Chromosomal Aneusomy (Chr 1, 11, 17) Detected by Fluorescence *In Situ* Hybridization May be a Prognostic Factor in Breast Cancer

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Abstract. *The relationship between clinicopathological findings and the long-term prognosis was investigated in 42 breast cancer patients in whom aneusomy was detected for chromosomes 1, 11 and 17. The frequencies of aneusomy of those chromosomes were 78.6%, 47.5% and 52.5%, respectively, and more than 90% of anomalies consisted of polysomy. The relationship between aneusomy and the clinicopathological findings showed a statistical correlation with a high histological grade in the case of polysomy of chromosome 17 compared with disomy, indicating a tendency for a high incidence of lymph node metastasis. Analysis of the survival data revealed that the prognosis was poor when there was polysomy of chromosomes 1 or 11. These results indicate the possibility that aneusomy of chromosomes 1, 11 and 17 can serve as prognostic factors of poor outcome in breast cancer patients.*

Various genetic alterations are involved in the onset and development of breast cancer. These alterations include both qualitative anomalies and quantitative anomalies. Microsatellite instability (MSI) and abnormal telomerase activity are examples of qualitative anomalies involved in canceration and the histological grade, while loss of hererozygosity (LOH) and overexpression of genes are examples of quantitative anomalies (1, 2). In breast cancer, high frequencies of LOH are seen in chromosomal regions such as 1p, 3p, 6q, 11p, 13q, 16q, 17p, 17q, 18q and 22q. In particular, the *p53* gene on 17p, the *BRCA2* and *RB* genes on 13q, and the *BRCA1* gene on 17q have been identified as tumor-suppressor genes involved in breast cancer (1-3). Overexpression of the *c-erbB-2* gene on 17q is known to be an important prognostic factor in breast cancer and is widely used in the clinic as a factor for predicting the therapeutic efficacy of Herceptin (4, 5). In addition, studies using DNA microarrays indicate the presence of yet unknown oncogenes and tumor-suppressor genes and it is anticipated that many such genes will be identified and put to clinical application (6, 7). However, in the clinic, the current most reliable prognostic factor is the classic status of axillary lymph node metastasis, in addition to the hormone receptor status, histological grade and Her2 expression. There has still been no establishment of therapeutic strategies tailored to individual patients on the basis of individual gene anomalies. On the other hand, aneusomy of chromosomes 1, 11 and 17 is commonly seen in breast cancer patients, and these anomalies were reported to show a correlation with the clinicopathological findings and are said to be related to the histological grade (8-11).

We investigated whether aneusomy of chromosomes 1, 11 and 17 could serve as a prognostic factor of breast cancer by analyzing the long-term prognosis of breast cancer patients who had been tested for these anomalies in the past using the fluorescence *in situ* hybridization (FISH) technique. This is the first published report of the

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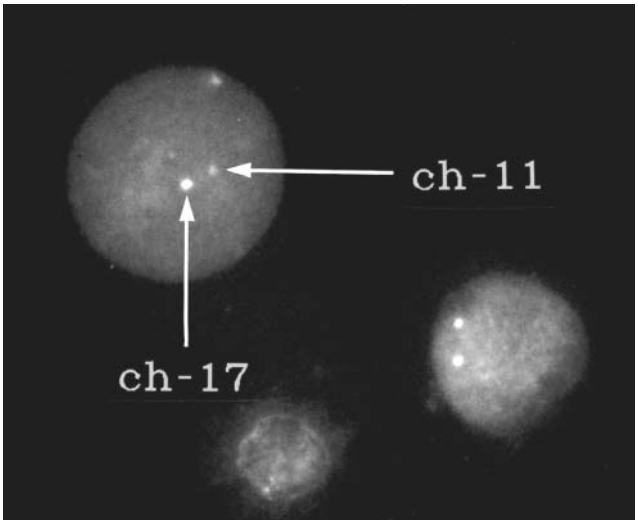

Figure 1. *Fluorescence in situ hybridization (FISH) analysis of fine-needle aspiration samples from breast tumors. Two-color FISH (chromosome 11, and chromosome 17) was conducted of fine-needle aspiration samples from a breast cancer. Two signals of chr-11 and one signal of chr-17 were seen in each nucleus from the malignant breast tumor. These findings were judged as disomy of chr-11 and monosomy of chr-17.*

relationship between aneusomy of chromosomes 1, 11 and 17 and the long-term prognosis of breast cancer.

Patients and Methods

Clinical samples. The study was carried out for 42 primary breast cancer patients for whom fine-needle aspiration cytology samples had been collected before surgery and tested for aneusomy of chromosomes 1, 11 and 17 and for whom long-term follow-up had been possible. The operations had been carried out at Higashi Tokushima National Hospital between May of 1995 and June of 1998. The duration of follow-up was at least 100 months for all surviving patients. Testing for chromosomal anomalies was carried out postoperatively using the FISH technique. The histological grade, lymph node metastasis, estrogen receptor (ER) status, progesterone receptor (PgR) status and Her2 were investigated. ER and PgR were evaluated using enzyme immunoassay (cut-off value: 10 fmol/mg/protein for both ER and PgR). Her2 was tested using an immunostaining method (Herceptest, Dako, Japan), and the results were graded using a scale of 0, 1+, 2+ and 3+ (0, no staining; 1+, weak partial membranous staining in more than 10% of cells; 2+, weak or moderate complete membranous staining in more than 10% of cells; 3+, strong complete membranous staining in more than 10% of cells).

FISH analysis. The numbers of chromosomes 1, 11, and 17 were examined using FISH using the probes specific for (peri)-centromeric regions (pUC1.77 for chromosome 1, cCR11 for chromosome 11, and CI 17-321 for chromosome 17), as described elsewhere (8-11). Hybridization signals were counted in more than 100 interphase nuclei with a fluorescence microscope. The criteria to define aneusomy are described elsewhere (8-11). Briefly, it was defined as follows: >15% of nuclei with one signal specific for (peri)centromeres of chromosomes of 1, 11, or 17 reported as "chromosome monosomy" (Figure 1), or >20% of nuclei with more than three signals specific for each chromosome 1, 11, 17, reported as "chromosomal polysomy" (Figure 2).

Statistics. The data for the presence/absence of chromosomal anomalies and the clinicopathological findings were tested for correlations using the Chi-square test; a p value of <0.05 was considered statistically significant. The survival rate was analyzed on the basis of the presence/absence of chromosomal aneusomy for each clinicopathological parameter. For analysis of the survival rates, survival curves were prepared using the Kaplan-Meier method and the log-rank test was used to determine significant differences. A p value of <0.05 was considered statistically significant. Statistical treatments were performed using the SPSS software (SPSS 11.0J with Advances Models, SPSS Inc. Japan).

Results

Aneusomy of chromosomes 1, 11 and 17 was detected in 33/42 patients (78.6%), 19/40 patients (47.5%) and 21/40 patients (52.5%), respectively and consisted of monosomy in 1, 2 and 4 patients and polysomy in 32, 17 and 17 patients respectively. The analysis of the clinicopathological findings revealed that the patients with polysomy of chromosome 17 had a significantly higher incidence of histological grade 3 disease compared to the patients with disomy and had a tendency for a higher incidence of lymph node metastasis. The patients with polysomy of chromosome 1 showed a tendency for a higher incidence of histological grade 3 disease and a higher incidence of lymph node metastasis. In addition, the patients with polysomy of chromosome 17 showed a significantly higher incidence of positivity for ER. On the other hand, aneusomy of chromosome 11 showed no correlations with the status of lymph node metastasis (Table I).

Twelve patients showed polysomy of all three of chromosomes and 5 patients showed disomy of all three. The clinicopathological findings showed lymph node metastasis in 5/12 of the all-polysomy patients but in only 1/4 of the all-disomy patients (Table II).

In the long-term follow-up, 5 of the 42 patients died due to their breast cancer. All 5 of these patients had polysomy of chromosome 1, while 3 showed polysomy of chromosome 11 and 4 showed polysomy of chromosome 17. None of the patients with monosomy died (Table III).

Analysis of the survival data for all 42 patients revealed that the prognosis was significantly worse when there was aneusomy of chromosome 11. Moreover, analysis of each of the clinicopathological parameters showed a significantly worse prognosis in stage II or higher disease when there was aneusomy of chromosome 1. In early-stage disease, chromosome 11 anomalies represented a poor prognosis (Table IV).

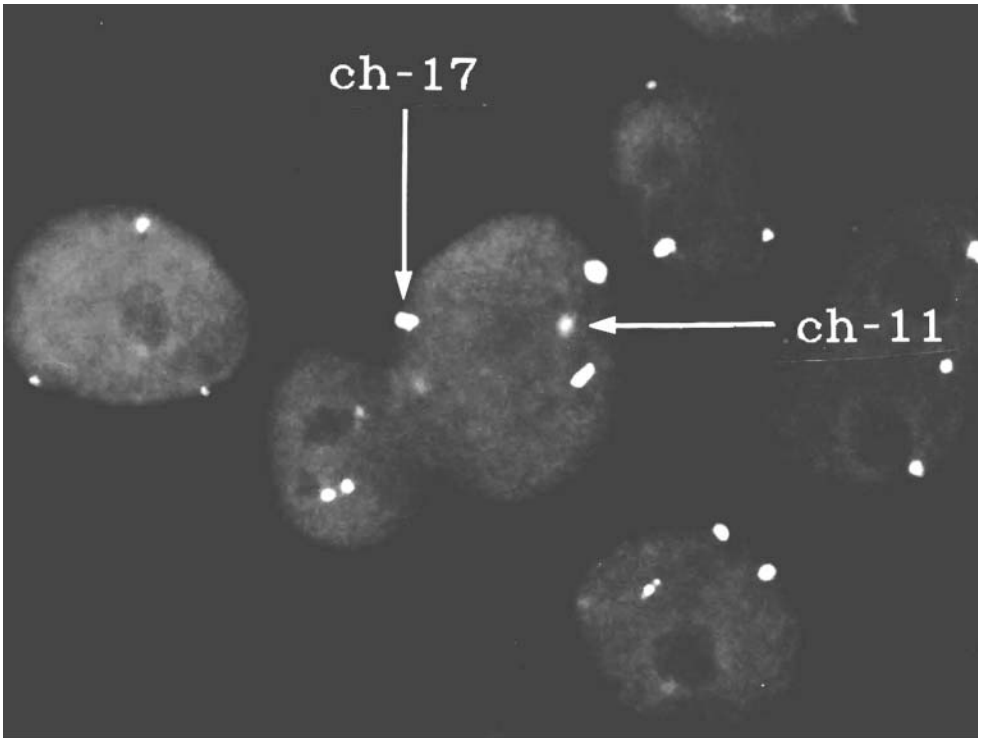

Figure 2. *Fluorescence in situ hybridization (FISH) analysis of fine-needle aspiration samples from breast tumors. Two-color FISH (chromosome 11, and chromosome 17) was conducted on fine-needle aspiration samples from a breast cancer. Two signals of chr-11 and more than two signals of chr-17 were seen in each nucleus from the malignant breast tumor. These findings were judged as the disomy of chr-11 and polysomy of chr-17.*

Table I. *Correlation of chromosomal aneusomy with clinicopathological findings.*

		Chromosome number								
		1			11			17		
		D	M	P	D	M	P	D	M	P
No. of aneusomy cases		9	1	32	21	2	17	19	4	17
Stage:	0, I	6	0	16	12	0	9	10	1	10
	II, III, IV	3	1	16	9	2	8	9	3	7
HG:	1	7	1	19	15	2	8	16	3	7
	2	2	0	4	3	0	3	3	0	3
	3	0	0	9	3	0	6	1	1	7*
N:	0	3	0	17	12	0	8	11	1	6
	(+)	1	1	14	7	2	6	5	3	9
ER:	(+)	4	0	15	12	1	5	2	2	6*
	(–)	0	1	10	4	1	5	11	2	5
PgR:	(+)	4	0	13	10	1	5	4	2	6
	(–)	0	1	12	6	1	5	9	2	5
Her2:	0, 1 (+)	6	1	26	14	2	15	15	4	12
	2 (+)	2	0	2	3	0	1	3	0	1
	3 (+)	1	0	4	4	0	1	1	0	4

D: disomy; M: monosomy; P: polysomy; HG: histological grade; *$p<0.05$ excluding cases with monosomy.

Table II. *Correlation between the clinicopathological findings and aneusomy in cases with all polysomy or all disomy.*

		All polysomy	All disomy
Number of cases		12	5
HG:	1	6	4
	2	2	1
	3	4	0
N:	0	7	3
	(+)	5	1
ER:	(–)	4	1
	(+)	3	1
Her2:	1 (+)	11	3
	2 (+)	1	2
	3 (+)	0	0

Table III. *Aneusomy patterns in death cases due to breast cancer.*

Case	Chromosome number		
	1	11	17
1	Polysomy	Disomy	Polysomy
2	Polysomy	Disomy	Disomy
3	Polysomy	Polysomy	Polysomy
4	Polysomy	Polysomy	Polysomy
5	Polysomy	Polysomy	Polysomy

Table IV. *P-values in survival results of chromosomal aneusomy in several clinicopathological parameters.*

		Chromosome 1 Aneusomy *vs.* disomy	Chromosome 11 Aneusomy *vs.* disomy	Chromosome 17 Aneusomy *vs.* disomy
All cases		0.5210	0.0369	0.5403
HG:	1	0.2553	0.2159	0.3778
	2	0.3270	0.1098	0.6068
	3	1.0000*	0.2383	0.2346
Her2:	3 (–)	0.1770	0.0770	0.2401
	3 (+)	0.1823	0.5151	0.0833
N:	0	0.0867	0.1167	0.9035
	(+)	0.0987	0.5436	0.3840
Stage:	0, I	0.7686	0.0093	0.2656
	II, III, IV	0.0053	0.9830	0.6137

*All were cases with polysomy.

Discussion

Mapping of the human genome has been completed and analysis of the functions of the genes is currently being carried out. Techniques such as DNA microarray analysis have made it possible to comprehensively analyze the expression of genes (6, 7). At present, this is possible for approximately 23,000 genes. The results of these analyses are likely to enable tailor-made therapeutic strategies for individual patients in the near future (6, 7). However, even today, the current most reliable prognostic factor available for use in the clinic for breast cancer is the status of axillary lymph node metastasis, the hormone receptor status, histological grade and, more recently, *Her2* gene expression.

Various authors have reported carrying out chromosomal analyses using the FISH technique for diagnosis of breast cancer and it has been reported that aneusomy of chromosomes 1, 11 and 17 is common in breast cancer. In addition, it has been reported that aneusomy of chromosomes shows a correlation with the clinicopathological findings in breast cancer as well as a correlation with the histological grade (8-11). Detection of aneusomy of chromosomes is comparatively easy by application of the FISH technique. Use of cytodiagnostic specimens also means that the invasiveness to patients is low, and since the generated information is obtained prior to surgery, it is useful for selection of the therapy if its clinical significance is established. We also carried out studies of aneusomy of chromosomes 1, 11 and 17 in breast cancer patients between 1995 and 1998. In the present study, we analyzed the long-term prognosis of these breast cancer patients and investigated whether information on the presence/absence of chromosomal aneusomy could be considered useful as prognostic factors.

Our results showed that the frequencies of aneusomy of chromosomes 1, 11 and 17 were 78.6%, 47.5% and 52.5%, respectively. The aneusomy consisted of monosomy in 7 chromosomes and polysomy in 66 chromosomes, showing that polysomy was much more common. The reports published by others also document that aneusomy of chromosome 1 is present in the large majority of breast cancer patients this concurs with our findings. In addition, most of the aneusomy events consisted of polysomy, with monosomy accounting for only 10% of the aneusomy. These results are in agreement with the reported findings (8-11). Tsukamoto *et al.* investigated anomalies of chromosomes 1, 11 and 17 and the clinicopathological findings in breast cancer (8). They reported finding a statistically significant correlation between a high histological grade and polysomy of chromosome 11 compared with disomy, and that there was also a significantly higher incidence of lymph node metastasis in patients with polysomy of all of chromosomes 1, 11 and 17 compared with disomy. Moreover, they reported that the incidence of lymph node metastasis increased as the incidence of polysomy increased. In addition, Ichikawa *et al.* reported finding a positive correlation between chromosome 17 anomalies and lymph node metastasis (10). Sneige *et al.* also reported that chromosomal anomalies increased together with the

histological grade (12), while Steinarsdottir *et al.* reported that the histological grade was high in patients with chromosome 1 anomalies (13).

Our present results also showed that there were more cases of lymph node metastasis in patients with polysomy of all three of chromosomes 1, 11 and 17, although the difference was not statistically significant. In 9 of 17 (52.9%) patients with polysomy of chromosome 11, the histological grade was 2 or 3, whereas the histological grade was 2 or 3 in only 6 of 21 (28.6%) patients with disomy of that chromosome. These results are similar to those reported by others, but we found no correlation with lymph node metastasis, which is at odds with earlier reports. In addition, although polysomy of chromosome 17 showed a significant correlation with a high histological grade, the incidence of lymph node metastasis was slightly higher but not statistically significant. Moreover, polysomy of chromosome 1 showed a tendency for slightly more cases of lymph node metastasis, but again the difference was not statistically significant. The limited number of patients in our present study can be considered one possible reason for the differences in our findings compared to the published literature, but it can also be surmised that for chromosome 11 there may also be genes that are related to the histological grade of breast cancer but are not involved in lymph node metastasis.

In this study we were also able to investigate the actual outcomes of the patients. Although the number of patients investigated in the study was small, this is the first published report of analysis for possible correlations between aneusomy of chromosomes 1, 11 and 17 and the prognosis of breast cancer. Our results showed that, with regard to the long-term survival outcome, aneusomy of chromosome 11 was associated with a significantly poor prognosis, and the findings showed the possibility that polysomy of chromosome 11 is an indicator of poor prognosis. In addition, the stratified analysis of the clinicopathological factors showed a significantly worse prognosis in stage II or higher disease when there was polysomy of chromosome 1 and also indicated the possibility that polysomy of chromosome 11 represents a poor prognosis in early-stage disease. Analysis of the patients who died showed that many had multiple polysomy, whereas none of the patients with monosomy died. These findings indicate that the prognosis is worse as the number of chromosomal anomalies increases and that multiplicity of genetic anomalies is an indicator of poor prognosis. In addition, it has been reported that LOH is common in breast cancer and it has been confirmed that there are tumor-suppressor genes such as *p53*. However, in terms of chromosomal anomalies, most of the anomalies are polysomy. Polysomy causes dysfunction of genes, but the fact that polysomy is common indicates the possibility that most of the genes involved in the canceration and progression of breast cancer are not tumor-suppressor genes but oncogenes. Moreover, it can be surmised that most of those oncogenes have not yet been identified.

With regard to possible relationships between chromosomal anomalies and markers such as ER and PgR, Farabegoli *et al.* reported that there were no correlations with chromosome 1 anomalies (14), but Tsukamoto *et al.* found that monosomy of chromosome 17 was associated with ER negativity (8). Conversely, our present study yielded results indicating a relationship between chromosome 17 polysomy and a positive ER status. The difference in these results may be attributable to the fact that the total number of patients in our study was small and that the number of patients with monosomy was small. Regarding Her2, the *Her2* gene (*c-erbB2*) is located on 17q, and although our results indicated that Her2 was over-expressed in many patients with 17 polysomy, the difference was not statistically significant.

Conclusion

Although the number of breast cancer patients included in this study was small, the results of analysis of the actual long-term patient outcomes demonstrated that aneusomy of chromosomes 1, 11 and 17 is a factor indicating a poor prognosis. In addition, most of those chromosomal anomalies consisted of polysomy and we surmise that yet-unknown oncogenes exist. We plan to continue this analysis of chromosomal anomalies in a larger number of breast cancer patients with the objective of determining statistical significance.

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