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ARTICLE

Lack of Correlation Between Survival and Allele Loss on Chromosome 7q31-32 in Primary Breast Cancer*

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High incidence of loss of heterozygosity (LOH), affecting the 7q31-32 chromosome region in sporadic primary human breast carcinomas suggests the presence of a tumor suppressor gene in this region which seems relevant to the development of breast cancer. To further determine the possible role of this region in the pathogenesis of human primary breast cancer and association with survival, LOH analysis was performed on 52 primary breast cancer patients using a set of highly polymorphic microsatellite markers. Our panel contained twenty biopsy cases of unknown survival, nineteen cases with more than five years survival and fourteen cases with less than two years survival. Corresponding normal and tumor DNAs were analyzed by polymerase chain reaction (PCR). The data presented here demonstrate that all patients were informative at least at one locus and 20 (38%) out of 53 cases showed LOH at one or more loci on chromosome 7q31-32. Relatively high incidence of LOH (34%) was detected at the D7S522 microsatellite marker located near to the cMet proto-oncogene while lower frequencies were observed at D7S523 (19%) and D7S495 (17%) loci, supporting the existence of a putative tumor suppressor gene at the chromosome 7q31.1 region. Our results suggest that allelic imbalance on 7q may occur at an early stage of breast carcinogenesis, as no correlation was observed between allelic loss and clinico-pathological data. (Pathology Oncology Research Vol 2, No1–2, 48–51, 1996)

Key words: chromosome 7q; tumor suppressor gene; loss of heterozygosity; primary breast carcinoma

Introduction

Human breast cancer is a heterogeneous and progressive disease, accounting for 19% of total cancer patients and is the most frequent malignancy among women showing an increasing rate worldwide.[1,3]

Growing evidence suggests that sequential accumulation of multiple alterations affecting tumor suppressor genes and proto-oncogenes are responsible for genesis and development of tumors.[10,19,23] Frequent observation of somatic allelic deletion or loss of heterozygosity (LOH) at a specific chromosomal locus in a particular tumor reveals the presence of a tumor suppressor gene.[15]

Previous cytogenetic and molecular genetic analyses of breast carcinoma highlighted involvement of LOH of different chromosomal regions including the long arm of chromosome 7.[5,10] In different studies, LOH frequency was detected in an average of 23% of primary breast cancer cases, ranging from 0 to 41%.[1,7,10,11,16,17] High incidence of LOH was reported on 7q in sporadic primary breast carcinomas indicating the presence of a putative tumor suppressor gene near to the *cMet* proto-oncogene (7q31-7q33).[2,9,21]

In order to determine the possible location of this putative tumor suppressor gene, extensive LOH studies were performed with a set of highly polymorphic genetic markers, now a widely accepted approach to indicate areas in a particular chromosome region where inactivation of a tumor suppressor gene may occur. Though significant chromosome 7 involvement in LOH has been reported primarily in breast cancer, several studies have revealed that consistent allelic losses for chromosome 7q regions are common in ovarian cancer,[20] in other types of epithelial

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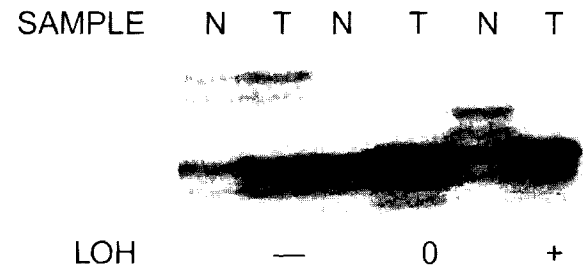

Figure 1. Representative results of LOH analyses of primary breast cancer patients at D7S522 locus. (T) and (N) indicate matched DNA samples isolated from tumor and normal tissues, respectively; — indicates no allele loss; 0 indicates uninformative case; + indicates LOH.

DNA extraction, PCR amplification, LOH analysis

DNA was extracted from 53 matching tumor and normal samples by standard methods. Corresponding archival normal section and peripheral lymphocyte DNA, respectively, were used as PCR templates. To determine the frequency of LOH three $(CA)_n$ microsatellite repeat markers (D7S522, D7S523, D7S495) were amplified in the 7q31-32 region. PCR reactions were performed at a final volume of 40 µl containing 100 ng genomic DNA, 20 pmol of each primer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 50 mM KCl, 10 mM Tris pH 8.3 and 1.5 U AmpliTaq DNA Polymerase (Perkin Elmer, Cetus). The 5'-primers of markers were end-labeled with [γ^{32}P]ATP using T4 Polynucleotide Kinase B. Samples were amplified in 30-35 cycles, each containing a denaturation step (45 sec at 90°C), an annealing step (1 or 1.5 min at appropriate temperature) and an extension step (70 sec at 72°C). In all cases, PCR cycles were preceded by an initial denaturation step (4 min at 94°C) and followed by a final elongation step (7 min at 72°C). PCR products were separated on 6% denaturing acrylamide sequencing gels. After fixation and drying, gels were autoradiographed for 1-14 days at –80°C. The samples were scored for LOH by comparing the autoradiographic signals of the corresponding normal and tumor tissue samples.

Statistical analysis

All comparisons were performed using Fischer's exact t-test.

Results and Discussion

Fifty-three clinically and pathologically characterized primary breast tumors and corresponding normal samples were screened for allele loss on the long arm of chromosome 7 using three polymorphic markers mapped to the 7q31-32 region (D7S522, D7S523, D7S495) (shown in *Table 1A*). The informativity of the markers and the frequencies of LOHs are listed in *Table 1B*. Selected example of LOH is given in *Fig. 1*.

All of our tumor samples (53 cases) were informative for at least one marker, and the overall frequency of LOH on chromosome 7q was relatively high (38%, 20/53). Allele loss frequently occurred at D7S522 (34%), while relatively lower frequency was observed at D7S523 (19%) and D7S495 (17%) loci. Allelic deletion was uncommon in biopsies, 15% of the informative tumors showed loss of heterozygosity compared to the observed 57% in short survival and 47% in long survival cases (*Table 1B*).

Reported values of LOH on 7q vary from 0 to 41% in different studies,[2,4,9,11,21] giving an average of 23% in breast cancer.[10] In our study, allelic loss occurred most frequently at the D7S522 locus which is the nearest microsatellite marker to the *cMet* proto-oncogene, often deleted in breast cancer as reported previously.[2,21] Recent studies revealed a consistent high frequency of LOH in the 7q31-32 region, not only in breast, but in other types of epithelial cancers.[26,27,28]

Seven tumors out of 20 (35%) showed loss at least at two loci. In two cases all three loci were deleted. The vast majority of such loss patterns was detected in biopsy tumor cases (3/3) and long survival cases (3/9). Occurrence of such a series of LOH in contiguous markers suggest deletion of the entire segment. Interstitial deletion affecting only one marker was observed in 65% (13/20) of primary breast tumors.

Currently there are several dozen putative breast cancer prognostic factors reported in humans.[1,12] In order to estimate the biological and/or clinical importance of LOH frequency at 7q, some of these factors (lymph node status, tumor size, ER status and TNM stage) were correlated to the observed genetic lesions (allelic losses), but no correlation was found.

Strong association between high frequency of LOH on chromosome 7q and poorer survival was reported.[2,6] However, in this study, LOH analysis did not reveal statistically significant differences in frequencies of chromosome 7q allele losses between patients with short and long survival time (57% vs. 47%).

The overwhelming majority of our samples were early stage tumors (stage I or II). 7q deletion was detected in 32% of informative cases. The relatively frequent allelic deletions may suggest that inactivation of a putative tumor suppressor gene(s) located in the 7q31 region occurs as a rather early step in tumorigenesis of the breast, and has less or no influence on tumor progression.

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Table 1 A. **LOH pattern of 7q31-32 in primary breast carcinoma patients**

Cases	ER status*	Chromosomal markers		
		D7S522	D7S523	D7S495
B1	+	-	-	0
B2	+	0	-	-
B3	-	-	-	-
B4	+	-	-	-
B5	-	+	+	+
B6	+	-	-	-
B7	-	-	-	0
B8	+	-	-	-
B9	-	-	-	-
B10	-	0	-	-
B11	+	-	0	-
B12	-	-	-	-
B13+	+	+	+	-
B14+	-	-	-	-
B15	-	+	-	-
B16		-	0	-
B17	-	0	-	-
B18	+	0	-	-
B19	+	-	0	-
B20	+	-	-	-
L1		-	-	-
L2		+	-	-
L3		+	+	+
L4	+	-	0	-
L5	+	-	0	ND
L6	+	+	ND	-
L7	+	-	-	ND
L8	+	ND	ND	-
L9		+	+	-
L10		+	-	ND
L11		-	0	-
L12		0	-	-
L13		+	-	-
L14		0	-	-
L15		-	-	+
L16		+	0	+
L17	+	-	0	0
L18		-	0	-
L19		+	0	-
S1		-	+	-
S2		+	0	-
S3	+	-	0	+
S4	-	-	-	-
S5	-	+	0	-
S6	-	-	0	-
S7	-	+	-	-
S8	+	-	-	-
S9	-	-	ND	-
S10	+	ND	-	-
S11	-	-	ND	+
S12		ND	ND	+
S13	-	+	0	+
S14		-	0	-
Total LOH		15/44 (34%)	6/32 (19%)	8/47 (17%)

B – biopsy cases; L – long survival paients; S – short survival patients; 0 – non-informative cases; ND – not determined;
ER* – estrogen receptor; B13+, B14+ – two tumors from the same patient (bilateral breast cancer)

tumors,[26,27,28] and less frequently, in non-epithelial tumors,[14,22] confirming the presence of a tumor suppressor gene in 7q31. The region of interest recently has been narrowed down to 1 cM in extent.[28]

Though the nature of the genetic alterations that are crucial at early stages of the tumorigenic process still remains obscure, recent microcell-fusion transfer and other in vitro studies on chromosome 7 demonstrated that genes affecting tumor suppression or senescence may be located on the long arm of chromosome 7.[18,25]

Bièche *et al.*[2] reported that somatic allelic deletions at the c*Met* proto-oncogene locus on chromosome 7q31 correlated with decreased survival in patients with primary breast cancer, contrary to previous observations.[3,7,8,16]

In order to contribute to the clarification of the biological significance of genetic alterations of chromosome 7q in primary breast cancer and to further examine how 7q31-32 allelic losses meet the criteria for the definition of a prognostic factor, the present study was designed to examine the frequencies of subchromosomal deletions on chromosome 7q and to determine their possible association with clinico-pathological parameters, some of them of prognostic value.

Materials and Methods

Samples

Fresh and archival tumor tissue samples were collected from 52 patients undergoing surgery for primary breast cancer, who had received no prior therapy. Histopathological classification of the tumors was carried out according to the WHO classification. The distribution of the breast tumor samples was as follows: 20 biopsy cases and 39 paraffin embedded archival specimens, of which 19 survived more than 5 years and 14 less than 2 years.

Table 1 B. **Informativity and LOH frequency of the markers used for detection of allele loss in breast cancer patients**

Locus	Informative cases (%)	LOH (%)			Informative/total cases (%)
		Short survival†	Long survival‡	Biopsy	
D7S495	47/50 (94%)	4/14 (29%)	3/15 (20%)	1/18 (6%)	8/47 (17%)
D7S522	38/44 (86%)	4/12 (33%)	8/16 (50%)	3/16 (19%)	15/44 (34%)
D7S523	32/48 (67%)	1/5 (20%)	2/10 (20%)	3/17 (18%)	6/32 (19%)
Total	53/53 (100%)	8/14 (57%)	9/19 (47%)	3/20 (15%)	20/53 (38%)

LOHs are presented as % of informative cases.
† data on patients with short survival (< 2yr)
‡ data on patients with long survival (> 5yr). Biopsy samples are unselected for survival.

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