Loss of heterozygosity at chromosome 6q correlates with tumor progression and patient survival

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Abstract. Several chromosome regions exhibit loss of heterozygosity (LOH) in human breast carcinoma and are thought to carry tumor suppressor genes. We have analysed human breast tumors with 9 polymorphic microsatellite markers that are specific to chromosome 6q. The mapping of smallest region of overlap (SRO) indicated location of candidate suppressor genes at 6q23 and 6q27. Variations in estrogen receptor (ER) expression were independent of the number of copies of the corresponding gene. Tumors with and without LOH on chromosome 6q were tested for association with clinicopathological factors. A significant association was found between LOH at 6q and the following: high S-phase, aneuploidy, deletions at chromosomes 3p and 9p and lower survival rate. In a multivariate model LOH at 6q is an independent prognostic variable and patients having tumors with LOH have approximately twofold increase in relative risk of death. It can be concluded that the 6q deletions give additional prognostic information that might be useful in breast cancer treatment.

Introduction

Breast cancer is the most common malignancy in women. It affects nearly 10% of all Caucasian women, its incidence is increasing worldwide. Irrespective of advances in therapy, the death rate has not decreased (1). Malignant transformation is envisioned as a multistep process. Activation of oncogenes and/or inactivation of tumor suppressor genes have been observed in the progression of many tumor types, including breast tumors.

The frequent finding of loss of heterozygosity (LOH) for a specific chromosomal marker in tumor DNA compared to normal DNA suggests the presence of a tumor suppressor gene. In primary breast tumors, frequent LOH has been detected on chromosome arms 1p (2), 1q (3), 3p (4,5), 6q (6), 11p and 11q (7), 13q (8), 16q (9), 17p (10), 17q and 18q (11). Some of these frequently deleted regions contain known tumor suppressor genes.

LOH on 6q has been reported in various cancer types other than breast tumors e.g. ovarian cancer (12,13), malignant melanoma (14), renal cell carcinoma (15,16), small cell lung cancer (17), primitive neuroectodermal tumors (18), and B-cell non-Hodgkins lymphoma (19,20).

Microcell mediated transfer of chromosome 6 into breast cancer cell lines lacking a normal chromosome 6 have been shown to induce alterations of *in vitro* growth properties and suppress tumorigenicity of the cells (21). Chromosome 6 has also been shown to suppress tumorigenicity of cell lines derived from endometrial carcinoma (22), and malignant melanoma (23) when transferred into these cells. Welch *et al* (24), showed that by injecting a normal chromosome 6 into a highly metastatic malignant melanoma cell line the ability of the cells to metastasize in athymic nude mice was suppressed but the cells remained tumorigenic.

The frequent LOH on 6q in several tumor types and the ability of a normal chromosome 6 to suppress tumorigenicity and metastatic properties of carcinoma cells suggests that this chromosome contains one or more tumor suppressor genes that play a role in tumor development and progression. This gives reason to focus on 6q in the search for putative tumor suppressor genes important in tumorigenesis.

In this study highly polymorphic DNA markers on 6q were used to screen 204 pairs of breast tumor DNA and normal DNA for loss of heterozygosity (LOH) in an attempt to find a common region of frequent deletions which could harbor a putative tumor suppressor gene. We also tested whether LOH correlated with certain clinicopathological variables such as tumor steroid receptor content, histological type, patient lymph node status at diagnosis, tumor size, age at diagnosis, S-phase fraction and ploidy and loss of heterozygosity on other chromosomal arms (3p, 9p, 11p, 11q, 16q, 17p and 17q).

Materials and methods

Samples and collection. Tumor samples from patients diagnosed with primary breast cancer were sent to our laboratory for estrogen receptor and progesterone receptor analysis, using a ligand binding assay. Blood samples were collected in EDTA from the patients. Tumor and blood samples were stored at -70°C until they are processed for DNA extraction. All information about the tumors e.g. tumor size, tumor type and node status was recorded by the

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Table I. Markers used and frequency of LOH.

Marker	Distance cM ^a	Location	Number of samples tested	Informative samples	Tumors with LOH %
D6S262	9	6q22.3-q23.1	174	135	21
D6S292	11	6q16.3-q23.2	86	67	36
D6S311	9	6q21-q23.3	182	166	25
D6S290	1	6q21-q25.2	180	117	28
ESR	10	6q25.1	165	145	18
D6S305	11	6q25.2-q27	116	92	22
D6S264	4	6q25.2-q27	177	126	29
D6S297	13	6q27	82	42	24
D6S281		6q27	156	113	23

^aMarkers ordered according to the genetic map provided by GDB.

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DNA extraction. Normal DNA was extracted from peripheral blood leukocytes according to the method described by Miller *et al* (25). Tumor DNA was extracted either from the nuclear pellet after cytosol removal for hormone receptor analysis or from the pulverized primary tumor itself according to the method described by Maniatis *et al* (26).

PCR analysis of microsatellites. Paired blood and tumor DNA was subjected to PCR analysis. Table I lists the microsatellite PCR primers used. They were obtained from the Nordic Primer Bank in Uppsala. Information about regional location of the primers was obtained from the Department of Clinical Genetics at the University Hospital in Uppsala and the Genome Data Base in Baltimore. The primers were end labelled with $\gamma^{32}\text{P}$ -ATP using T4-PNK. DynaZyme™ polymerase was used in the buffer solution supplied with the enzyme. PCR products were electrophoresed on 6.5% denaturing polyacrylamide gels and then subjected to autoradiography and interpreted by 2 reviewers.

Statistical analysis. The Chi-square test was used to determine whether any correlation exists between allelic imbalance on 6q and certain clinicopathological variables. These clinicopathological variables and the cut-off levels used are listed in Table II. Survival analysis was made according to the Kaplan Meier method. Survival curves were compared by the log-rank test. The Cox's partially non-parametric regression model was used for multivariate analysis.

Results

Loss of heterozygosity (LOH) on 6q. We analysed pairs of normal DNA and tumor DNA from 204 breast cancer patients for LOH, using 9 polymorphic DNA markers localized to the

Figure 1. Dinucleotide repeat polymorphism in selected matched normal (N) and tumor (T) tissue from breast cancer patients. The microsatellite markers D6S292 (a) and D6S262 (b) were used in PCR analysis and the ^{32}P -labelled amplification products separated in 6.5% polyacrylamide, 8 M urea denaturing gels. Case numbers are shown at the top. Numbers to the right indicate allele sizes in nucleotides (nt). LOH can be seen with D6S292 in case 1189 and with D6S262 in cases 632 and 1086, indicated by arrows. Case 1182 is homozygous with marker D6S292 while other cases are normal heterozygotes.

Figure 2. Patterns of allelic deletions on chromosome 6q for 54 breast carcinomas. These are selected tumors with partial and interstitial deletions on chromosome 6q. Group A is concordant with a smallest region of overlap (SRO) between markers D6S311 and D6S290. Group B is concordant with a SRO between markers D6S264 and D6S297. Group C is consistent with SRO at both regions.

Table II. Chi-square analysis comparing loss of heterozygosity (LOH) on 6q of tumor DNA to other categorized prognostic factors.

Variable	LOH/Total	%	P
All	84/204	41	
S-phase			
low (<7)	32/105	31	<0.001***
high (>7)	47/81	58	
no information	5/18		
Ploidy			
diploid	25/77	33	0.029*
aneuploid	56/116	48	
no information	3/11		
Node status			
negative	44/122	36	0.071
positive	40/82	49	
no information	1/1		
Histological type			
ductal	78/183	43	0.169
lobular	5/19	26	
no information	1/2		
PgR fmol/mg protein			
≥ 25	42/111	38	0.345
< 25	37/83	45	
no information	5/10		
ER fmol/mg protein			
≥ 10	59/149	40	0.445
< 10	22/48	46	
no information	3/7		
Tumor size			
< 2 cm	35/82	43	0.870
≥ 2 cm	49/118	42	
no information	0/4		
Age			
< 50year	30/72	42	0.916
≥ 50year	54/132	41	

***=99.9% confidence interval. *=95% confidence interval.

long arm of chromosome 6. Fig. 1 shows representative autoradiograms documenting LOH detected in breast tumor samples.

Eighty-four of the 204 samples showed LOH with at least one marker used in the study (41%). Frequencies of LOH at each of the 9 markers tested at chromosome 6 are summarized in Table I. LOH for individual markers ranged from 18-36%. The markers ESR located at 6q25.1 and D6S305 located at 6q25.2-q27 gave the lowest percentage of abnormality.

A high percentage of abnormality was detected with markers localized at 6q22-q23 and 6q27 (Table I). Of the 84 tumors, 23 showed LOH for all informative markers on 6q, suggesting a major loss of the long arm of chromosome 6. The remaining 61 tumors showed partial and interstitial deletions (Fig. 2). In view of the high proportion of LOH (Table I) and the smallest region of overlap (SRO) (Fig. 2), two regions with deletions on chromosome 6q are detected. These two SROs are located between markers D6S311 and D6S290 and markers D6S264 and D6S297, respectively.

Loss of heterozygosity on 6q and correlation to clinicopathological variables. The clinicopathological variables and the cut-off levels used are listed in Table II. A significant association was found between LOH on 6q and high tumor S-phase fraction with 58% of tumors with high S-phase having LOH on 6q but 31% of tumors with low S-phase ($p<0.001$). A significant correlation was also found between LOH on 6q and aneuploidy, with 48% of aneuploid tumors with LOH on 6q but 33% of diploid tumors ($p=0.029$). There was no significant association between LOH on 6q and the other parameters tested (Table II).

LOH on 6q and association with LOH on other chromosome arms. At our laboratory chromosome regions 3p, 9p, 11p, 11q, 16q, 17p and 17q have been analyzed in sporadic breast cancer with microsatellite markers. A strong significant association was found between LOH on 6q and LOH on 3p and 9p and a significant association was also observed between LOH on 6q and LOH on 11q, 17p and 17q (Table IV).

Survival analysis. A significant prognostic separation of survival curves was detected in relation to LOH at 6q (Fig. 3).

Table III. Multivariate analysis (proportional-hazard (Cox) regression) of survival in patients with breast cancer.

Factor	Number of patients=158		
	Univariate p-value	Multivariate p-value	RR (with 95% CI)
Tumor size	0.004	0.021	2.40 (1.14-5.05)
6q LOH	0.013	0.039	1.95 (1.03-3.66)
PgR	0.040	0.110	1.65 (0.89-3.05)
Axillary nodal involvement	0.018	0.187	1.51 (0.82-2.78)
S-phase fraction	0.014	0.690	1.14 (0.61-2.13)

Factors were categorized as shown in Table II. According to this model tumor size and 6q LOH are independent predictors of hazard. RR=relative hazard of dying.

Figure 3. Overall survival. The number of patients at risk at time 0, 2.5 years and 5 years is shown for both categories. —, patients without 6q LOH; ---, patients with 6q LOH.

After 5 years of follow-up there was 38% mortality among patients with LOH at 6q but only 20% among those without 6q LOH ($p=0.013$). The possible clinical relevance of 6q LOH as a prognostic factor in breast cancer was evaluated with multivariate analyses. The patients having tumors with 6q LOH had a 1.95 fold increase in relative mortality rate compared with patients without 6q LOH (Table III).

Discussion

In this study LOH on chromosome 6q was detected with at least one marker in 41% of the tumors examined. These observations are in accordance with the findings of other researchers (6). The elevated frequency of LOH with certain

Table IV. Correlation of LOH at 6q and LOH at other chromosome regions.

	p value	n
3p	<0.001***	117
9p	<0.001***	140
11p	0.165	70
11q	0.029*	85
16q	0.757	152
17p	0.022*	75
17q	0.016*	79

(n=number of samples tested). ***=99.9% confidence interval. *=95% confidence interval.

markers and the mapping of smallest region of overlap (SRO) suggests two regions with deletions, at 6q21-23 and 6q25-27, indicating the location of candidate tumor suppressor genes. These two regions are also deleted in ovarian tumorigenesis, further supporting the presence of tumor suppressor genes (27).

Estrogen receptor content of a breast tumor is an indicator of differentiation. High ER content predicts improved disease-free survival and the likelihood of benefit from tamoxifen therapy. A number of estrogen receptor variants have been described in breast tumors (28). We found no significant association between loss of heterozygosity on 6q and estrogen receptor content of the tumor. These results are in accordance with earlier reports (29,30). This seems to compare to similar observations regarding LOH at chromosome 11q and lack of association with PgR gene expression (7,29,30). One copy of these hormone receptor genes seems to be sufficient for the receptor expression. Mechanisms other than loss of heterozygosity on chromosome 6q seem to cause loss of estrogen receptor

expression in breast cancer cells. Abnormal methylation of a certain region in the ER gene is a mechanism that has been proposed (31). A low percentage of abnormality was detected with the marker ESR which is located in the estrogen receptor gene (32). We can not conclude from our results that the gene for the estrogen receptor plays a role as a tumor suppressor gene.

Measures of the proliferation activity of tumor cells have prognostic value in patients with node-negative breast cancer (33). LOH on 6q was found to be significantly correlated with high tumor S-phase fraction and tumor aneuploidy. These findings suggest that LOH at 6q is associated with cell proliferation and an instable genome. We found no significant association between LOH at 6q and common prognostic factors such as patients age at diagnosis, tumor size, progesterone receptor content, histological type and degree of axillary involvement at diagnosis.

A significant separation of survival curves was detected in relation to LOH at 6q, patients with LOH at 6q having worse prognosis. In a multivariate analysis LOH at 6q and tumor size were found to be independent prognostic variables and patients having tumors with LOH were found to have approximately a twofold increase in relative risk of death from the disease. This suggests that LOH at 6q might be useful as an independent prognostic factor.

Some studies have revealed that certain LOH events occur in preferred combinations e.g. 3p and 17p (6), 13q and 17p (1,34,35), 11p and 17p (36). LOH on 6q was significantly correlated with LOH on chromosome arms 3p and 9p. These combinations of LOH events in cells could result in a growth selection during cancer progression. Preferred combinations of tumor suppressor gene deletions might result in enhanced tumor growth. These results should however be interpreted cautiously in functional terms since as Devilee and Cornelisse (37) have pointed out they might be coincidental with the fact that some of the regions mentioned above are among the most intensively studied in breast cancer.

Our results show that LOH at 6q is common in breast cancer. LOH has significant correlation with some of the clinicopathological factors and has an independent prognostic value. It can be concluded that 6q deletions give additional prognostic information. Further investigation should reveal whether this finding can assist in treatment of the disease.

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References

1. 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.
2. Genuardi M, Tsihira H, Anderson DE and Saunders GF: Distal deletion of chromosome 1p in ductal carcinoma of the breast. *Am J Hum Genet* 45: 73-82, 1989.
3. Gendler SJ, Cohen EP, Craston A, Duhig T, Johnstone G and Barnes D: The locus of the polymorphic epithelial mucin (PEM) tumor antigen on chromosome 1q21 shows a high frequency of alteration in primary human breast tumors. *Int J Cancer* 45: 431-435, 1990.
4. Eiríksdóttir G, Bergthorsson JT, Sigurdsson H, Gudmundsson J, Skirnisdóttir S, Egilsson V, Barkardóttir RB and Ingvarsson S: Mapping of chromosome 3 alterations in human breast cancer using microsatellite PCR markers: correlation with clinical variables. *Int J Oncol* 6: 369-375, 1995.
5. Bergthorsson JT, Eiríksdóttir G, Barkardóttir RB, Egilsson V, Arason A and Ingvarsson S: Linkage analysis and allelic imbalance in human breast cancer kindreds using microsatellite markers from the short arm of chromosome 3. *Hum Genet* (In press).
6. Devilee P, Van Vliet M, Van Sloun P, Kuipers-Dijkshoorn NJ, Hermans J, Pearson PL and Cornelisse CJ: Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. *Oncogene* 6: 1705-1711, 1991.
7. Gudmundsson J, Barkardóttir RB, Eiríksdóttir G, Arason A, Egilsson V and Ingvarsson S: Correlation of prognostic factors with genetic alterations on chromosome 11 in human breast cancer: two possible deletion regions on 11q. *Br J Cancer* (In press).
8. Larsson C, Byström C, Skoog L, Rotstein S, Nordenskjöld M: Genomic alterations in human breast carcinomas. *Genes Chromosomes Cancer* 2: 191-197, 1990.
9. Skirnisdóttir S, Eiríksdóttir G, Baldursson T, Barkardóttir RB, Egilsson V and Ingvarsson S: High frequency of allelic imbalance at chromosome region 16q22-q23 in human breast cancer: correlation with high PgR and low S-phase. *Int J Cancer* 64: 112-116, 1995.
10. McKay J, Steel CM, Elder PA, Forrest APM and Evans HJ: Allele loss on the short arm of chromosome 17 in breast cancer. *Lancet* 2: 1384-1385, 1988.
11. Cropp CS, Lidereau R, Campell G, Champeme MH and Callahan R: Loss of heterozygosity on chromosomes 17 and 18 in breast carcinoma: two additional regions identified. *Proc Natl Acad Sci USA* 87: 7737-7741, 1990.
12. Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K and Nakamura Y: Fine scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. *Cancer Res* 52: 5815-5817, 1992.
13. Foulkes WD, Ragoussis J, Stamp GWH, Allan GJ and Trowsdale J: Frequent loss of heterozygosity on chromosome 6 in human ovarian carcinoma. *Br J Cancer* 67: 551-559, 1993.
14. Millikin D, Meese E, Vogelstein B, Witkowski C and Trent J: Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. *Cancer Res* 51: 5449-5453, 1991.
15. Morita R, Ishikawa J, Tsutsumi M, Hikiji K, Tsukada Y, Kamidono S, Maeda S and Nakamura Y: Allelotype of renal cell carcinoma. *Cancer Res* 51: 820-823, 1991.
16. Morita R, Saito S, Ishikawa J, Ogawa O, Yoshida O, Yamakawa K and Nakamura Y: Common regions of deletion on chromosome 5q, 6q and 10q in renal cell carcinoma. *Cancer Res* 51: 5817-5820, 1991.
17. Merlo A, Gabrielson E, Mabry M, Vollmer R, Baylin S and Sidransky D: Homozygous deletion on chromosome 9p and loss of heterozygosity on 9q, 6p and 6q in primary human small cell lung cancer. *Cancer Res* 54: 2322-2326, 1994.
18. Thomas G and Raffel C: Loss of heterozygosity on 6q, 16q and 17p in human central nervous system primitive neuroectodermal tumors. *Cancer Res* 51: 639-643, 1991.
19. Gaidano G, Hauptschein RS, Parsa NZ, Offit K, Rao PH, Lenoir G, Knowles DM, Chaganti RSK, Dalla-Favera R: Deletions involving two distinct regions of 6q in B-cell non-Hodgkin lymphoma. *Blood* 80: 1781-1787, 1992.
20. Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, Jhanwar SC, Dalla-Favera R, Chaganti RS: 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. *Blood* 82: 2157-2162, 1993.

21. Negrini M, Sabbioni S, Possati L, Rattan S, Corallini A, Barbanti-Brodano G and Croce C: Suppression of tumorigenicity of breast cancer cells by microcell-mediated chromosome transfer: studies on chromosomes 6 and 11. *Cancer Res* 54: 1331-1336, 1994.
22. Yamada H, Wake N, Fujimoto S, Barret J and Oshimura M: Multiple chromosomes carrying tumor suppressor activity for a uterine endometrial carcinoma cell line identified by microcell-mediated chromosome transfer. *Oncogene* 5: 1141-1147, 1990.
23. Trent JM, Stanbridge EJ, McBride HL, Meese EU, Casey G, Araujo DE, Witkowski CM, Nagle RB: Tumorigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. *Science* 247: 568-571, 1990.
24. Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Stanbridge EJ, Weissman BE: Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. *Oncogene* 9: 255-262, 1994.
25. Miller SA, Dykes DD and Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl Acids Res* 16: 1215, 1988.
26. Maniatis T, Fritsch EF and Sambrook J: Molecular cloning: A laboratory manual. Cold Spring Laboratory Press, New York, 1982.
27. Orphanos V, McGown G, Hey Y, Thorncroft M, Santibanez-Koref M, Russell SEH, Hickey I, Atkinson RJ and JM Boyle: Allelic imbalance of chromosome 6q in ovarian tumors. *Br J Cancer* 71: 666-669, 1995.
28. McGuire WL, Chamness G and Fuqua S: Estrogen receptor variants in clinical breast cancer. *Mol Endocrinol* 81: 1571-1575, 1991.
29. Magdelenat H, Gerbault-Seureau M, Laine-Bidron C, Prieur M and Dutrillaux B: Genetic evolution of breast cancer: II. Relationship with estrogen and progesterone receptor expression. *Breast Cancer Res Treat* 22: 119-127, 1992.
30. Magdelenat H, Gerbault-Seureau M and Dutrillaux B: Relationship between loss of estrogen and progesterone receptor expression and of 6q and 11q chromosome arms in breast cancer. *Int J Cancer* 57: 63-66, 1994.
31. Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB and Davidson NE: Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. *Cancer Res* 54: 2552-2555, 1994.
32. Senno L, Aguiari G and Piva R: Dinucleotide repeat polymorphism in the human estrogen receptor (ESR) gene. *Hum Mol Genetics* 1: 354, 1992.
33. Sigurdsson H, Baldetorp B, Borg A, Dalberg M, Fernö M, Killander D and Olsson H: Indicators of prognosis in node-negative breast cancer. *New Engl J Med* 322: 1045-1053, 1990.
34. Andersen TI, Gaustad A, Farrants GW, Nesland JM, Tveit KM and Borresen AL: Genetic alterations of the tumor suppressor gene regions 3p, 11p, 13q, 17p and 17q in human breast carcinoma. *Genes Chromosomes Cancer* 4: 113-121, 1992.
35. Borg A, Zhang QZ, Alm P, Olsson H and Sellberg G: The retinoblastoma gene in breast cancer: allele loss is not correlated with loss of gene protein expression. *Cancer Res* 52: 2991-2994, 1992.
36. Takita KI, 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 arm of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes. *Cancer Res* 52: 3914-3917, 1992.
37. Devilee P and Cornelisse CJ: Somatic genetic changes in human breast cancer. *Biochim Biophys Acta* 1198: 113-130, 1994.