Frequent Loss of Heterozygosity on Chromosomes 6q, 11, and 17 in Human Ovarian Carcinomas

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ABSTRACT

Recently, tumor-specific allele loss has been shown to be an important characteristic of some tumors. When such loss includes one or more growth-regulatory genes, it may allow the expression of tumorigenicity. Using Southern blots, we analyzed normal and tumor DNA samples from 19 ovarian cancer patients, using a series of polymorphic DNA probes that map to a variety of chromosomal loci. Of 14 informative cases, tumor-specific allelic loss was observed in nine (64%) at the estrogen receptor (ESR) gene locus on chromosome 6q. On chromosome 17p at the D17S28 and D17S30 loci, allelic losses were also detected in 6 of 8 (75%) and 9 of 14 (64%) cases, respectively. Allelic loss at the HRAS gene locus on chromosome 11p occurred in 5 of 11 (46%) informative cases. The relatively high incidence of these allelic losses observed on chromosome 6q represents the first implication by molecular genetic analysis of this chromosomal region in a human malignancy, and it thus appears to be a genetic change specific to ovarian carcinoma. DNA sequence losses on 11p and 17p, also reported for other cancers, may reflect the presence of tumor- or growth-suppressor genes on these chromosomes that are important in the genesis of many tumor types, including ovarian malignancies.

INTRODUCTION

Although ovarian carcinoma is the most frequent cause of death from gynecological malignancies in the Western World (1), the histogenesis and biological characteristics of these tumors are not well understood. Furthermore, little is known about potential molecular genetic alterations that may contribute to development of such tumors or to disease progression.

It is now well established that molecular genetic aberrations can alter the expression of genes and may play a role in malignant transformation (2). Recent studies indicate that loss or inactivation of genes at specific chromosomal loci may contribute to the development of certain childhood tumors such as retinoblastoma (3), Wilms’ tumor (4), and rhabdomyosarcoma (4). It has been hypothesized that loss of heterozygosity at a specific locus may indicate loss of genetic material that suppresses the tumor phenotype. Such negative growth-regulating genes have been called “tumor-suppressor genes” or “anti-oncogenes” (5). Because these allelic losses occur not only in hereditary but also in nonhereditary tumors, it has been suggested that negative growth-regulating genes are commonly lost in adult malignancies (6–12). It has been further suggested that the inactivation of a gene is achieved more readily than its activation through specific mutations. Consequently, the loss of an important negative growth-regulating gene may occur even more frequently during tumorigenesis than activation of a positive growth-regulating gene (13).

Even though the identification of consistent chromosomal aberrations in fresh ovarian tumors has been very limited, nonrandom structural or numerical aberrations have been reported for several different chromosomes (14–17). We, therefore, analyzed 19 fresh ovarian cancers for possible tumor-specific allelic loss at 18 different chromosomal loci. Because of cytogenetic reports of 6q deletions in this disease, we were particularly interested in the 6q region, as well as in other chromosomal regions that frequently show allelic loss in other tumors.

MATERIALS AND METHODS

Patients and Tissues. Primary and metastatic malignant ovarian tumors from a total of 19 patients at the M. D. Anderson Cancer Center (Houston, TX) were obtained from surgical pathology immediately after surgery. The fresh solid tissues were dissected free of fat and necrotic tissue. The specimens were stored at −70°C until DNA was extracted. The histological diagnosis of the tumors studied was established by the Pathology Department, from paraffin-embedded sections. The histological grading was performed according to a system described by Mauch et al. (18). Peripheral blood samples were obtained from the same patients from whom the tumor tissues were procured. Stages of disease were assigned according to the classification scheme accepted by the general assembly of International Federation of Gynecology and Obstetrics (19).

Preparation of High Molecular Weight DNA. Tumor tissue, pulverized in liquid nitrogen as described (20), and lymphocytes from peripheral blood samples were lysed in EDTA/sodium dodecyl sulfate solution, proteinase K digested, phenol/chloroform extracted, ethanol precipitated, resuspended in sterile water, and spectrophotometrically quantified as previously described (20).

Allelic Loss Analysis. Fifteen μg of high molecular weight DNA were digested to completion with the appropriate restriction endonuclease as recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, MD). The restriction fragments generated were separated by electrophoresis, neutralized, and transferred to a nylon filter (21). Hybridization to oligo-primed 32P-labeled (22) probes was conducted at 42°C for 48–72 h. The filters were then washed at 60°C for 60 min in 0.1 × saline sodium citrate (SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate and autoradiographed at −70°C using intensifying screens. The following probes and restriction enzymes were used: chromosome 1, pAT3 (PstI) (23); chromosome 2, pYNZ15 (Taql) (24) and IMR-6 (EcoRI) (25); chromosome 5, pHGRI (BclI) (26); chromosome 6, pHH1157 (BamHI) (27), pHM2.6 (EcoRI) (28), and pOR3 (PvuII) (29); chromosome 7, HER-A64-1 and HER-A64-3 (HindIII) (30); chromosome 11, c-Ha-ras1 (BamHI) (31), pHNS300 (EcoRI) (32), and SS6 (BamHI) (33); chromosome 12, pSW480-cDNA-5 (Taql) (34); chromosome 17, pYNZ22 (MpiI or BamHI) (36), and cEFD52 (PvuII) (37); chromosome 19, pHP450 (SauI) (38); and chromosome 22, L335 (HindIII) (39).

Patients’ constitutional DNA samples that were informative, i.e., heterozygous, for a given marker displayed restriction fragments corresponding to each of two alleles in the resultant autoradiograph. Allelic loss from the matched tumor DNA was indicated by the disappearance of restriction fragments specific for either allele.

RESULTS

Loss of heterozygosity occurred frequently at several chromosomal loci in ovarian carcinomas. We examined a total of
18 loci from 10 different chromosomes. Representative results are displayed in Figs. 1–3. Loss of heterozygosity was observed at loci on seven different chromosomes: chromosomes 2, 6, 7, 11, 17, 19, and 22. The frequency of allele loss at individual loci ranged from one of eight (13%) at the MYB locus on chromosome 6q to six of eight (75%) at the D17S28 locus on chromosome 17p (Table 1). Loss of heterozygosity at one or more loci was detected in 75% (14 of 19) of ovarian carcinomas. The incidence of loss of heterozygosity was remarkably high on chromosomes 6q (64% at the ESR gene locus), 17p (75% and 64% at the D17S28 and D17S30 loci, respectively), and 11p (46% at the HRAS1 gene locus).

Allelic Losses on Chromosome 6. To characterize allelic loss on chromosome 6, we used three different polymorphic DNA probes: one for the short arm (D6S29 locus) and two for the long arm (MYB and ESR loci). Loss of heterozygosity occurred at high frequency in the terminal region of the long arm of chromosome 6, the area to which the ESR gene maps (Table 1 and Fig. 3). The frequencies of loss of heterozygosity were similar in both primary tumors (63%) and metastatic lesions (67%) at the ESR locus. Of six individuals heterozygous at both the ESR and MYB loci, five (83%) showed an allelic loss at only the ESR locus. A polymorphic DNA probe (pHH1157) for the D6S29 locus on the short arm detected nine informative individuals, none of whom revealed an allelic loss.

Allelic Losses on Chromosome 17. On chromosome 17p at the D17S28 and D17S30 loci, loss of an allele was observed in 6 of 8 (75%) informative individuals and in 9 of 14 (64%) informative patients, respectively (Table 1 and Fig. 2). Among 14 samples that showed loss of heterozygosity on the short arm of chromosome 17 (D17S28 or D17S30) and that also were informative at the D17S26 locus on the long arm, six (43%) showed concurrent allelic losses. Of eight samples from informative individuals who revealed ESR gene allelic loss and also were informative at a chromosome 17p locus (D17S28 or D17S30), five (63%) showed simultaneous allelic losses on both chromosomes.

Allelic Losses on Chromosome 11p. Among the 19 normal/tumor DNA sample pairs we examined, 11 (58%) individuals were heterozygous and thus informative. In 5 of these 11 (46%) cases, loss of heterozygosity occurred. The fact that we did not observe any allelic loss at the INT2 locus (on 11q) suggests that the HRAS1 allelic loss was largely due to subchromosomal structural changes.

Allelic Losses on Other Chromosomes. To determine whether these restriction fragment length polymorphism allelic losses were specific for chromosomes 6q, 11p, and 17p in ovarian
cancers and not simply a result of widespread random losses of genetic material, we employed other DNA probes capable of detecting restriction fragment length polymorphisms at 14 additional loci on chromosomes 1, 2, 5, 6, 7, 11, 12, 19, and 22 (see Table 1). Among these, at each chromosomal locus where constitutional heterozygosity was observed, heterozygosity was also maintained in the matched tumor tissue in most cases.

DISCUSSION

The demonstration of loss of heterozygosity at the HRAS1 locus, as well as other 11p markers, in a variety of human cancers (reviewed in Ref. 40) including ovarian carcinomas (20) suggests that their development or progression may involve loss or inactivation of normal genes on 11p. Previously we reported that tumor-specific allele loss occurs at the HRAS1 locus on chromosome 11p in 50% of ovarian carcinomas (four primary tumors, eight metastases, and five ascites samples) (20). The current study extends that observation at about the same frequency (46%) to an expanded sample, cases 1–9 in Table 1, which includes five additional primary ovarian carcinomas, and cases 10–19 in Table 1, which includes two additional metastases.

More significantly, our study clearly shows that tumor-specific allele loss occurs in ovarian carcinoma at an even higher frequency for markers on chromosomes 6q (64% at ESR) and 17p (75% at D17S28, 64% at D17S30) than for 11p. This is the first report of tumor-specific allele loss from chromosomes 6 and 17 in ovarian carcinomas, suggesting the potential existence on these chromosomes of tumor- or growth-suppressor genes whose loss promotes this disease.

Deletion of part of chromosome 6q has been observed in several cytogenetic studies of ovarian carcinoma (14–17), which prompted us to investigate the long arm of chromosome 6 at the molecular level. Our finding of such a high incidence of tumor-specific allele loss in both primary and metastatic tumor tissues at the ESR gene locus (6q24–q27) (41) suggests that this loss might be a genetic event specific to ovarian cancer. Because 6q deletions have been observed cytogenetically in early gynecological malignancies, including a noninvasive ovarian carcinoma (42) and an ovarian granulosal cell tumor (43) (a borderline malignancy), loss of DNA sequences from 6q might indicate that inactivation of a tumor- or growth-suppressor gene followed there is an earlier event in ovarian tumorigenesis. However, this hypothesis needs to be tested by looking for tumor-specific 6q allele loss in the DNA of such early and borderline cancers before trying to classify such genetic changes as early or late events.

The fact that the more distal ESR allele marker on 6q is lost more frequently (65%), compared to the more proximal MYB marker (13%), suggests that the ESR locus is closer to the region possibly uniquely lost in ovarian cancer. This notion is supported by the fact that the only tumor sample showing loss of heterozygosity at the MYB locus had concordant loss of heterozygosity at the ESR locus. Of further interest, other investigators in our laboratory have analyzed breast cancer DNA samples from 15 informative patients and observed an ESR gene allele loss in tumor DNA from only one patient, demonstrating that loss of 6q sequences is not common to all cancer types.

In addition to the cytogenetic reports of chromosome 6q deletions, an isochromosome for the long arm of chromosome 17 (i[17q]) was also reported in early gynecological malignancies, indicating possible deletion of the short arm of chromosome 17 (42). Our results suggest that the mechanism of allele loss from chromosome 17p resulted from partial or total loss of chromosome 17, because in 6 of 14 (43%) instances there were concurrent allele losses at both short and long arm loci. Because others have shown that tumor-specific allelic loss from chromosome 17 occurs in several different tumor types (12, 44–46) and because our data imply frequent chromosome 17 losses in ovarian cancer, genes on this chromosome might be involved in a cooperative role with other genes on 6q (or 11p) in mechanisms of tumorigenesis or disease progression. Further, the high incidence of concordant allelic losses from chromosomes 6q and 17p in 7 of 14 (50%) patients informative for both loci was, in our study, very impressive. Within the limits of the present study, it would be very difficult to determine the timing of these allelic losses from different chromosomes. The target genes in ovarian cancer may be located on both chromosomes.

In conclusion, whereas allele loss from chromosome 11p has 3 E. H. Koh et al., personal communication.
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