Multiple regions of chromosome 6q affected by loss of heterozygosity in primary human breast carcinomas

ZM Sheng1, A Marchetti2, F Buttitta2, M-H Champeme3, D Campani2, M Bistocchi2, R Lidereau3 and R Callahan1

Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD 20892; 2Institute of Pathological Anatomy and Histology, University of Pisa, Pisa, Italy; 3INSERM, Centre Rene Huguenin, 92211 Saint Cloud, France.

Summary A total of 80 primary human breast carcinoma DNAs were analysed for loss of heterozygosity (LOH) on the long arm of chromosome 6, using microsatellite markers whose location has been defined physically and by linkage analysis. Loss of heterozygosity was observed in 38 of 80 (48%) tumours that were informative for at least one locus. The analysis revealed partial or interstitial deletions of chromosome 6q. Detailed mapping of chromosome 6q in these tumour DNAs identified two and perhaps three commonly deleted regions. One of these is located between markers D6S251 and D6S252 (6q14-9q16.2), another between D6S268 and D6S261 (6q16.3-q23) and a third between D6S287 and D6S270 (6q23-2q23.1).

Keywords: breast cancer; deletion; chromosome 6q; tumour-suppressor gene

Cytogenetic (Dutrillaux et al., 1990; Lu et al., 1993; Thompson et al., 1993; Trent et al., 1985, 1993) and molecular analyses (reviewed in Callahan et al., 1993) of primary human breast carcinomas have documented frequently occurring genetic alterations that take place during the evolution of tumour development. It is thought that these mutations either inactivate normal growth controls or give the tumour some selective advantage. At the molecular level, loss of heterozygosity (LOH) is the most frequent type of genetic alteration in primary human breast tumours (Callahan et al., 1993). LOH at specific chromosomal loci has been taken as evidence for the presence of putative tumour-suppressor genes within the affected regions (Knudson, 1989). In sporadic primary human breast carcinomas LOH has been detected on at least 12 different chromosome arms (Callahan et al., 1993). However only in the case of chromosome 17p13 has the target gene for LOH (TP53) been identified (Hollstein et al., 1991). Generally one allele of the target gene is lost and the remaining allele contains a nonsense or missense mutation. The involvement of chromosome 6q in breast carcinomas has been noted in cytogenetic analysis of primary tumours (Dutrillaux et al., 1990; Lu et al., 1993; Thompson et al., 1993; Trent et al., 1985, 1993). Similarly, molecular analysis of primary breast tumour DNAs has shown that chromosome 6q is frequently affected by LOH (Devilee et al., 1991). In this report, we describe studies aimed at defining the location of putative tumour-suppressor gene(s) on chromosome 6q in primary breast tumour DNAs. We have screened 80 pairs of matched breast tumour and normal DNAs using sequence-tag sites (STSs) whose location has been defined by linkage analysis and have constructed a detailed deletion map of this chromosome arm.

Materials and methods

Thirty invasive ductal carcinomas (IDCs) of the breast and matching peripheral lymphocytes were collected at the Centre Rene Huguenin, Saint Cloud, France (tumour panel 1), and another 50 pairs of IDCs and matched lymphocytes were collected from University of Pisa, Pisa, Italy (tumour panel 2). Patients corresponding to each of the tumour panels had received no prior therapy.

Genomic DNA was extracted and diluted to 100–200 ng μl−1. Polymerase chain reaction (PCR) was performed with 100–200 ng of template DNA, 10 mM Tris-HCl, 1.5 mM magnesium chloride, 50 mM potassium chloride, gelatin 0.1 mg ml−1, 200 μM each dNTP, 0.5 U Taq polymerase (Boehringer Mannheim) and 50 pmol of each primer in a total volume of 10 μl or 25 μl. The PCR product was identified by end labelling primers with [γ-32P]ATP or the PCR product was internally labelled with [α-32P]dCTP. All PCR reactions were performed on a Perkin Elmer Cetus PCR system with denaturation for 6 min at 94°C followed by 30 cycles of denaturation at 94°C for 1 min, annealing temperature (Table 1) for 1 min, and extension at 72°C for 1 min. The primers for the STS loci that were examined (Gyapta et al., 1994; Volz et al., 1994), their annealing temperatures and the references describing them are shown in Table 1.

The PCR products were diluted with loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), heated denatured and rapidly cooled. Samples were run in pairs (tumour and lymphocyte PCR product from the same patient) on a denaturing gel (7% acrylamide, 32% formamide, 6 M urea, 1 x TBE) at a constant 30–35 W. After electrophoresis the gel was transferred to 3MM Whatman paper and autoradiography performed with Kodak X-Omat AR film at ~70°C. When the signal of an allele in tumour DNA was less than 50% of intensity observed in matching normal DNA from a heterozygous patient, LOH was considered to have occurred (Bieche et al., 1993).

Results

Preliminary results obtained at 6 loci (D6S254, D6S251, D6S252, D6S249, ARG1 and D6S255) on chromosome 6q in 30 breast tumour DNAs (tumour panel 1) suggested that LOH on this arm of chromosome 6 was a frequent event (Table II). LOH was detected in nine tumour DNAs. In three tumour DNA samples all informative loci were affected by LOH. Six other tumour DNAs had loss of one allele at D6S251 or D6S252 or D6S249. However, the data set was too small to determine with any precision the location of the target region(s). Therefore, this study was extended to include another 50 primary breast carcinoma DNAs (tumour panel 2) with eight additional microsatellite markers whose localisa-
Table I STS loci and primers on chromosome 6q

| Locus/STS | Annealing temperature (°C) | Primers (5’-3’) | References |
|-----------|---------------------------|-----------------|------------|
| D6S254    | 55                        | AGAGAGGCTGAAGACCAATCC TCCCCATAGCCTCAGGGACACT | Wilkie et al. (1993) |
| D6S252    | 55                        | CCCCGGCATGCTCACCAGCCC | Gyapa et al. (1994) |
| D6S249    | 53                        | AAGCACTTCTGACCTTGCTCTTG | Wilkie et al. (1993) |
| D6S251    | 55                        | ATATTTTGTTAAAGTAAGTTCVAC | Wilkie et al. (1993) |
| D6S250    | 50                        | CTAGTTACCATTCATCGTACGA | Wilkie et al. (1993) |
| D6S248    | 50                        | AAAAGGAAGCTTATAATATCG | Gyapa et al. (1994) |
| D6S252    | 55                        | TTTCTTTTGGGATTTAATGTTCC | Gyapa et al. (1994) |
| D6S297    | 50                        | GAGGTTTATGTGAAAGCCAG | Gyapa et al. (1994) |
| D6S262    | 50                        | ATATTAGTGCTTTAGTCTCTTG | Gyapa et al. (1994) |
| D6S270    | 55                        | GTGAAAACCTCTCATCGTC | Gyapa et al. (1994) |
| ARG1      | 55                        | TTAGATGATTTTTAAGCAGGA | Wilkie et al. (1993) |
| D6S255    | 55                        | TGAACCATGTTTGATTTGGAAGCCAG | Wilkie et al. (1993) |

Table II LOH on chromosome 6q in breast tumour panel 1

| Regional assignment | Locus | n | LOH/inf. (%) | 14 | 21 | 22 | Tumour DNA No. | 25 | 48 | 51 | 54 | 55 | 66 |
|---------------------|-------|---|--------------|----|----|----|----------------|----|----|----|----|----|----|
| 6q1.3               | D6S254| 30 | 0/5 (0)      | NI | NI | NI | H   | NI | NI | NI | NI | NI |  
| 6q14-q16.2          | D6S254| 30 | 0/6 (21)     | D  | H  | D  | D  | D  | D  | D  | D  |  
| 6q2.2               | D6S254| 30 | 0/16 (25)    | D  | D  | D  | D  | D  | D  |  
| 6q16.3-6q21         | D6S249| 30 | 0/5 (14)     | D  | H  | D  | D  | D  | D  |  
| 6q22.3-q23.1        | D6S254| 30 | 2/12 (17)    | D  | D  | D  | H  | D  |  

The genotypes of nine tumour DNA samples from tumour panel 1 at STS markers between D6S254 and D6S255 that were tested and their regional locations are listed. The genetic order of the STS loci is according to published linkage studies (Gyapa et al., 1994; Volz et al., 1994). n, total number of tumour DNA samples examined for each marker; LOH/inf., fraction of tumours from informative patients that showed LOH at each marker; the number in parenthesis is the percentage of tumours having LOH at each locus; D, LOH; H, STS loci that were informative but unaffected; NI, STS loci that were not informative.

Table III summarised in Table III for tumour DNA samples 63, 224 and 204 are also consistent with the presence of a tumour-suppressor gene located in the 5.2 cM interval between D6S268 and D6S261 (region 2). Evidence for a third region affected by LOH was found in tumours DNA 204, 99, 208, 49, 114, and 83 (Table III). In these tumour DNA samples D6S262 was affected by LOH, whereas the more centromeric locus D6S287 was informative and unaffected. The telomeric boundary of this region could be D6S270 since in tumour DNA 204 and 99 it was unaffected by LOH.

Several of the tumour DNAs were remarkable in that more than one region of chromosome 6q was affected by LOH. For instance tumour DNA samples 82, 263 and 281 exhibited independent LOH of regions 1 and 2 whereas in tumour DNA 204 regions 2 and 3 were independently affected by LOH (Table III). Similarly in tumour DNA sample 208 regions 1 and 3 were affected by LOH and in sample 49, each of the three regions were independently affected by LOH. Cytogenetic analysis of metastatic breast carcinomas have also identified tumours with multiple chromosome 6 alterations (Dutrillaux et al., 1990; Lu et al., 1993; Thompson et al., 1993; Trent et al., 1993). In several cases it was not possible to unambiguously determine which
Table III  LOH on chromosome 6q in breast tumour panel 2

| Regional assignment | Locus  | Recombination frequency (cM) | n  | LOH/inf. (%) | 127 | 28 | 91 | 304 | 281 | 263 | 82 | 63 | 224 | 204 | 99 | 208 | 49 | 114 | 83 | 351 | 86 | 50 | 178 | 394 | 125 | 272 | 14 | 376 | 321 | 62 | 396 | 376 | 267 |
|---------------------|--------|-------------------------------|----|--------------|-----|----|----|-----|-----|-----|----|----|-----|-----|----|-----|----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 6q14–q15            | D6S284 | 0                             | 9  | 1/5 (20)     | D   | N  | I  | H   | N   | I   | H   | N   | I   | H   | N   | I   | H   | N   | D   | N   | H   | N   | N   | D   | N   | H   | N   | N   | D   |
| 6q14–q16.2          | D6S286 | 1.1                           | 21 | 4/11 (36)    | D   | N  | I  | N  | I  | N  | I  | D   | N  | I  | N  | H   | H   | D   | N   | I   | H   | N   | D   | D   | N   | D   | N   | D   | D   | N   |
| 6q16.3–q21          | D6S251 | 8.9                           | 50 | 10/39 (26)   | D   | D  | D  | N  | I  | D  | D   | H   | H   | H   | H   | H   | D   | D   | H   | H   | N   | N   | D   | D   | N   | D   | N   | D   | D   | N   |
| 6q21–q23            | D6S252 | 3.3                           | 50 | 1/3 (22)     | H   | H  | H  | D  | H  | N  | H   | H   | H   | H   | H   | N   | D   | N   | D   | D   | D   | N   | H   | H   | N   | H   | D   | N   | D   |
| 6q22.3–q23.1        | D6S249 | 10.3                          | 26 | 4/14 (29)    | H   | H  | N  | I  | N  | I  | N  | I  | N  | I  | N  | I  | H   | N   | I   | N   | H   | D   | N   | N   | N   | H   | N   | D   | D   |
| 6q21–q23            | D6S261 | 2.6                           | 1  | 5/25 (20)    | N   | I  | N  | I  | H  | D  | N  | I  | H  | H  | H  | H   | H   | H   | N   | D   | N   | D   | N   | D   | N   | D   | N   | D   |
| 6q22.3–q23.1        | D6S268 | 2.6                           | 40 | 10/28 (36)   | N   | I  | N  | I  | H  | N  | I  | H  | N  | I  | D   | D   | D   | D   | D   | D   | H   | H   | N   | H   | N   | D   | N   | D   |
| 6q22.3–q23.1        | D6S287 | 4                             | 50 | 4/33 (12)    | N   | I  | H  | H  | N  | I  | H  | H  | H  | H  | H   | H   | H   | H   | H   | N   | H   | D   | N   | D   | N   | N   | D   | N   |
| 6q22.3–q23.1        | D6S262 | 5                             | 1  | 1/5 (20)     | H   | H  | H  | N  | I  | H  | N  | I  | D   | D   | D   | D   | D   | H   | H   | D   | H   | N   | D   | N   | D   | D   |

The genotypes of 29 tumour DNAs from tumour panel 2 that had LOH at one or more informative STS loci are shown. The regional location of the STS loci and the recombination frequency between them in centimorgans (cM) is according to published studies (Gyapaa et al., 1994; Volz et al., 1994). n, total number of tumours DNA samples examined for each marker; LOH/inf, fraction of tumours from informative patients that showed LOH at each marker; the number in parenthesis is the percentage of tumours having LOH at each locus; D, LOH; H, STS loci that were informative but unaffected; N, STS loci that were not informative; vertical bars highlight the smallest regions affected by LOH. Tumours not tested at a particular locus are blank.
region was the target for LOH. Thus in tumour DNAs 351 and 86 either region 1 or region 2 could have been the target for LOH. Similarly, in samples 178, 394, 125, 272, 14, 150, and 62 either region 2 or region 3 could contain the target tumour-suppressor gene.

Discussion

Our results confirm the findings of Devielle et al. (1991) that LOH on chromosome 6q is a frequent event in primary human breast carcinomas. They detected LOH at MYB and/or D6S37 in 50% of the tumour DNA samples. The MYB locus is 5 cm telomeric of D6S270 (Gyapa et al., 1994; Volz et al., 1994) and D6S37 is at the distal end of chromosome 6q. Our study extends their results by defining three regions of chromosome 6q that are independently affected by LOH. During the course of our work Orphanos et al. (1995) reported two regions on chromosome 6q that are affected by LOH in human breast tumour DNAs. One of these spans 6q13–q21 and probably corresponds to regions 1 and 2 in our data set. The second region in their study was located at 6q21–q27 and is distal to region 3 in our study. LOH on chromosome 6q is not unique to breast carcinomas, it has been detected in 40% of melanomas in the region 6q16–q23 (Millikin et al., 1991). Similarly, Saito et al. (1992) found that 51% of ovarian carcinomas had LOH at one or more of nine loci on chromosome 6q24–q27. In this study the commonly deleted region was 6q26–q27.

At the present time there are few, if any, candidate target genes for LOH on chromosome 6q. However, Negrini et al. (1994) have shown that microcell-mediated transfer of chromosome 6 into the human breast tumour cell line MDA-MB-231 inhibits its tumorigenecity in BALB/c-nu/nu mice as well as causing the cells to age in culture. An analysis of polymorphic loci which identifies the portions of the transferred chromosome 6 that were retained in the cell line, suggested that at least two functional regions of 6q are important for tumour suppression. One functional region was at the distal end of 6q near D6S458. Based on current linkage maps of chromosome 6q (Gyapa et al., 1994; Volz et al., 1994) the second functional region is located between the CNR locus (6q14–q15) and D6S310 (9 cm telomeric of D6S270, see Table III). This second functional region is consistent with regions 1, 2 and possibly 3 presented in our study. Clearly the development of a physical map of the polymorphic STSs on chromosome 6q should lead to further definition of the regions affected by LOH and to the target gene(s) in primary breast tumours.

Acknowledgements

This study was supported by the Ligue Nationale de la Lutte Contre le Cancer (LNCC), the Comités Regionaux des Hauts de Seine, du Val d'Oise and des Yvelines, Association pour la Recherche sur le Cancer (ARC) and CNR, ACRO no. 94.01084.39.

References

BIECHE I, CHAMPEME M-H, MATIFAS F, CROPP CS, CALAHAN R AND LIDEREAU R. (1992). Two distinct regions involved in 1p deletion in human primary breast cancer. Cancer Res., 53, 1990 – 1994.

CALLAHAN R, CROPP C, MERLO GR, DIELLA F, VENESIO T, LIDEREAU R AND CAPPA APM. (1993). Genetic and molecular heterogeneity of breast cancer cells. Clin. Chim. Acta, 217, 63 – 73.

DEVIJLE P, VAN VLIET M, VAN SLOUP P, KUIPERS DKJUKSHORN N, HERMANS J AND PEARSON PL. (1991). Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. Oncogene, 6, 1705–1711.

DUTRILLAUX B, GEBRATULLE-SEUREAU AND ZAFRANI B. (1990). Characterization of chromosomal abnormalities in human breast cancer. Cancer Genet. Cyto. Genet., 49, 203 – 217.

FRIEND SH, BERNARDS R, ROGELJ S, WEINBERG RA, RAPAPORT JM, ALBERT DM AND DRYA TP. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature, 323, 643.

GYAPA G, MORISSETTE J, VIGNAL A, DIB C, FIZAMES C, MILLASSEAU P, MARC S, BERNARDI G, LATHROP M AND WEISSENBACH J. (1994). The 1993 – 1994 Genethon human genetic map. Nature Genet., 7, 246 – 339.

HOLSTEIN M, SIDRANSKY D, VOGELSTEIN B AND HARRIS CC. (1991). p53 mutations in human cancers. Science, 253, 49 – 53.

KNUDSON AG. (1989). Hereditary cancers: clue to mechanisms of carcinogenesis. Br. J. Cancer, 59, 661 – 666.

LU Y-J, XIAO S, YAN Y-S, FU S-B, LIU Q-Z AND LI P. (1993). Direct chromosome analysis of 50 primary breast carcinomas. Cancer Genet. Cyto. Genet., 69, 91 – 99.

MILLIKIN D, MESEE E, VOGELSTEIN B, WITKOWSKI C AND TRENT JM. (1991). Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. Cancer Res., 51, 5433 – 5449.

NEGRINI M, SABBIONI S, ROSSATI L, RATTAN S, CORALLINI A, BARBANTI-BRODANO G AND CROCE CM. (1994). Suppression of tumorigenicity of breast cancer cells by microcell-mediated chromosome transfer: Studies on chromosomes 6 and 11. Cancer Res., 54, 1331 – 1336.

ORPHANOS V, MCGOWN G, HEY S, BOYLE JM AND SANTIBANEZ-KOREF M. (1995). Proximal 6q, a region showing allelic loss in primary human breast cancer. Br. J. Cancer, 71, 290 – 293.

SAITO S, SAITO H, KOOS S, SAGAE S, KUDO R, SAITO J, NODA K AND NAKAMURA Y. (1992). Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. Cancer Res., 52, 5815 – 5817.

THOMPSON F, EMERSON J, DALTO J-M, MCGEE D, VILLAR H, KNOX S, MASSEY K, WEINSTEN R, BHATTACHARYYA A AND TRENT J. (1993). Clonal chromosome abnormalities in human breast carcinomas I. Twenty-eight cases with primary disease. Genes, Chrom. Cancer, 7, 185 – 193.

TRENT JM. (1985). Cytogenetic and molecular biological alterations in human breast cancer: a review. Breast Cancer Res. Treat., 5, 221 – 229.

TRENT J, JIN-MING Y, EMERSON J, DALTO W, MCGEE D, MASSEY K, THOMPSON F AND VILLAR H. (1993). Clonal chromosome abnormalities in human breast carcinomas II. Thirty-four cases with metastatic disease. Genes, Chrom. Cancer, 7, 194 – 203.

VOLZ A, BOYLE JM, CANN HM, COTTINGHAM JF, O'GR HJ AND ZIEGLER A. (1994). Report of the second workshop on human chromosome 6. Genomics, 21, 464 – 472.

WILKIE P, POLYMEROPoulos MH, TRENT J, SMALL KW AND WEBER JL. (1993). Genetic and physical map of 11 short tandem repeat polymorphisms on human chromosome 6. Genomics, 15, 225 – 227.