HIGH FREQUENCY OF ALLELIC IMBALANCE AT CHROMOSOME REGION 16q22-23 IN HUMAN BREAST CANCER: CORRELATION WITH HIGH PgR AND LOW S PHASE

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The loss of genetic material from a specific chromosome region in tumors suggests the presence of tumor-suppressor genes. Loss of heterozygosity (LOH) or allelic imbalance (AI) on the long arm of chromosome 16 is a known event in sporadic breast cancer. To locate the commonly deleted regions, and therefore (a) candidate tumor-suppressor gene(s), a deletion map of chromosome 16 was made, using 10 microsatellite markers on 150 sporadic breast tumors. The 3 smallest regions of overlap (SRO) were detected on the long arm of chromosome 16. Allelic imbalance was observed with at least one marker in 67% of the tumors. One marker, D16S421, at the 16q22-23 region, showed the highest allelic imbalance, 58%. Tumors with and without AI on 16q were tested for correlation with clinico-pathological features of the tumors such as estrogen- and progesterone-receptor content (ER and PgR), age at diagnosis, tumor size, node status, histological type, S-phase fraction, AI on chromosome 3p, and ploidy. A correlation was found between AI on 16q and high PgR content, also low S-phase fraction (99% confidence limits). A comparison of tumors with and without AI at the D16S421 marker locus revealed a slight correlation with high PgR content. The survival data showed no difference between patients with AI on 16q and those with a normal allele pattern on the long arm of chromosome 16.

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Breast-cancer incidence has been increasing worldwide in recent decades. Nearly 10% of Caucasian women and 2% of Japanese women are likely to develop breast cancer. It is the most common malignancy in the Western world and has the highest death rate of any malignancy. Chromosomal alterations, usually deletions, could imply genes that play an important role in cell division and growth. Frequent allelic imbalance (AI) in breast cancer has been detected on chromosome arms 1p, 1q, 3p, 6q, 11p, 11q, 13q, 16q, 17p and 17q (Borg *et al.*, 1992; Devilee *et al.*, 1989, 1991; Larsson *et al.*, 1990; Lundberg *et al.*, 1987; Pandis *et al.*, 1993; Sato *et al.*, 1991a; Takita *et al.*, 1992).

AI on 16q has been reported in various cancer types, such as breast, prostate, ovary and hepatocellular carcinomas, Wilms' tumor and myeloid malignancy, suggesting a tumor-suppressor gene or group of genes (Betts *et al.*, 1992; Carter *et al.*, 1990; Maw *et al.*, 1992; Nishida *et al.*, 1992; Sato *et al.*, 1991b; Tsuda *et al.*, 1990).

CA-microsatellite markers are highly informative and well distributed along chromosome 16q. They are more informative than RFLP probes and require less DNA material. Therefore, CA-microsatellite markers are ideal for AI mapping. Our aim was to map AI on 16q in sporadic breast cancer, using polymorphic microsatellite markers. We compared tumors with and without AI on 16q with respect to the clinico-pathological features of the tumors and the patients.

MATERIAL AND METHODS

Samples

Tumors and blood samples were collected from breast-cancer patients treated in the University and National Hospital of Iceland during the period 1986 to 1993. All information about the tumors, e.g., size, type and node status, was recorded

by the Department of Pathology, National Hospital of Iceland. Estrogen- and progesterone-receptor (ER and PgR) analyses by the dextran-coated charcoal method were done in our laboratory. The histology was systematically examined by a trained pathologist to evaluate the proportion of normal cells and we used only samples with a majority of tumor cells for statistical analysis, giving a total of 150 cases of primary breast cancers. This was done to avoid false-negative results due to contamination of normal cells. Blood samples were collected in EDTA.

DNA extraction

All tissue samples were immediately frozen at -70°C . Genomic DNA was extracted either from the nuclear pellet from the cytosol preparations after hormone-receptor analysis or from the pulverized primary tumor directly. The powder was suspended in 0.5 to 5 ml of nuclear-lysis buffer, containing 400 mM NaCl, 2 mM EDTA, 10 mM Tris-HCl (pH 8.2), depending on the amount of tissue. Proteinase K (100 $\mu\text{g}/\text{ml}$) was added immediately, followed by 0.5% SDS and the samples were incubated overnight at 50°C . Genomic DNA was purified by phenol:chloroform:isoamyl alcohol (25:24:1) extraction. After 2-hr incubation at 37°C with RNase (100 $\mu\text{g}/\text{ml}$), the DNA was extracted by the salting-out procedure (Miller *et al.*, 1988), precipitated with ethanol and dissolved in $1 \times \text{TE}$ (10 mM Tris-HCl and 1 mM EDTA). Normal DNA was isolated from blood lymphocytes. RBC in 15 to 20 ml of blood were lysed in cold lysis buffer containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4), 5 mM MgCl_2 and 1% Triton X-100. The nuclear pellet was then suspended in 6 ml nuclear-lysis buffer, 0.5% SDS and proteinase K. As with the tumor DNA, the salting-out procedure was used and the DNA was precipitated with ethanol and suspended in $1 \times \text{TE}$.

Markers and PCR technique

The microsatellite PCR markers used are listed in Table I. They were obtained from Oligos Etc. (Wilsonville, OR) and the Department of Clinical Genetics, University Hospital, Uppsala, Sweden. End-labelling was done with T4-PNK using γATP^{32} (Amersham, Aylesbury, UK). After 35 cycles of DNA amplification by PCR (at annealing temperatures of 55°C or 60°C) in a buffer provided by the manufacturer of the polymerase (Finnzymes, Espoo, Finland), the DNA was subjected to 5 to 6% polyacrylamide urea gels (6 M) as blood/tumor pairs and detected by autoradiography. Markers used in this study span about 130 cM of chromosome 16, but linkage mapping of chromosome 16 suggests a genetic length of about 150 cM. DNA sequence of the oligos used are: D16S423, AACAGGCTTGAAAGTCTCTGTC, GCCTATTTGATAATGCTGTACG; D16S261, AAGCTTGATCTTTCTCAGG, ATCTACCTTGCTGTCTATTG; D16S260, GGTTGAGATGCTGACATGC, CAGGGTGGCTGTTATAATG; D16S265, CCAGACATG-

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GCAGTCTCTA, AGTCCTCTGTGCACTTTGT; D16S421, ACATGAACCGATTGGACTGA, CCGTTCCTATATTT-CCTGG; D16S266, AGCTTTACAGATGAGACCAG, CAGCCAATTTCTTGAGTCCG; D16S422, CAGTGTAACCTGGGGGC, CTTTCGATTAGTTTAGCAGAATGAG; D16S402, TTTTGTAACCATGTACCCCC, ATTTATAGGGCCATGACCAG; D16S413, ACTCCAGCCCCGAGTAA, GGTACAGGTGGGTTC; D16S305, CCTCCAGGTTTCA-GGCAATTCCTCT, TAGGCGACAGAGTGGGACTCCATTA. The nucleotide sequence and regional localization of the markers were obtained from the Genome Data Base (GDB), Baltimore, MD, and the Department of Clinical Genetics, University Hospital, Uppsala, Sweden. In an independent study, we have determined LOH at the chromosome 3p region in the same tumors, also by using highly polymorphic microsatellite markers.

Statistical methods

The Chi-squared test was used for the comparison of AI with clinico-pathological variables. The 2-tailed *t*-test was also used for average grades for further substantiation of the Chi-squared analysis. Survival analysis was made according to the Kaplan-Meier method and compared by the log-rank test. For comparing survival in several groups, the Cox-Hazard model was used (Statistica/Mac, 1992, StatSoft, Tulsa, OK).

RESULTS

Chromosomal mapping of 16q

We analyzed 150 tumors with 4 polymorphic markers (D16S265 and D16S421 at the 16q21–22.1 region, D16S402 and D16S413 at the 16q24.2–24.3 region). We scored for AI by determining the reduction of allele signals in tumor DNA in comparison with normal DNA, using highly informative microsatellite markers. Figure 1 shows the autoradiograms of the AI data in a few of the tumors. AI was found in 43 to 58% of the tumors (Table I). Marker D16S421 showed the highest AI of the markers tested. Of the 150 tumor samples, 101 showed AI with at least 1 of the 4 markers. These 101 samples were analyzed with 5 other markers on 16q and one on 16p (to test for complete loss of chromosome 16q in the tumor). This enabled mapping of the deleted region(s) on 16q and finding of the region(s) showing the highest AI. Of the 101 tumors, 48 showed AI for all informative markers on 16q, from D16S261 to telomere, suggesting a complete loss of the long arm of chromosome 16. The remaining 53 tumors showed partial and interstitial deletions.

AI in the 101 tumors ranged from 60 to 85% at each marker locus (Table I), the highest frequency being at the D16S421 locus. Five informative tumors had a normal allele pattern for D16S421 but showed AI for other marker(s) at the 16q23–24 region (Fig. 2, tumors 576, 1086, 686, 1184 and 1085). The results are summarized at each marker locus in Table I.

In view of the high proportion of AI (Table I) and the smallest regions of overlap (SRO) (Fig. 2), there is more than one region with deletion on chromosome 16q. The strongest evidence for SRO is between markers D16S421 and D16S266. Only one tumor (576, Fig. 2, group C) has an informative and normal pattern for both markers. There is also strong evidence for a SRO between markers D16S413 and D16S305 (Fig. 2, groups C, D and E). There is also evidence for the third SRO that could be located between markers D16S261 and D16S260. However, no individual tumors show AI for this region only. Group A and D are consistent with SRO in the D16S261–D16S260 region and in tumors 944, 1098, 686, 1050 and 1187 it is separated from the SRO at the D16S421–D16S266 location.

Correlation with clinical and pathological variables

Table II shows the Chi-squared analysis comparing tumors showing allelic loss on 16q with clinical and pathological

FIGURE 1 – AI on 16q. The autoradiograms show normal DNA (lane N) and tumor-tissue DNA (lane T) with the marker D16S413. The case numbers are indicated above each autoradiogram. Complete loss of band is seen in tumor 938. AI in seen in tumors 1050 and 1032. The fact that there is not a complete loss of band in tumors 1050 and 1032 is most likely due to contamination from normal DNA in the tumor sample. This pattern of AI is more common than complete loss as in tumor 938. The tumors 966, 871 and 827 have normal allele patterns.

variables. There was a correlation between AI on 16q and high PgR content (95% confidence interval) and between AI on 16q and low S-phase fraction (99% confidence interval). There was no significant correlation between AI and the other parameters tested: node status, tumor size, histological type of the tumor, ER content, family history of breast cancer, age at diagnosis, ploidy, and AI on 3p. Of the tumors analyzed, 20% (21/107) showed AI both on the long arm of chromosome 16 and on the short arm of chromosome 3 (AI on 3p is 34%, data not shown). Table III shows correlation results by the 2-tailed *t*-test. This test also reveals correlation between AI on 16q and high PgR content (99% confidence interval).

Correlation analysis with the same clinical and pathological parameters was also done for the locus which showed the highest AI (marker D16S421). There was only a significant correlation between AI and high PgR content ($p = 0.042$, 95% confidence interval).

Survival

No association was observed between AI on 16q (with at least one marker) and survival. The same is seen when AI with marker D16S421 was tested as the only variable. Table IV shows the multivariate analysis (proportional-hazard Cox regression model) of prognostic factors for survival. The follow-up time varied from several months to more than 7 years. The analysis shows that patients with AI on 16q have a low relative risk of mortality compared with other factors such as positive node status, 3p AI and tumor size.

DISCUSSION

Chromosome 16 is about 3% of the human genome and could contain 1500 to 3000 genes (Callen *et al.*, 1992). Only a small proportion is likely to manifest a disease phenotype or genes that play an important role in cell division or differentiation. Studies indicate 2 to 3 tumor-suppressor genes in breast cancer on the long arm of the chromosome (Cleton-Jansen *et al.*, 1994; Sato *et al.*, 1991a; Tsuda *et al.*, 1994). The 3 reported regions on 16q are 16q22.2–24.2, 16q22.1 and 16q24.3.

TABLE 1 - MARKERS USED AND AI RESULTS

Marker ¹	Location	All patients		AI patients ²	
		Number of informative patients (%)	Number of patients with AI (%)	Number of informative patients (%)	Number of patients with AI (%)
D16S423	16p13.3			46 (58%)	7 (15%)
D16S261	16q12.1			58 (64%)	35 (60%)
D16S260	16q22.2-q22.3			51 (57%)	42 (82%)
D16S265	16q21	110 (81%)	47 (43%)	74 (81%)	45 (61%)
D16S421	16q22.1	60 (48%)	35 (58%)	41 (46%)	35 (85%)
D16S266	16q23.2			48 (55%)	34 (71%)
D16S422	16q24.1			76 (84%)	57 (75%)
D16S402	16q24.2	97 (74%)	45 (46%)	65 (71%)	45 (69%)
D16S413	16q24.3	116 (81%)	53 (46%)	75 (79%)	53 (71%)
D16S305	16q24.3			74 (86%)	56 (76%)

¹Markers ordered according to the genetic map provided by GDB. ²Patients who showed AI with at least one marker on 16q.

FIGURE 2 - Patterns of allelic deletions on chromosome 16 for 46 breast carcinomas. These are selected tumors with partial and interstitial deletions on chromosome 16q. Groups A and B are concordant with a smallest region of overlap (SRO) between markers D16S421 and D16S266. Group C is concordant with a SRO between D16S413 and D16S305. Group D and E are consistent with SRO at both regions. Furthermore, group A and D are consistent with SRO at the third region, between markers D16S261 and D16S260.

Our results show that 67% of the tumors have AI on the chromosomal arm 16q with at least one marker. This is a slightly higher percentage than reported earlier on a larger number of tumors (Cleton-Jansen *et al.*, 1994; Tsuda *et al.*, 1994). The highest AI, 58%, with one marker, was at the 16q22.1 region (marker D16S421), and there is strong evidence for a SRO in the region. Our findings are in line with those of Cleton-Jansen *et al.* (1994), giving reason to focus on this region in search for a suppressor gene. Mapping of AI on 16q in prostate cancer, hepatocellular carcinomas and Wilms' tumor has also indicated tumor-suppressor gene(s) at the 16q22.1 region (Carter *et al.*, 1990; Maw *et al.*, 1992; Tsuda *et al.*, 1994). This suggests that a single gene at this region could be important in suppressing these cancer types as well as breast cancer. The most distal marker tested, D16S305, showed elevated level of AI and a SRO was mapped at this location. This could indicate an additional suppressor gene in breast cancer on the telomere of the chromosome as suggested by Callen *et al.* (1992) and Cleton-Jansen *et al.* (1994).

We found weak but significant correlation between AI on 16q and high PgR content and between AI and low S-phase

fraction. However, we found no correlation between survival and AI on 16q. A Swedish study by Fernö *et al.* (1992) showed that the age group (40 to 49 years) with the best prognosis of all patients had the highest PgR content in their tumors and also the lowest median S-phase fraction in relation to other age groups. Patients with low S-phase fraction have about 19% higher survival rate than patients with high S-phase fraction.

There was no significant correlation between the frequencies of AI on 16q and the other parameters tested: node status, tumor size, histological type of the tumor, ER content, family history of breast cancer, age at diagnosis, AI on 3p, and ploidy. Other reports are in line with ours (Sato *et al.*, 1991a,c; Takita *et al.*, 1992; Cleton-Jansen *et al.*, 1994), although Cleton-Jansen *et al.* (1994) found a weak correlation between AI on 16q and positive estrogen-receptor content. No correlation was found between AI on 3p and 16q, which is in line with the findings of Sato *et al.* (1991a). AI on 3p correlated with low ER content, DNA non-diploidy, low PgR status and high percentage of cells in S-phase (data not shown), whereas AI on 16q correlated with high PgR content and low S-phase. Whether

TABLE II – AI ON 16q (WITH AT LEAST ONE MARKER) IN TUMOR DNA IN RELATION TO OTHER CLINICAL AND PATHOLOGICAL PARAMETERS BY CHI-SQUARED ANALYSIS

Variable	AI/total 101/150	67 %	p value
Node status			
Negative	57/78	73	0.0522
Positive	35/61	57	
No data	9/11		
Tumor size			
< 2 cm	43/61	70	0.4935
≥ 2 cm	56/86	65	
No data	2/3		
Histological type			
Ductal	85/129	66	0.2157
Lobular	13/16	81	
No data	3/5		
ER			
fmol/mg protein			
> 10	80/115	70	0.2770
≤ 10	19/32	59	
No data	2/3		
PgR			
fmol/mg protein			
> 25	71/96	74	0.0276*
≤ 25	28/50	56	
No data	2/4		
Relatives with breast cancer			
First-degree relatives	11/23	48	0.2046
Other relatives	30/44	68	
No relative	33/49	67	
No data	27/34		
S-phase			
Low (< 7%)	31/37	84	0.0082**
High (> 7%)	41/70	59	
No data	29/43		
Ploidy			
Diploid	29/44	66	0.7566
Non-diploid	44/64	69	
No data	28/42		
Age at diagnosis			
< 50 years	31/48	65	0.6223
≥ 50 years	70/102	69	
AI on 3p			
AI on 3p	21/34	62	0.6881
3p normal	48/73	66	
No data	32/43		

*95% confidence interval.—**99% confidence interval.

these results can provide information towards determining the primary events in tumor development remains to be seen.

We found no association between AI on 16q and survival. Although 16q has one of the highest deleted regions in breast cancer, patients with this factor do not have a higher relative risk of mortality than patients with the normal allele pattern on 16q in their tumors (Table IV). Node status, tumor size and 3p imbalance remain the best predictors of hazard, with a high relative risk of mortality according to the model in Table IV. Lindblom *et al.* (1993) have shown that patients in 79 families with AI on 16q are more likely to get distant metastases and have lower survival. It remains to be investigated whether

TABLE III – AI ON 16q (WITH AT LEAST ONE MARKER) OF TUMOR DNA IN RELATION TO OTHER CLINICAL AND PATHOLOGICAL PARAMETERS BY 2-TAILED *t*-TEST ANALYSIS

Variable	Mean	2-tailed p value
ER	88.4 fmol/mg	0.0924
PgR	95.6 fmol/mg	0.0063**
Tumor size	3.11 cm	0.0318*
Age at diagnosis (years)	57.7	0.5041

*95% confidence interval.—**99% confidence interval.

TABLE IV – MULTIVARIATE ANALYSIS (PROPORTIONAL-HAZARD COX REGRESSION MODEL) OF PROGNOSTIC FACTORS FOR SURVIVAL¹

Variable	p value	Relative risk of mortality (with 95% CI)
Node status	0.0078	4.69 (1.50–14.68)
AI at 3p	0.0060	4.40 (1.53–12.67)
Tumor size	0.0556	4.33 (0.96–19.40)
S phase	0.2498	3.17 (0.44–22.92)
ER	0.3876	1.96 (0.43–8.95)
PgR	0.6954	1.32 (0.33–5.18)
Histological type	0.7970	1.31 (0.17–10.12)
AI at 16q	0.6198	1.30 (0.46–3.72)
Ploidy	1.2326	0.84 (0.27–2.62)

¹Number of patients, 92.

there could be a difference of survival in hereditary and in sporadic breast cancer in patients with AI on 16q in the tumor.

We have analyzed chromosome regions 3p, 6q, 11p, 11q, 17p and 17q in sporadic breast cancer with microsatellite markers. AI at these regions is lower than at 16q, observed in 32 to 45% of the tumors. Chromosome regions 17p and 17q show the highest imbalance after 16q, giving 45% and 42% AI respectively (data not shown).

AI on 16q was found to be very high in sporadic breast cancer; in particular, the D16S421 locus was the most frequently deleted. A correlation was found between 16q AI and high PgR content and low S-phase fraction. The survival data showed that patients with AI on 16q do not have lower survival than those with a normal allele pattern on the long arm of chromosome 16. The results suggest that a putative tumor-suppressor gene is located at the 16q22.1 region and that 16q is not an independent prognostic factor.

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REFERENCES

- BETTS, D.R., ROHATINER, A.Z.S., EVANS, M.L., RASSAM, S.M.B., LISTER, T.A. and GIBBONS, B., Abnormalities of chromosome 16q in myeloid malignancy: 14 new cases and a review of the literature. *Leukemia*, **6**, 1250–1256 (1992).
- BORG, A., ZHANG, Q.X., OLSSON, H. and WENNGREN, E., Chromosome 1 alterations in breast cancer: allelic loss on 1p and 1q is related to lymphogenic metastases and poor prognosis. *Genes, Chrom. Cancer*, **5**, 311–320 (1992).
- CALLEN, D.F., HILDEBRAND, C.E. and REEDERS, S., Report of the second international workshop on human chromosome-16 mapping. *Cytogenet. Cell Genet.*, **60**, 158 (1992).
- CARTER, B.S., EWING, C.M., WARD, W.S., TREIGER, B.F., AALDERS, T.W., SCHALKEN, J.A., EPSTEIN, J.I. and ISAACS, W.B., Allelic loss of chromosomes 16q and 10q in human prostate cancer. *Proc. nat. Acad. Sci. (Wash.)*, **87**, 8751–8755 (1990).

- CLETON-JANSEN, A.M., MOERLAND, E.W., KUIPERS-DIJKSHOORN, N.J., CALLEN, D.F., SUTHERLAND, G.R., HANSEN, B., DEVILEE, P. and CORNELISSE, C.J., At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer. *Genes, Chrom. Cancer*, **9**, 101–107 (1994).
- DEVILEE, P., VAN DEN BROEK, M., KUIPERS-DIJKSHOORN, N., KOLLURI, R., KHAN, P.M., PEARSON, P.L. and CORNELISSE, C.J., At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. *Genomics*, **5**, 554–560 (1989).
- DEVILEE, P., VAN VLIET, M., VAN SLOUN, P., KUIPERS-DIJKSHOORN, N., HERMANS, J., PEARSON, P.L. and CORNELISSE, C.J., Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. *Oncogene*, **6**, 1705–1711 (1991).
- FERNÖ, M., BALDETORP, B., BORG, A., OLSSON, H. and SIGURDSSON, H., Flow-cytometric DNA index and S-phase fraction in breast cancer in relation to other prognostic variables and to clinical outcome. *Acta oncol.*, **31**, 157–165 (1992).
- LARSSON, C., BYSTRÖM, C., SKOOG, L., ROTSTEIN, S. and NORDENSKJÖLD, M., Genomic alterations in human breast carcinomas. *Genes, Chrom. Cancer*, **2**, 191–197 (1990).
- LINDBLOM, A., ROTSTEIN, S., SKOOG, L., NORDENSKJÖLD, M. and LARSON, C., Deletions on chromosome 16 in primary familial breast carcinomas are associated with development of distant metastases. *Cancer Res.*, **53**, 3707–3711 (1993).
- LUNDBERG, C., SKOOG, L., CAVENEE, W.K. and NORDENSKJÖLD, M., Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. *Proc. nat. Acad. Sci. (Wash.)*, **84**, 2372–2376 (1987).
- MAW, M.A., GRUNDY, P.E., MILLOW, L.J., ECCLES, M.R., DUNN, R.S., SMITH, P.J., FEINBERG, A.P., LAW, D.J., PATERSON, M.C., TELZEROW, P.E., CALLEN, D.F., THOMPSON, A.D., RICHARDS, R.I. and REEVE, A.E., A third Wilms' tumor locus on chromosome 16q. *Cancer Res.*, **52**, 3094–3098 (1992).
- MILLER, S.A., DYKES, D.D. and POLESKY, H.F., A simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16**, 1215 (1988).
- NISHIDA, N., FUKUDA, Y., KOKURYU, H., SADAMOTO, T., ISOWA, G., HONDA, K., YAMAOKA, Y., IKENAGA, M., IMURA, H. and ISHIZAKI, K., Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma. *Int. J. Cancer*, **51**, 862–868 (1992).
- PANDIS, N., JIN, Y., LIMON, J., BARDI, G., IDVALL, I., MANDAH, N., MITELMAN, F. and HEIM, S., Interstitial deletion of the short arm of chromosome 3 as a primary chromosome abnormality in carcinomas of the breast. *Genes, Chrom. Cancer*, **6**, 151–155 (1993).
- 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 (1991a).
- SATO, T., SAITO, H., MORITA, R., KOI, S., LEE, J.H. and NAKAMURA, Y., Allelotype of human ovarian cancer. *Cancer Res.*, **51**, 5118–5122 (1991b).
- 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 (1991c).
- TAKITA, K., SATO, T., MIYAGI, M., WATATANI, M., AKIYAMA, F., SAKAMOTO, G., KASUMI, F., ABE, R. and NAKAMURA, Y., Correlation of loss of alleles on the short arms of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes. *Cancer Res.*, **52**, 3914–3917 (1992).
- TSUDA, H., CALLEN, D.F., FUKUTOMI, T., NAKAMURA, Y. and HIROHASHI, S., Allele loss on chromosome 16q24.2-qter occurs frequently in breast cancer irrespectively of differences in phenotype and extent of spread. *Cancer Res.*, **54**, 513–517 (1994).
- TSUDA, H., ZHANG, W., SHIMOSATO, Y., YOKOTA, J., TERADA, M., SUGIMURA, T., MIYAMURA, T. and HIROHASHI, S., Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. *Proc. nat. Acad. Sci. (Wash.)*, **87**, 6791–6794 (1990).