Frequent Allelic Loss at 6q26-27 in Breast Carcinomas of the Solid-tubular Histologic Type

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To characterize the effect that inactivation of a putative tumor suppressor gene on 6q appears to have on breast carcinogenesis, we examined loss of heterozygosity in 528 primary breast carcinomas with a polymorphic marker located at 6q26-27, a frequent target region of allelic loss in ovarian and breast cancers. Of the 56 informative tumors of the solid-tubular type, 29 (52%) showed allelic loss at 6q26-27; however, only 10 of 42 papillotubular carcinomas (23%) and 34 of 86 scirrhous carcinomas (39%) showed allelic loss (p=0.0215). These results are consistent with reported alterations at 6q26-27 in serous and mucinous types of ovarian cancers, in that they suggest that inactivation of one or more genes in that region may affect carcinogenic mechanisms in a histologic type-specific manner in neoplasms of the female reproductive organs.


Key words: Breast cancer, Chromosome 6, Loss of heterozygosity, Tumor suppressor gene, Ovarian cancer

Recent advances in molecular genetic research have indicated that carcinomas develop through accumulation of genetic changes within a cell lineage1. Several genetic alterations that activate oncogenes and/or inactivate tumor-suppressor genes have been documented in breast cancers. Frequent observations of loss of heterozygosity (LOH) at specific chromosomal loci in human tumors are generally understood to signal the presence of tumor-suppressor genes in the affected chromosomal regions. Accumulating evidence from various LOH studies indicates that chromosomal region 6q26-27 harbors a gene in which inactivation is associated with female endocrine neoplasms. Frequent LOH on the long arm of chromosome 6 was first observed in ovarian cancers3. Saito et al3 later performed a fine-scale deletion mapping study in ovarian cancers and mapped a commonly deleted region at 6q26-27. In those cases, LOH was observed frequently in the serous type of ovarian carcinoma but only rarely in the mucinous type; those results suggested that inactivation of a tumor suppressor gene at this location exerts its effect in a histologic type-specific manner. Although frequent LOH on 6q has also been observed in breast cancers4,5, no histologic type-specificity of 6q loss in this type of female neoplasm has been documented so far.

Breast cancer is now considered to represent a heterogeneous group of neoplasms that develop in the breast and possess distinct pathologic, clinical, and genetic features6. Most breast cancers are classified as ductal carcinomas, which are divided into four histologic types: non-invasive ductal (1a), invasive papillotubular (well-differentiated, a1), invasive solid-tubular (moderately poorly-differentiated, a2), and invasive scirrhous carcinoma (poorly-differentiated, a3). The incidence of other types is low in Japan. To characterize the effect of loss of the putative tumor suppressor gene at 6q26-27 as a contributor to breast carcinogenesis, we examined LOH with a microsatellite marker at 6q26-27 in 528 patients with primary breast cancer, and analyzed relationships between LOH and several clinicopathological parameters including histologic classification.
Materials and Methods

Samples and DNA Preparation

Tumor and corresponding normal tissues were obtained from 528 patients with primary breast cancers who underwent surgery at the Cancer Institute Hospital. Informed consent were obtained from all participating patients prior to surgery. DNAs were extracted according to procedures described previously.7

LOH Analysis

LOH analysis at 6q26-27 was carried out with microsatellite marker D6S503. Polymorphisms were amplified by the polymerase chain reaction (PCR) using 20 ng of genomic DNA, 30 mM of Tris-HCl (pH 8.8), 50 mM of KCl, 2 mM of MgCl₂, 5 mM of 2-mercaptoethanol, 100 mM of dNTPs, 1.6 pmol each of [gamma-3²P]ATP-end-labeled primer and non-labeled primer, and 0.25 units of Taq polymerase in a total volume of 10 ml, according to procedures described previously. Cycling conditions were 94°C for 4 min, then 30 cycles of 94°C for 30 sec, 55-64°C for 30 sec, and 72°C for 30 sec, with a final extension step of 5 min at 72°C in a Gene Amp PCR 9600 System (Perkin Elmer Cetus, USA). PCR products were electrophoresed in 0.2 mm-thick, 6% polyacrylamide denaturing gels containing 36% formamide and 8 M urea, at 2000 volts for 2-4 hours. Gels were transferred to filters, dried at 80°C, and exposed to autoradiographic film at room temperature for 16-20 hours.

Definition of LOH

Signal intensities of polymorphic alleles were quantified by a Hoefer GS-300 scanning densitometer; peak areas corresponding to each signal were calculated by electric integration using the GS-370 electrophoresis data system (Hoefer Scientific Instruments, San Francisco, CA, USA). The signal intensities of alleles of tumor-tissue DNAs were compared to those of corresponding normal-tissue DNAs. We judged a reduction in signal intensity greater than 50% to be loss of heterozygosity. We distinguished LOH from chromosome multiplication by normalizing each signal to the signal obtained when the same DNA was analyzed with markers for loci on other chromosomes.

Clinicopathological Parameters

The following parameters were examined in our panel of cancers: histologic type, tumor stage, tumor size, and the presence of lymph node metastasis. Tumors were classified by pathologists according to the histologic TNM classification and the histologic typing scheme of The Japanese Breast Cancer Society (1989). Estrogen receptor (ER) and progesterone receptor (PgR) were measured by radioreceptor assay according to a standard dextran-coated charcoal (DCC) method, using [³°I]-estradiol as labeled ligand on homogenates of fresh-frozen tissue (Otsuka Pharmaceutical, Tokyo, Japan). All samples containing more than 5 fmol of ER or PgR per mg protein were considered receptor-positive. The chi-square test and Fisher's exact test were used for statistical analysis of the results. One-tailed P values of less than 0.05 were considered statistically significant.

Results

A total of 528 primary breast-cancer DNAs paired with their corresponding normal DNAs were analyzed for the presence or absence of LOH at a microsatellite locus (D6S503) located at 6q26-27. Figure 1 shows representative autoradiograms from LOH analyses with microsatellite D6S503, demonstrating allelic differences between tumor (T) and normal (N) tissues in four patients with breast cancer. Abscissa, tumor identification number.