carcinomas. *Cancer Res.*, **49**, 6675-6679 (1989).
- Maeda, S., Watatani, M., Nagayama, K., Kadota, K., Ozaki, K., Wada, T. and Yasutomi, M. Immunohistochemical analysis of overexpression of *c-erbB-2* protein in node-positive breast carcinoma. *J. Jpn. Surg. Soc.*, **94**, 86-92 (1993) (in Japanese).
- Borg, A., Tandon, A. K., Sigurdsson, H., Clark, G. M., Ferno, M., Fuqua, S. A. W., Killander, D. and McGuire, W. L. HER-2/*neu* amplification predicts poor survival in node-positive breast cancer. *Cancer Res.*, **50**, 4332-4337 (1990).
- Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C. and Vogelstein, B. Mutations in the *p53* gene occur in diverse human tumor types. *Nature*, **342**, 705-708 (1989).
- Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A. and Friend, S. H. Germ line *p53* mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, **250**, 1233-1238 (1990).
- Coles, C., Thompson, A. M., Elder, P. A., Cohen, B. B., Mackenzie, I. M., Cranston, G., Chetty, U., Mackay, J., Macdonald, M., Nakamura, Y., Hoyheim, B. and Steel,

- C. M. Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. *Lancet*, **336**, 761-763 (1990).
- 17) Sato, T., Akiyama, F., Sakamoto, G., Kasumi, F. and Nakamura, Y. Accumulation of genetic alterations and progression of primary breast cancer. *Cancer Res.*, **51**, 5794-5799 (1991).
- 18) Davidoff, A. M., Humphrey, P. A., Iglehart, J. D. and Marks, J. R. Genetic basis for p53 overexpression in human breast cancer. *Proc. Natl. Acad. Sci. USA*, **88**, 5006-5010 (1991).
- 19) Davidoff, A. M., Kerns, B.-J. M., Iglehart, J. D. and Marks, J. R. Maintenance of p53 alterations throughout breast cancer progression. *Cancer Res.*, **51**, 2605-2610 (1991).
- 20) Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.*, **50**, 7184-7189 (1990).
- 21) Steeg, P. S., Bevilacqua, G., Kopper, L., Thorgeirsson, U. P., Talmadge, J. E., Liotta, L. A. and Sobel, M. E. Evidence for a novel gene associated with low tumor metastatic potential. *J. Natl. Cancer Inst.*, **80**, 200-204 (1988).
- 22) Varesco, L., Caligo, M. A., Simi, P., Black, D. M., Nardini, V., Casarino, L., Rocchi, M., Ferrara, G., Solomon, E. and Bevilacqua, G. The *NM23* gene maps to human chromosome band 17q22 and shows a restriction fragment length polymorphism with *Bgl*II. *Genes Chromosomes Cancer*, **4**, 84-88 (1992).
- 23) Hall, J. M., Lee, M. K., Newman, B., Morrow, J. E., Anderson, L. A., Huey, B. and King, M.-C. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science*, **250**, 1684-1689 (1990).
- 24) Sato, T., Saito, H., Swensen, J., Olifant, A., Wood, C., Danner, D., Sakamoto, T., Takita, K., Kasumi, F., Miki, Y., Skolnick, M. and Nakamura, Y. The human prohibition gene located on chromosome 17q21 is mutated in sporadic breast cancer. *Cancer Res.*, **52**, 1643-1646 (1992).