Allelotype of Endometrial Carcinoma


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Abstract

An allelotype analysis of endometrial carcinoma was undertaken to identify chromosomal loci that are relevant to this tumor type. A total of 70 highly polymorphic microsatellite markers, distributed among all nonacrocentric chromosome arms, were examined for evidence of loss of heterozygosity or allelic imbalance in DNA samples from matched normal and tumor tissues. An average of 21 informative tumor cases were obtained for each marker. Allelic deletions or imbalance were observed on 31 of 41 chromosome arms with no marker showing an allelic loss ratio of greater than 83%. Those chromosome arms most frequently involved were 3p, 8p, 9p, 14q, 16q, and 18q. There was a strong correlation between loss of heterozygosity on chromosome 14q and death from disease. These data indicate that the molecular genetic character of endometrial carcinoma is complex and that a relatively large number of different chromosomal loci are likely to play a role in the etiology and progression of this tumor type.

Introduction

Carcinoma of the uterine endometrium is the most frequently diagnosed gynecological malignancy in the United States, accounting for approximately 34,000 new cancer cases per year (1). The age-specific incidence rate for this cancer suggests that approximately six genetic alterations are required for tumorigenesis (2). As for most human cancers, the involvement of ras oncogenes and the p53 tumor suppressor gene have been thoroughly studied in endometrial cancer. An activating mutation of K-ras codon 12 is found in 10–30% of premalignant hyperplasias and invasive cancers (3–6). Mutational inactivation of the p53 gene, often accompanied by allelic deletion, occurs in 10–20% of advanced stage endometrial carcinomas (7–9) but not endometrial hyperplasias (10). The erbB-2 oncogene is overexpressed in approximately 10% of advanced endometrial cancers and correlates with a poor prognosis (11, 12), although gene amplification is apparently not associated with the observed overexpression (13). Microsatellite instability associated with defective mismatch repair is found in approximately 20% of sporadic endometrial carcinomas (14–16). Taken together, these findings provide only a small fraction of the complete molecular genetic profile of endometrial carcinoma. Further studies are clearly indicated for a better understanding of this cancer type.

Toward this end, we undertook an allelotyping analysis of endometrial carcinoma. The purpose of such a study is to define chromosomal regions that have sustained allelic deletion as manifested by LOH, implicating mutant tumor suppressor genes at the affected loci (17). Two previous studies have provided variable degrees of allelic loss data for endometrial carcinoma, with significant LOH observed on chromosomes 17p and 18q (7, 18). Our goal was to analyze a sufficiently large sample size with highly polymorphic markers such that informative results could be obtained for all chromosome arms. Microsatellite repeat markers were chosen for their high informativity and used to assess LOH in normal/tumor DNA pairs from cancer cases representative for grade, stage, invasion, metastasis, histological subtype, and clinical outcome. The cumulative allelotype presented here provides clues to the location of additional genetic loci relevant to endometrial tumorigenesis.

Materials and Methods

Tissue Samples and DNA Preparation. Seventy-nine endometrial carcinomas were accessioned for this study from the Duke University Medical Center in Durham, NC, and the Jikei University Medical Center in Tokyo, Japan. Tumors consisted of an approximately equal mixture of frozen tissues and formalin-fixed, paraffin-embedded tissues obtained between 1987 and 1991 and representative for grade, stage, invasion, metastasis, histological subtype, and clinical outcome. For DNA isolation, areas of tumors were selected based on histological evidence of a high tumor cellularity (>75–80%), and microdissection techniques were used to enrich further for malignant cells. Corresponding normal tissues were all from paraffin blocks, and generally consisted of nonmalignant myometrium or other reproductive tract tissue obtained at hysterectomy. Standard procedures were used for DNA preparation from both fixed and frozen tissues, as described previously (6).

Allelotyping. Primers for PCR amplification of microsatellite markers were obtained from Research Genetics (Huntsville, AL) or synthesized on an ABI Model 394 DNA synthesizer (Applied Biosystems, Foster City, CA). Primer sequence and marker mapping information was obtained from the Genome Data Base (Welch Medical Library, Johns Hopkins University, Baltimore, MD). Seventy markers distributed among all 41 chromosome arms were analyzed and are listed in Table 1. For each marker, tumor cases were selected at random from the pool of 79 cases. For technical and practical reasons, no effort was made to examine every tumor case using every marker; our intent was to assess the potential involvement of each marker locus in endometrial carcinoma generally, rather than attempting to score the extent of allelic loss for a given tumor or that associated with a particular clinical parameter. Such an analysis would be inherently inaccurate as a result of the inevitable lack of informativity of a given normal/tumor pair with 30–40% of the markers studied. The study design was based on a goal of 20 informative tumor cases per marker. Less than 20 cases were sometimes obtained as a result of low heterozygosity or poor resolution for some markers, and more than 20 cases were often examined for those markers subject to more frequent LOH. All tumors with evidence of microsatellite instability were excluded from this analysis.

PCR reactions were carried out in a total volume of 20 μL containing 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, 200 μM concentrations of each deoxynucleotide triphosphate, 1 μM concentrations of each primer, 0.5 unit AmpliTaq polymerase (Perkin Elmer/Cetus), and 10–50 ng of genomic DNA. Prior to PCR amplification, one primer was end-labeled with [γ-32P]ATP by polynucleotide kinase using the Kinase-It kit (Stratagene) and column purified. Thirty cycles were performed, consisting of 30 s at 94°C, 30 s at 52–60°C, and 45 s at 72°C, with a final extension for 7 min at 72°C. The PCR products were electrophoresed through a 5% polyacrylamide gel (acrylamide:bisacrylamide 19:1) at 60 W for 4 h. Gel bands were visualized by autoradiography and recorded as present or absent. The data were scored independently by two observers, and differences were resolved by discussion.
30 s at 72°C, followed by a 7-min extension at 72°C, using a Perkin Elmer polyacrylamide gels containing 8.3 M urea for 2-3 h at 70 W. Gels were fixed (Table 1) distributed throughout the genome. Representative examples deletion were subjected to densitometric quantitation using the Molecular in 10% methanol/10% acetic acid, dried, and exposed to Phosphorlmager min; and placed on ice. Five jxl of tn's solution were electrophoresed in 6% xylene cyanol FF, and 0.02% bromophenol blue; then denatured at 90°C for 2 qualitative signal is often seen for markers subject to LOH and results from polymorphism and applicability to PCR-based techniques, but residual signal is seen for one allele in tumor samples. Subject to this qualification, allelic imbalance as defined above will hereafter be referred to as allelic deletion, or LOH.

Individual markers showing the highest percentage of LOH were D8S87 at 8p12, D9S104 at 9p21, TCRD at 14q11.2, D16S186 and D16S289 at 16q22-24, and DCC at 18q21. The results were also expressed as the percentage of informative tumor cases showing LOH at any locus on each chromosome arm (Fig. 3). All chromosomes except 2 and 21 were subject to LOH in at least one tumor, but no chromosome arm exhibited LOH in greater than one-third of the informative cases. Those chromosome arms displaying the highest percentage of tumors with LOH (>15%) were 3p, 8p, 9p, 14q, 16q, and 18q.

As indicated in “Materials and Methods,” the experimental design of the allelotype analysis did not provide for an extensive correlation of the allelotype data with clinical parameters. However, there was a conspicuous association of LOH on chromosome 14q with a poor clinical prognosis. Nine of 47 informative tumor cases displayed LOH on chromosome 14q, and of these 9 cases, 6 were from patients who had died of their disease (n = 4) or who had recurrent disease (n = 2) (Table 2). All of the remaining 38 informative tumor cases without 14q LOH were from patients with no evidence of disease; the follow-up period for both groups was the same. Of the nine cases exhibiting 14q LOH, three were of the biologically aggressive papillary serous subtype, two were advanced stage (IVB) cancers of the endometrioid histology, and the remaining four were early stage adenocarcinomas. Using Fisher’s exact test, this correlation between poor clinical outcome and 14q LOH was highly significant (P < 0.00001).

## Discussion

Comprehensive allelotype analyses in which all chromosome arms were examined for LOH have now been described for several types of human carcinomas, including those of the colon (19), breast (20), prostate (21), lung (22), ovary (23), bladder (24), and head and neck (25), providing clues concerning the potential involvement of known or novel tumor suppressor genes in these cancers. Partial allelic loss data have been reported for endometrial carcinoma, implicating chromosome 18q and the p53 locus on chromosome 17p as sites of frequent deletion (7, 18). Our data confirm these findings and further implicate loci on chromosomes 3, 8, 9, 14, and 16 as sites of potential tumor suppressor gene localization with relevance to endometrial cancer.

Unlike most cancers that have been allelotted, endometrial carcinoma does not appear to be characterized by one or more loci that show involvement in a majority of cases. The greatest frequency of LOH was observed at the DCC locus on chromosome 18q21, with 33% of the tumors analyzed exhibiting apparent allelic deletions in the region of this putative human tumor suppressor gene (26). These deletions were partial or interstitial and confined to a relatively small region of chromosome 18q; no evidence for LOH was observed at the D18S35 marker distal to DCC on 18q21.2-21.3. This finding is consistent with that of Imamura et al. (18), who found that the frequency of LOH at the more distal marker D18S5 on chromosome...
18q22 was significantly lower than that observed in the 18q21 DCC region. However, we have been unable to detect any mutations through either Southern blotting or single strand conformation polymorphism analysis of the 29 known exons of the DCC gene (27) in 60 endometrial carcinomas. Mutations may exist in intronic or promoter regions of the DCC gene or, alternatively, the LOH may be driven by a simple gene dosage phenomenon (reduction to hemizygosity). The observed LOH on chromosome 18q may also occur subsequent to the mutation of an as yet unidentified tumor suppressor gene other than DCC. The observation of 18q LOH at loci distal to or proximal to but not including DCC in some colorectal tumors (27) is consistent with this possibility.

Chromosome 16q was affected in 21% of the cancers examined. In addition to those markers indicated in Table 1, the analysis of several additional widely spaced 16q markers indicated that the entire arm is probably lost in those tumors with LOH. The E-cadherin gene on chromosome 16q22.1 is a plausible candidate tumor suppressor gene that could underlie the observed LOH, but an extensive survey of this gene in gynecological cancers indicates that coding region mutations are rare (28). All of the caveats discussed above for the DCC/chromosome 18q analysis apply to this relationship as well, but additional tumor suppressor loci on chromosome 16q should be considered. Carcinomas of the prostate (29), liver (30), and breast (31) also demonstrate frequent LOH on chromosome 16q.

Although the goals of this study did not include a clinical correlation, the association of chromosome 14q LOH with a poor clinical prognosis was striking. All of the tumors used in this study that were from patients who had died from disease or had recurrent disease sustained 14q LOH. Among these were several papillary serous carcinomas and advanced stage adenocarcinomas. Further studies focused on chromosome 14q and endometrial carcinoma will be necessary to confirm and extend this finding. Subsequent studies in our laboratory have defined a minimal region of deletion for these tumors to chromosome 14q32. Other cancer types for which 14q LOH has been described include neuroblastoma (32), ovarian carcinoma (23), and advanced stage colorectal carcinomas (33).

LOH was also observed at a significant frequency on chromosomes 3p, 8p, and 9p in endometrial carcinomas. Allelic deletions on chromosome 3p occur in many human cancers, and there are at least three distinct regions that are likely to harbor tumor suppressor genes on this chromosome arm (34). The VHL tumor suppressor gene on 3p25-26 has now been identified as one of these genes (35) but would appear to be specific for renal carcinomas. A more proximal region at 3p13–21.1 has been shown by deletion mapping to harbor a tumor suppressor locus commonly involved in carcinomas of the uterine cervix (36). The recently identified hMLH1 gene encoding a DNA mismatch repair protein is localized to chromosome 3p21–23 (37, 38) and represents a reasonable candidate gene for endometrial carcinomas with 3p LOH, especially in light of the microsatellite instability phenotype observed in some of these cancers (14–16).

Similarly, chromosome 8p appears to contain at least two distinct tumor suppressor gene loci (39). In addition to colorectal cancers (19, 39), homozygous deletion and frequent LOH at 8p22 have recently been described for prostate carcinoma (40). Although two endometrial tumors demonstrated LOH at 8p22, the more proximal locus at 8p12 was most frequently deleted in our study.

Chromosome 9p is the most frequent site for LOH as determined by allelotypes of bladder cancer (24) and head and neck cancer (25). A putative tumor suppressor gene has been localized to the 9p21–22 region based on fine mapping of LOH in head and neck cancers (41) and mapping of homozygous deletions in melanomas (42) and lung cancers (43, 44). The recently described p16/MTSI/CDK4I gene may

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3 J. I. Risinger and J. Boyd, unpublished data.


Figs. 1A–1F. Representative autoradiograms of microsatellite markers examined for LOH. Lane T, DNA from tumor; Lane N, DNA from corresponding normal tissue. A, D3S1038 (chromosome 3p); B, TNFβ (chromosome 6q); C, D8S87 (chromosome 8p); D, TCRD (chromosome 14q); E, D14S76 (chromosome 14q); F, D16S186 (chromosome 16q). Open arrows, alleles scored as deleted.
A2 to the right. Tumor 1 was scored as LOH for the lower allele, while tumor 2 contained between tumor 2 and normal 2 (no LOH).

bands showing allele intensity. C, integration of area under curves shows 3-fold difference the same two alleles but was scored as retention of heterozygosity. *.

peak analysis of diagram of PCR products for microsatellite marker DCC. Alleles are indicated by A1 and BRCA1 region of chromosome 17q21 (48), in which 30 tumors were

ing involvement in endometrial carcinoma. Among these was the

coding region.5

represent affected tumor suppressor gene in this region (45–47); however, preliminary data from our laboratory indicate that endometrial cancers with 9p LOH do not contain mutations in the MTS1/p16 coding region.5

Several chromosomal regions were notable for their lack of apparent involvement in endometrial carcinoma. Among these was the BRCA1 region of chromosome 17q21 (48), in which 30 tumors were uniformly negative for evidence of LOH. Likewise, no LOH was observed at the WTI locus on chromosome 11p13. Only 1 of 22 cases displayed LOH at the APC locus on chromosome 5q21; we have also failed to find any evidence of APC mutation in a thorough mutational survey of endometrial carcinomas.5 Only 11% of the tumors in this study (3 of 28) sustained LOH on the p53 locus on chromosome 17p13.1, which is in agreement with our previous finding that 14% of endometrial carcinomas contain a mutation in the p53 gene that is not necessarily accompanied by LOH (9).

These data suggest that multiple tumor suppressor genes are involved in the etiology of endometrial carcinoma and that the molecular genetic profile of this tumor type is likely to be complex. There were insufficient data from individual tumor cases to attempt an analysis based on the number of deleted loci per case and possible clinical correlates. Further studies which focus on particular deleted loci should be more conducive to this type of evaluation. For example, we are particularly interested in pursuing the possibility that one or more genes on chromosome 14q may be associated with biologically aggressive endometrial carcinomas. This allelotype provides a rational foundation upon which further efforts to define the molecular genetics of endometrial cancer may be based.

References


7. Okamoto, A., Samehashima, Y., Yamada, Y., Teshima, S-I., Terashima, Y., Terada, M.,
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