Detection of 6q deletions in breast carcinoma cell lines by fluorescence in situ hybridization

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Abstract Dual-color fluorescence in situ hybridization was performed to detect the frequency and extent of 6q deletions in ten breast carcinoma cell lines. In five cell lines, the 6q deletions involved large regions extending from 6q12–q16 to 6q27, and in one the deletion extended from the region distal to YAC 751G10 at 6q25.1 to 6q27. In two cell lines, 6q deletions occurred only in cells with polysomy 6, indicating that such deletions might be secondary chromosomal aberrations and reflect late genetic changes in breast carcinomas. In addition, an overrepresentation of 6q21–q22.2 was detected in one cell line.

Chromosomal deletions and loss of heterozygosity (LOH) affecting the long arm of chromosome 6 (6q) are frequent genetic changes in breast cancer, ovarian carcinoma, and melanoma as well as in hematological neoplasms (Trent et al. 1993; Bieche and Linereau 1995; Theile et al. 1996). Therefore, it is assumed that at least one tumor suppressor gene is localized on 6q. To detect the frequency and extent of 6q deletions in ten breast carcinoma cell lines (R103, BRC230, MDA-MB-231, T-47D, CAMA1, MCF-7, CAL51, R30, R104 and BT-20), dual-color fluorescence in situ hybridization was performed as previously described (Schlegelberger et al. 1998) using 37 biotinylated yeast artificial chromosome (YAC) probes hybridizing from 6q12 to 6q27 and, as internal control, a digoxigenin-labeled D6Z1 probe specific for the centromere of chromosome 6. On the basis of extensive control studies on phytohemagglutinin-stimulated peripheral lymphocyte cultures from five healthy donors, the cut-off limit for detecting 6q deletions was set at 10%, which was above the highest cut-off level calculated as the mean of false-positive cells in control studies plus three standard deviations for any of the analyzed 6q YACs. 6q deletions were detected in six cell lines (Fig. 1). In five cell lines, the 6q deletions involved large regions extending from 6q12–q16 to 6q27. In MCF-7 the deletion extended from the region distal to YAC 751G10 at 6q25.1 to 6q27. The proportions of cells with deletions ranged from 20% to 100% (Table 1). Deletions affecting 6q25–q27 or LOH at this region have frequently been detected in breast carcinomas and other solid tumors by chromosomal analysis, comparative genomic hybridization (CGH) and LOH studies (Trent et al. 1993; Theile et al. 1996; Nishizaki et al. 1997). These findings are confirmed by our study on breast carcinoma cell lines. Several genes, such as LOT-1, SEN6 and SOD2, have been mapped to this region and seem to be promising candidate tumor suppressor genes. Both the SEN6 and SOD2 genes have been associated with senescence induction and suppression of tumorigenicity in different immortal human fibroblasts and tumor cell lines (Sandhu et al. 1994). In two cell lines (MDA-MB-231 and CAMA1) that contained two heterogeneous cell populations with different copy numbers of chromosome 6 (Table 1), 6q deletions occurred only in cells with polysomy 6, but not in cells with disomy 6, indicating that 6q deletions might be secondary chromosomal aberrations and reflect late genetic changes in breast carcinomas.

In addition, an overrepresentation of 6q21–q22.2 was detected in BT-20. More than 90% of cells showed two hybridization signals for D6Z1 and three, up to six hybridization signals for the YAC probes hybridizing to this region (Fig. 1). Amplifications or gains of 6q, most of them involving 6q21–q23, have been detected in about 15% of breast cancers by chromosomal analysis and CGH studies (Trent et al. 1993; Nishizaki et al. 1997). Several genes, such as SYR, FYN, ROS1, MYB, ARG1 and MACS, are probably localized within this region. Further studies are needed to investigate which genes are indeed overexpressed in this cell line.
Fig. 1  A Dual-color fluorescence in situ hybridization (FISH) studies on breast carcinoma cell line MCF-7. Biotinylated yeast artificial chromosome (YAC) 700H1 hybridizing to 6q25.2 and digoxigenin-labeled D6Z1 were visualized by Cy3 (red) and fluorescein isothiocyanate (FITC) (green), respectively. Cells with three green hybridization signals for D6Z1 had only two red hybridization signals for the YAC, indicating a deletion of 6q25.2 on one chromosome 6. B Dual-color FISH studies on breast carcinoma cell line BT-20. Biotinylated YAC 763D6 hybridizing to 6q22.1 and digoxigenin-labeled D6Z1 were visualized by Cy3 (red) and FITC (green), respectively. Cells displayed two green hybridization signals for D6Z1 and five to six red hybridization signals for the YAC, indicating an overrepresentation of 6q22.1.

Table 1 Detection of 6q deletions in ten breast carcinoma cell lines by FISH. Chromosome arm 6q is depicted diagrammatically on the left. Cell lines R103, R30 and R104 were established in the Max Delbrück Center for Molecular Medicine (Berlin, Germany). BCR230 was kindly provided by Dr. W. Zoli (Cologne, Germany), MDA-MB-231, T-47D, CAMA1, MCF-7 and BT-20 were purchased from ATCC (Rockville, Md., USA) and CAL51 was obtained from the Centre Antoine Lacassagne (Nice, France). YACs yWPR108 and 19I11F were kindly provided by Dr. D. Schlessinger (St. Louis, Mo., USA) and ICRF (Cambridge, UK), respectively. The other YACs were obtained from CEPH (France) and the German Resource Centers (Berlin, Germany). D6Z1 was purchased from Oncor (Gaithersburg, Md., USA). The signal ratio of YAC/D6Z1 is a comparison of the hybridization signal numbers of 6q-specific YAC probes with those of D6Z1 in dual-color FISH. For example, 4/4: 80% and 3/4: 20% in R103 means that all analyzed cells contained four hybridization signals for D6Z1. However, 80% of the cells showed four, and 20% of the cells showed three hybridization signals for the YACs hybridizing to the region of 6q16.3–q27, indicating that in 20% of the cells one of four chromosomes 6 has a deletion of 6q16.3–q27. (n no deletion, d deletion, over overrepresentation)