Allele Loss at the c-Ha-ras1 Locus in Human Ovarian Cancer

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ABSTRACT

Recent reports have shown allele loss at the c-Ha-ras1 locus on the short arm of chromosome 11 in some types of tumors. To determine whether loss of heterozygosity occurs at the c-Ha-ras1 locus in uncultured human ovarian carcinomas we used Southern blot analysis to study DNA from 17 pairs of ovarian tumors and matched white blood cell samples from the same patients. In one of these 17 tumors, the c-Ha-ras1 locus was rearranged, and in five tumor DNAs from ten informative patients, a c-Ha-ras1 allele was lost. This loss, of relatively high incidence, appears to be an important characteristic of human ovarian cancer and may provide a useful tool for understanding its biological behavior.

INTRODUCTION

Ovarian cancer is the most frequent cause of death from the gynecological malignancies in the western world. The overall 5-yr survival rate is 25 to 30% (1). The prognosis of patients with ovarian cancer is largely dependent on the clinical stage as well as tumor grade. However, the pathological classification and grading present difficulties due to lack of agreement among pathologists (2). The histogenesis and biological characteristics of these tumors are also not well understood. There is currently no evidence to incriminate any single etiological factor for this group of tumors. Cytogenetic and epidemiological studies of ovarian cancer have suggested that some genetic alterations are implicated in the development of these tumors (3–5); however, the precise role of these defects and the molecular mechanisms involved still remain unknown.

Recently, tumor-specific allele loss has been shown to play an important role in tumors such as retinoblastoma (6), Wilms’ tumor and rhabdomyosarcoma (7), bladder carcinoma (8), and colon carcinoma (9, 10). It has been suggested that loss of normal growth-regulatory genes, so-called “tumor suppressors” or “antioncogenes,” potentially allow the expression of tumorigenicity (11, 12). The c-Ha-ras1 gene, the human homologue of the transforming gene of the Harvey murine sarcoma virus, has been found to be highly polymorphic and shows RFLP2 allelic loss in several types of tumors, including one case of human ovarian carcinoma (13, 14). The data presented here show that, in 50% of ovarian tumor DNAs from constitutionally heterozygous patients, one c-Ha-ras1 allele was lost. This loss, of relatively high incidence, appears to be an important characteristic of human ovarian cancer.

MATERIALS AND METHODS

Patients and Tissues. Primary and metastatic malignant ovarian tumors from a total of 17 patients from 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 type and grade of the tumors studied were established on paraffin-embedded sections by the Department of Pathology at the M. D. Anderson Cancer Center. Ascites samples were obtained by paracentesis into vacuum bottles containing preservative-free heparin (10 units/ml). Routine cytological examinations of ascites were done by the Department of Cytology. Peripheral blood samples were obtained from the same patients from whom the ascites or tumor was procured.

Preparation of High-Molecular-Weight DNA. Histologically proven tumor tissues were immersed in liquid nitrogen in a ceramic mortar and pulverized to a fine powder using a pestle. The pulverized tissue and cells obtained from ascitic fluid or peripheral blood were then suspended in 20 ml of 1× standard sodium citrate plus 0.01 M EDTA, pH 8, in 1% sodium dodecyl sulfate solution. The cells lysed completely in approximately 5 to 10 min with gentle rocking at room temperature. These suspensions were then incubated with proteinase K overnight at 37°C with gentle shaking. The next morning, the DNA was extracted with phenol and chloroform as described previously (15), and ethanol was precipitated. The purified high-molecular weight DNAs were resuspended in sterile water and quantified spectrophotometrically.

Blot Hybridization. Fifteen μg of high-molecular-weight DNA were digested to completion with the appropriate restriction endonucleases as recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, MD) and size-fractionated in a 0.8% agarose gel, denatured, neutralized, and transferred to a nylon filter (16). Hybridization to an oligo-primed 32P-labeled (17) c-Ha-ras1 probe was conducted at 42°C for 48 to 72 h. The filters were then washed at 60°C for 60 min in 0.1× standard sodium citrate containing 0.1% sodium dodecyl sulfate and autoradiographed at −70°C using intensifying screens.

Probes Used. Probes used included: a 2.9-kilobase SacI fragment of the human c-Ha-ras1 gene (18); two EcoRI subclones of the epidermal growth factor receptor gene complementary DNA (HER-A-64) (19); and a BamHI subclone (pL335) of the c-sis gene (20).

RESULTS

Loss of Heterozygosity at the c-Ha-ras1 Locus. We prepared DNAs from 4 primary and 8 metastatic ovarian tumors, as well as 5 malignant ascitic samples plus matched normal tissue DNAs (WBC) from the same individuals (Table 1). The histological grading was performed according to a system described by Mauch et al. (21). DNAs were digested with BamHI, blotted, and hybridized to a radiolabeled c-Ha-ras1 probe. Each sample displayed either a single band or a doublet, which represented various fragment sizes ranging from 6.5 to 8.8 kilobases. A typical Southern blot is presented in Fig. 1. Our results are summarized in Table 1. Among the 17 pairs of samples, 10 (58.8%) normal tissues were heterozygous and thus informative. Five of these 10 (50%) revealed loss of heterozygosity; that is, in the WBC DNA there were two hybridizing bands, whereas in the ovarian tumors from these patients, there was only one band (Fig. 1). This indicates that DNA from chromosome 11p, where the c-Ha-ras1 gene is located, is lost in 50% of the tumors from the informative patients analyzed. As shown in Table 1, a large proportion (6 of 10) of the informative patients had serous adenocarcinoma. A total of 4 of 6 such patients showed tumor-specific loss of heterozygosity at the c-Ha-ras1 locus, whereas only 1 of 4 informative patients with other ovarian cancer types showed such allele loss. It is also of note that, in 4 instances of loss of heterozygosity, the tumors were obtained from metastatic sites (including 1 ascites sample).
Table 1  Loss of c-Ha-ras1 heterozygosity in ovarian cancer

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* Histological grading was performed according to a system described by Mauch et al. (21). N, normal WBC DNA; T, tumor DNA; OV, ovarian tumor; AD, adenocarcinoma; EC, endometrioid carcinoma; TCC, transitional cell carcinoma; OM, omental metastasis; MMT, malignant mixed mesodermal tumor; MC, Mullerian carcinoma; IL, ileal metastasis; AB, abdominal metastasis; ASC, ascites; ND, not done.

To determine whether this RFLP allele loss was specific for chromosome 11p and not simply from widespread random loss, we also examined RFLPs at the epidermal growth factor receptor gene locus on chromosome 7 (22) and the c-sis gene locus on chromosome 22 (23). These data are summarized in Table 1. We did not observe any loss of alleles at these loci when we compared normal and tumor DNAs.

Rearrangement of c-Ha-ras1 Gene. Among 17 pairs of samples analyzed for RFLPs at the c-Ha-ras1 locus, following digestion with either EcoRI or HindIII, one tumor DNA showed a rearrangement in the form of a smaller fragment in addition to the single fragment seen in normal WBC DNA from the same patient and in other control DNAs (Fig. 2).

DISCUSSION

To detect the somatic loss of c-Ha-ras1 locus alleles in ovarian cancer DNA samples, we studied a RFLP that occurs when using the c-Ha-ras1 gene probe and the BamHI restriction enzyme (14, 24). The basis of this polymorphism is the variable amplification or deletion of a 28-base pair tandem repeat element located approximately 1.5 kilobases 3' to the last c-Ha-ras1 coding region (14, 25). In DNAs of heterozygous individuals, two different allelic fragments are seen. Comparison of DNA from normal and tumor tissue reveals whether either the paternal or maternal allele is lost in tumors.

Yokota et al. (13) have studied c-myc gene amplification and allelic alterations of c-Ha-ras1 and c-myb gene loci in a variety of tumors and suggested a correlation of the alterations of these genes with progression of malignant disease. In their report, the overall incidence of loss of one c-Ha-ras1 allele was 21% in metastases and 15% in primary tumors. In addition, Theillet et al. (26) suggested that loss of a c-Ha-ras1 allele is highly associated with more aggressive breast carcinomas. To date, the specific incidence of loss of heterozygosity has not been studied with regard to ovarian cancer. The present study of 17 pairs of matched tumor and WBC DNAs from the same patients with ovarian cancer revealed that 10 (58.8%) of these were heterozygous and that in 5 instances (50%), one c-Ha-ras1 allele was lost. This observation is higher than the general incidence of a c-Ha-ras1 allele loss in a mixed group of malignancies reported by Yokota et al. (13). As shown in Table 1, there were some variations in the pathological subtypes we studied. Four of 6 (66.7%) informative patients with serious adenocarcinoma showed tumor-specific loss of an allele at the c-Ha-ras1 locus, whereas such allele loss was seen in only 1 of 4 other ovarian cancers. To date, there has been no report demonstrating genetic alterations specific for pathological subtypes. Tumor DNAs from the metastatic lesions (including ascites) showed a higher incidence (4 of 7) of loss of heterozy-

Fig. 1. Southern blot analysis demonstrating loss of one allelic c-Ha-ras1 restriction fragment in human ovarian cancer. Lanes 1 to 7 represent the normal tissues (patients' WBCs) and ovarian tumors from different patients. In Lanes 1 to 5, the normal tissues (N) contain two bands (6.5 and 8.8 kilobases), whereas the tumor tissue (T) has lost one band in each instance. Lanes 6N and 6T demonstrate that the heterozygous state is present in both normal and tumor tissue. Lanes 7N and 7T demonstrate that the homozygous state is present in both the normal and tumor tissue.

Fig. 2. Southern blot analysis of c-Ha-ras1 gene in WBC (IV) and tumor DNAs ('/') in patients with ovarian carcinoma. Arrows indicate rearranged bands in tumor DNA from Patient 14 in Table 1. DNA samples at top were digested with HindIII, and at bottom, with EcoRI.
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Heterozygosity than those from primary lesions (1 of 3). This could indicate that 11p allele loss is a relatively late event in progression of ovarian cancer.

At present, the mechanisms leading to the loss of a c-Ha-ras allele in ovarian cancer are unknown. The c-Ha-ras gene is located on chromosome 11p15.1-pter (27-29). Even though loss of one chromosome 11 has been occasionally observed in fresh ovarian carcinomas or in ovarian tumor cell lines (30, 31), this is not a consistent finding. In addition, one of the constitutive fragile sites is located in the vicinity, on Band p13 on chromosome 11 (32). Other studies have demonstrated loss of heterozygosity at the c-Ha-ras locus in different types of tumors, including Wilms’ tumor (55%) (33), rhabdomyosarcoma and hepatoblastoma (7), carcinomas of bladder (42%) (8) and breast (27%) (26), and adrenal adenoma (34). Taken together, these observations suggest that loss or inactivation of normal genes on 11p may be involved in the etiology or progression of a variety of human cancers including ovarian carcinoma (35). Alternatively, perhaps loss of the normal allele from some tumor cells leaves a mutated c-Ha-ras allele within them, giving a growth advantage over the cells that retain both a mutated and a wild-type allele (8). The case of a rearranged c-Ha-ras gene in an ovarian tumor DNA sample could be a rare local mutation or may be indicative of a larger scale rearrangement on 11p that functionally inactivates a normal tumor-suppressor gene located there.

In conclusion, the present data, though based on a relatively small sample size and limited number of additional chromosome markers, demonstrate that a relatively high incidence of loss of heterozygosity at the c-Ha-ras 11p locus on chromosome 11 is a characteristic of ovarian cancer.

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