A Region of Deletion on Chromosome 22q13 Is Common to Human Breast and Colorectal Cancers

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Abstract

Chromosomal allelic losses have varying frequency in breast cancer, with key regions including chromosomes 1, 3p, 7q, 9p, 16q, 17, and 22q. Recently, we have been able to map a new target region of allelic loss on chromosome 22q involved in colorectal cancer. The aim of the current investigation was to determine whether this target region may also be involved in human breast carcinogenesis. Thirty-six pairs of matched normal and tumor specimens from breast cancer patients, as well as eight breast cancer-derived cell lines, were genotyped using 17 microsatellite markers spanning chromosome 22q. Allelic deletion was found in 19 of 36 tumors (53%), and the pattern observed in those cases with partial losses was consistent with a region flanked by D22S1171 and D22S928. This interval overlaps that identified in colorectal cancer and comprises nearly 1.1 Mb. This study provides evidence of a common region of deletion on chromosome 22q13 involved in both breast and colorectal cancers and underscores the existence of putative tumor suppressor gene(s) at this location.

Introduction

Breast cancer is the most common malignancy among women, and it represents the cause of death in approximately 20% of all females who succumb to cancer in developed countries (1). Many investigations have led to the elucidation of some of the genetic mechanisms involved in carcinogenesis. Inactivation of tumor suppressor genes, usually by point mutation in one allele and deletion of the other allele, seems to play a critical role (2). Identification of such genes has been usually by point mutation in one allele and deletion of the other allele, thus allowing differentiation of allelic loss in tumor samples. In this context, chromosomal allelic losses have varying frequency in breast cancer, with key regions including chromosomes 1, 3p, 7q, 9p, 16q, 17, and 22q (3).

Allelic loss on 22q is a common event in breast cancer, with a frequency varying between 11% and 66% (3–8). However, no tumor suppressor gene on chromosome 22q involved in this neoplasm has been identified thus far. NF2, the gene whose mutant forms are responsible for the neurofibromatosis type 2 syndrome, maps to 22q12–13 (9). Its role in the acoustic form of neurofibromatosis has made it a likely candidate for involvement in the carcinogenesis of 22q-related neoplasms. In addition to germ-line mutations in this gene in neurofibromatosis type 2 patients, somatic mutations are known to occur in NF2-related tumors, such as sporadic meningiomas and vestibular schwannomas, thus suggesting that the NF2 gene is a tumor suppressor gene. However, malignant mesotheliomas are the only non-NF2-related tumors in which a high rate of NF2 mutations has been reported (10).

Chromosome 22q allelic deletion is a common somatic alteration not only in breast cancer but also in other neoplastic processes such as colorectal (11), ovarian (12), brain (13), oral (14) and pancreatic endocrine tumors (15). Recently, we have been able to map a new target region of allelic loss on chromosome 22q involved in human colorectal cancer (11). This area is limited by markers D22S1171 and D22S928, which correspond to the cytogenetic location 22q13 (11). Interestingly, Lida et al. (7) were able to map a 2-cM region of allelic loss in breast cancer to the same cytogenetic band. Similarly, the pattern of allelic loss observed by Bryan et al. (12) in ovarian cancer was consistent with an interval proximally flanked by D22S276, which is located close to the centromeric boundary of the area delimited in colorectal tumors. Finally, in oral squamous cell carcinoma, allelic deletion seems to be restricted to D22S274 (14), a marker included within the region of deletion identified in our previous study.

Recently, the human chromosome 22 sequence has been completed and released into the public domain (16). This information will be extremely useful for the eventual identification of those genes previously mapped on this chromosome by either linkage analysis in inherited diseases or allelic deletion assays in sporadic neoplasms. Polymorphic markers are now physically mapped at a nucleotide-level resolution, thus providing a unique opportunity to combine all available data in reference to the potential involvement of a common tumor suppressor gene in these neoplastic processes. As part of this effort, the aim of the current investigation was to determine whether the target region of allelic loss on chromosome 22q found in colon cancer may also be involved in breast cancer.

Materials and Methods

Tissues and Cell Lines. Thirty-six paired normal and breast tumor specimens were obtained from the Massachusetts General Hospital Tumor Bank. Tumors were classified as invasive ductal carcinoma in 28 patients, invasive lobular carcinoma in 4 patients, ductal carcinoma in situ in 3 patients, and medullar carcinoma in the remaining patient. Genomic DNA was obtained from frozen tissues by phenol-chloroform extraction and ethanol precipitation (17).

Additionally, eight breast cancer-derived cell lines obtained from American Type Culture Collection (HS-578, MDA-MB-231, MDA-MB-453, MCF7-ADR, DU4475, SK-BR-3, ZRB, and MCF7) were grown under standard conditions. Thereafter, genomic DNA was obtained from each cell line, according to standard procedures (17).

Microsatellite DNA Analysis. Evaluation of chromosome 22q allelic loss was carried out by a PCR-based DNA polymorphism analysis at 17 loci distributed across chromosome 22q (D22S2868, D22S274, D22S276, D22S282, D22S283, D22S284, D22S426, D22S421, D22S928, D22S1140, D22S1141, D22S1153, D22S1160, D22S1168, D22S1169, D22S1170, and D22S1171). These loci correspond to dinucleotide (CA), repeats, and their location was based on the Genethon linkage map (18). PCR was performed with normal and tumor DNA templates as described previously (11). Sense and antisense
primers for these loci are available in the Genome Database.\(^3\) PCR products were separated in denaturing 6% polyacrylamide sequencing gels at 70 W for 2 h. Gels were dried and exposed to X-OMAT AR film (Kodak, Rochester, NY) overnight without an intensifying screen. Alleles were scored as described previously (11).

All breast cancer-derived cell lines, with the exception of HS-578, were analyzed for homozygous deletions. For HS-578, DNA from the normal counterpart was available, and DNA polymorphism analysis was performed in a manner similar to that described for tissue samples.

Physical mapping of polymorphic markers involved in the minimal region of deletion was done in accordance with the DNA sequence of human chromosome 22\(^4\) (16).

Results and Discussion

Microsatellite DNA analysis identified 19 of 36 tumors (53%) displaying allelic loss in at least one marker (Fig. 1). Of the 19 tumors, 10 cases exhibited losses in all informative loci, suggesting that one copy of chromosome 22 had been completely lost. The remaining nine tumors showed variable patterns of partial loss on 22q with overlapping markers D22S1140, D22S1168, and D22S274. Based on our analysis, the minimal region of allelic deletion is flanked on the centromeric side by D22S1171 and on the telomeric side by D22S928 (Figs. 1 and 2). This corresponds to the cytogenetic location 22q13, and it comprises 1080 kb according to the physical map of human chromosome 22 (16).

The high frequency of allelic loss on chromosome 22q established in the present investigation, consistent with previous studies (7, 8), suggests that this chromosomal alteration may be involved in the development of breast cancer. Indeed, this figure represents one of the highest rates of allelic imbalance in breast cancer along with other chromosomal regions such as 1p (58%), 7q (54%), 17p (51%), 17q (49%), 3p (46%), 16q (45%), and 9p (40%) (3–5, 19–21). In addition, the frequency of chromosome 22q allelic loss in breast cancer is even higher than that observed in other neoplasms in which a region of deletion in such a location has been determined (11–15).

The present study represents the first investigation to identify precisely a region on chromosome 22q with a high likelihood of containing putative tumor suppressor gene(s) involved in breast carcinogenesis. The novelty of this finding rests on the relatively small size of the identified region, which is especially noteworthy considering that it corresponds to a relatively GC-rich area. Interestingly, in a previous effort, Iida et al. (7) were able to map a minimal region of allelic loss between the IL2RB and D22S279 loci. This interval is also located in the cytogenetic region 22q13, but its relatively large size (more than 2.5 Mb) made it unsuitable for targeted analysis of candidate genes. Similarly, Allione et al. (8) reported six regions along chromosome 22q, ranging from 3–6 cM in size, one of which overlapped those intervals identified in the current study, and another which overlapped the interval noted in the study by Iida et al. (Fig. 3).

Unfortunately, none of the seven breast cancer cell lines exhibited homozygous deletion in any loci, and allelic deletion was not observed in the HS-578 cell line. The isolation of cancer-derived cell lines carrying homozygous deletions in specific markers has been extremely helpful in some investigations, leading to the identification of new tumor suppressor genes, such as Smad4 (22). However, in the vast majority of studies, it is typical that homozygous deletions with

\[^3\]http://gdbwww.gdb.org.
\[^4\]http://www.sanger.ac.uk/HGP/chr22.
specific microsatellite markers are not found, as noted in our previous analysis of colorectal cancer cell lines (11). The lack of homozygous deletion may suggest alternative inactivating mechanisms of putative tumor suppressor genes.

When the breast tumors were classified histologically, allelic deletion on 22q was observed in 15 of 28 (54%) invasive ductal carcinomas, 3 of 4 (75%) invasive lobular carcinomas, and in the single medullar carcinoma. By contrast, none of the three ductal carcinomas in situ exhibited allelic loss in any loci. Although the relatively small number of cases corresponding to some of these histological subtypes precludes definitive conclusions, it is tempting to hypothesize that candidate gene(s) in this region may be involved in advanced stages of tumor progression, especially for ductal carcinoma. This notion may be supported by other studies in which allelic loss on 22q was rarely observed in ductal carcinoma in situ (23) but was observed in a high proportion of either invasive ductal (up to 66%) or lobular (75%) carcinoma (7, 8, 24). Nevertheless, in a study based on comparative genomic hybridization in lobular carcinoma in situ, allelic loss on 22q was observed in 52% of cases (25). Taking into account all these considerations, it is possible that mutations in one putative 22q tumor suppressor gene may represent a late event for tumor progression in the majority of breast carcinomas, as well as an early event in those less prevalent neoplasms with lobular differentiation. However, the involvement of different genes located in the same or nearby regions cannot be ruled out.

Allelic loss on chromosome 22q is a common phenomenon not only in breast cancer but also in other neoplasms. Whereas cytogenetic and genome-wide allelotyping studies allow one to estimate the frequency of 22q deletion, identification of the specific region of deletion has relied on refined mapping analysis. At present, this information is available in breast, colon, and ovarian cancers, as well as oral squamous cell carcinomas and astrocytomas (Refs. 7, 8, and 11–14; Fig. 3). It is important to note that the recent completion of human chromosome 22 (16) allows, for the first time, to map precisely all markers involved in each region of deletion. This comprehensive approach indicates that the interval identified in the present investigation is consistent with data derived from colon (11), ovarian (12),...
and oral (14) cancer studies, thus delimiting a minimal region of deletion flanked by markers D22S1171 and D22S928. In addition, a larger region of deletion involved in astrocytomas (13), breast (8), and ovarian cancers (12) could be limited by markers D22S284 and D22S282.

After defining a 1-Mb minimal region of deletion, our effort has been focused on identifying genes mapped in that area. As an initial consideration, searches through the Sanger Center as well as the Human Genome Database and the National Center for Biotechnology Information were conducted. Unfortunately, no known gene was located between the above-mentioned markers. The completion of the chromosome 22q sequencing project permitted the prediction of unknown genes using computer-based approaches. Following this strategy, the Sanger Center predicted the existence of eight genes and four pseudogenes between markers D22S1171 and D22S928 (16), and we have also been able to identify four additional genes (Fig. 4). These predicted genes match EST sequences or exhibit similarities with some known genes. Nevertheless, it is important to note that before undertaking mutational and functional analyses, it is essential to verify the existence of these predicted genes by means of experimental approaches, a process that is ongoing in our laboratory.

In conclusion, this study provides evidence of a common region of deletion on chromosome 22q13 involved in both breast and colorectal processes, and it reinforces the existence of putative tumor suppressor gene(s) in this location that may be involved in different neoplasms. The identification of a 1-Mb minimal region of deletion may serve as the basis for the isolation of such a gene by positional cloning and/or computer-based gene prediction approaches.

References
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