Deletion Mapping of Two Potential Chromosome 14 Tumor Suppressor Gene Loci in Ovarian Carcinoma

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Abstract

Previous allelotyping studies of epithelial ovarian carcinoma suggest that loss of heterozygosity on chromosome 14q may be a common genetic alteration in this tumor type. The purpose of this study was to determine a precise frequency of chromosome 14q allelic loss in ovarian carcinomas and to define a minimal region(s) of deletion. Seventy-six ovarian carcinomas representative of the complete spectrum of grade, stage, and histological subtype were selected for PCR-based deletion mapping analysis using 15 highly polymorphic microsatellite markers spanning the length of this chromosome arm. Loss of heterozygosity was observed in 49% of the tumors studied, placing 14q among the most frequently affected chromosomal regions in ovarian cancer. Deletions were observed in all tumor grades and stages and in all histological subtypes except tumors of low malignant potential. Deletion of the entire chromosome arm was rare; the majority of tumors displayed partial losses, providing an informative basis for detailed deletion mapping. Two distinct minimal regions of deletion were delineated. One region was defined by markers D14S65 and D14S267 at 14q32. These data implicate the involvement of two tumor suppressor genes on chromosome 14q in a substantial fraction of ovarian carcinomas.

Introduction

Epithelial ovarian carcinoma is the fourth most common cause of cancer death in women in the United States and leads to greater mortality than all other gynecological cancers combined (1). To impact upon the survival rates associated with this disease, a thorough understanding of the molecular genetic alterations causing ovarian cancer is essential. Multiple genetic alterations are required for the development of all human solid tumors (2, 3); a mathematical model based on age-specific incidence rates implies four to eight mutations as rate-limiting steps in the development of some of the most common malignancies (4). A small fraction of ovarian carcinomas is associated with genetic predisposition arising from inheritance of a mutant tumor suppressor allele, e.g., BRCA1, but somatic mutations in these genes do not seem to occur in a significant number of sporadic ovarian cancers (5). Acquired alterations in the K-ras and ERBB-2 oncogenes and the p53 tumor suppressor gene are among the most well-documented contributors to some ovarian cancers (6), but clearly, the great majority of molecular genetic features for this malignancy are yet to be determined.

Although genetic linkage analysis has proven successful in the identification of genetic loci representing the first rate-limiting alteration in highly penetrant hereditary cancers, other techniques must be used to search for the somatically mutated genes involved in both hereditary and sporadic cancers. Knudson's two-hit model established the paradigm for tumor suppressor gene recessivity at the cellular level, wherein both alleles of the affected gene must be inactivated to contribute to the tumor phenotype (7). Allelic deletion manifesting as LOH at polymorphic loci has become recognized as a hallmark of tumor suppressor gene mutation at the corresponding allele. Furthermore, it is well established that deletions are the most common detectable genetic aberrations in epithelial solid tumors and that LOH analysis is more efficient than cytogenetic analysis for detecting deletions at sites of known tumor suppressor genes (8).

One strategy to search for the involvement of tumor suppressor genes in a particular tumor type is an allelotype analysis, which represents a genome-wide survey of LOH at polymorphic markers mapped to known chromosome regions. Such analyses have now been performed for many of the most common solid tumor types, including ovarian carcinoma (9—12). Deletions on chromosome 17 are consistently the most common finding, with several other chromosomal regions displaying high frequency LOH in some but not all studies. The acrocentric chromosome 14 was reported to sustain LOH in nearly half of all ovarian carcinomas tested in two studies (10, 12), but two other studies reported much lower estimates for the involvement of this chromosome (9, 11). Such discrepancies are not unusual when LOH estimates for a particular chromosome arm are based on results obtained from one marker only.

The purpose of this study was to examine a large representative sample of ovarian carcinomas for LOH at markers spanning the length of chromosome 14q, with the goal of obtaining a reasonably precise estimate of the extent to which deletions on this chromosome are involved in ovarian cancer. An additional aim was to determine a minimal region(s) of deletion to contribute to the eventual localization of potential tumor suppressor gene loci on this chromosome.

Materials and Methods

Tissue Acquisition and DNA Preparation. Seventy-six epithelial ovarian carcinomas were obtained at the Hospital of the University of Pennsylvania or from the Gynecologic Oncology Group/Cooperative Human Tissue Network ovarian tissue bank (Columbus, OH). An institutional review board approved informed consent was obtained from each patient before tissue acquisition. The tumors used in this study were primary site cancers that had not been previously treated with chemotherapeutic drugs and were representative for grade, stage, histological subtype, and clinical outcome. Corresponding normal tissues consisted of either peripheral lymphocytes or uninvolved tissue from the reproductive tract removed at hysterectomy. Tissues were flash-frozen in liquid nitrogen after pathological examination, and genomic DNA was prepared using standard procedures (13).

LOH Analysis. Allelic deletions on chromosome 14q were assessed using the following 15 microsatellite markers from the Généthon genetic linkage
map (14): D14S261, D14S80, D14S70, D14S75, D14S286, D14S288, D14S285, D14S290, D14S284, D14S256, D14S258, D14S61, D14S265, D14S267, and D14S260. Information regarding primer sequences and cytogenetic localization of the markers was obtained from the Genome Data Base (The Johns Hopkins University School of Medicine, Baltimore, MD) at http://gdbwww.gdb.org. PCR reactions were carried out in a volume of 10 μl containing 50 ng of genomic DNA, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), each deoxynucleotide triphosphate at 200 μM, each primer at 0.8 μM, and 0.75 unit of Taq polymerase (Perkin-Elmer Corp., Foster City, CA). One primer was end-labeled with [γ-32P]ATP by polynucleotide kinase, using the KinAce-It kit (Stratagene, La Jolla, CA) and column-purified before the PCR reaction. Thirty PCR cycles were performed, consisting of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, followed by a 7-min extension at 72°C. The PCR products were processed by diluting 1:1 in denaturing loading buffer (95% formamide, 10 mM NaOH, 0.05% xylene cyanol FF, and 0.05% bromophenol blue) and heated at 90°C for 2 min, and 5 μl were electrophoresed in 6% polyacrylamide gels containing 8.3 M urea for 2–3 h at 70 W. The gels were fixed in 10% methanol/10% acetic acid, dried, and exposed to Reflection autoradiography film (DuPont New England Nuclear, Boston, MA) for 3–24 h at room temperature.

For a given marker, LOH was scored in informative (heterozygous) individuals by comparing the ratio of autoradiographic allele intensity in tumor DNA to that of normal DNA. Our criteria and rationale for the quantitative assessment of LOH have been described previously in detail (15).

Results

Unambiguous evidence of LOH at one or more chromosome 14q markers was observed in 37 of 76 (49%) ovarian carcinomas. There was no correlation between either the extent or chromosomal region of LOH and tumor grade, stage, histologic subtype, or clinical outcome. The one exception to this observation was that 4 of 76 tumors displayed loss of the entire chromosome arm, 2 tumors with partial LOH were used to define minimal regions of deletion, and these data are summarized in Fig. 1.

Two minimal regions of deletion were identified by this analysis. The first region was bounded by marker D14S50 proximally and by marker D14S70 distally. This markers have been cytogenetically mapped to chromosome 14q12 and 14q12—13, respectively. The second region was bounded by marker D14S65 proximally and by marker D14S267 distally. This markers have been cytogenetically mapped to chromosome 14q32.1 and 14q32.1—32.2, respectively. Examples of autoradiographic LOH data from several tumors that proved critical in defining the minimal regions of deletion are shown in Fig. 2.

Discussion

These data demonstrate that approximately half of all ovarian cancers have sustained an allelic deletion on chromosome 14q. Only chromosome 17 has consistently been shown by allelotype analyses to undergo LOH in a larger fraction of ovarian cancers (9–12). Putative tumor suppressor loci on chromosome 14 are thus likely to play a relatively significant role in ovarian tumorigenesis. Although tumors of all grades, stages, and histologic subtypes were examined, we were unable to discern any clinicopathological correlates with 14q deletions except that tumors of low malignant potential (also known as borderline ovarian tumors) do not seem to be affected by deletions on this chromosome. This observation is consistent with the categorization of these tumors as distinct biological entities, with their less aggressive clinical behavior and with a lower frequency of LOH than generally observed in invasive epithelial ovarian carcinomas (16).

Other solid cancer types in which a significant degree of 14q LOH has been observed include endometrial carcinoma (15), renal cell carcinoma (17), colorectal carcinoma (18), neuroblastoma (19), and bladder carcinoma (20). In these studies, statistically significant clinical correlates include an association with poor prognosis in endome-

![Fig. 1. Summary of chromosome 14q deletion mapping in ovarian carcinoma. The microsatellite markers used in this study and their cytogenetic locations are shown to the left. Individual ovarian tumors that showed partial LOH are labeled across the top. Minimal regions of deletion are illustrated by bars to the right.](cancerres.aacrjournals.org)
in the same tumor set at 14q12–21 (20). Although the proximal region defined in our study did not overlap precisely with the proximal region defined in the bladder cancer study, the boundaries of the respective regions are separated by only 4 cM, suggesting that both of these studies may be detecting deletions reflecting the same tumor suppressor locus.

Considered together, the above data strongly support the hypothesis that two novel tumor suppressor genes exist on chromosome 14, one at 14q12–13 and another at 14q32. Furthermore, these loci seem to play significant roles in several common solid tumor types. Assuming that the deletions observed at 14q12–13 in bladder and ovarian cancers are driven by the same gene and that the deletions observed at 14q32 in endometrial, colorectal, bladder, and ovarian cancers are also driven by the same gene, the identification and characterization of these tumor suppressor loci would be expected to represent a substantial advance in the field of cancer molecular genetics. Although these regions are not sufficiently small to plausibly consider positional cloning efforts, it may be possible to identify smaller minimal regions of deletion through the identification of additional microsatellite markers in these regions and LOH analysis of a larger number of these tumors with markers saturating the affected regions.

References

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