Advances in Brief

A Distinct Region of Chromosome 19p13.3 Associated with the Sporadic Form of Adenoma Malignum of the Uterine Cervix

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Abstract

Adenoma malignum (AM) is known to be one of the malignant tumors that is commonly associated with Peutz-Jeghers syndrome. Recently, the genetic locus of Peutz-Jeghers syndrome was mapped to the telomeric region of chromosome 19p. We analyzed nine sporadic cases of AM with high-density loss of heterozygosity to study the region of chromosome 19p13.2–13.3 using eight microsatellite markers. Our deletion mapping data revealed a distinct region with 100% loss of heterozygosity frequency at marker D19S216. This result indicates that a putative tumor suppressor gene for AM is located at D19S216 on chromosomal band 19p13.3 and plays an important role in AM tumorigenesis.

Introduction

The incidence of primary adenocarcinoma has been variously assessed at 5–8% of all epithelial malignancies of the cervix (1). Histologically, there are many variations in both the cell type and growth pattern. On occasional instances, lesions classified as AM or minimal deviation adenocarcinomas have been reported. This peculiar type of lesion is currently regarded as an extremely well-differentiated adenocarcinoma with a deceptively innocent histological appearance masking highly aggressive behavior (2, 3). To date, there has been no report on the genetic alterations involved in the carcinogenesis of AM, due to its rarity and the difficulty in selectively procuring malignant glandular cells without using microdissection techniques.

AM is a rare autosomal dominant disorder with varying degrees of penetrance characterized by gastrointestinal hamartomatous polyps and mucocutaneous melanin pigmentation (4, 5); these polyps are not thought to be malignant, but a disturbance of superfluous tissue. Such patients are, however, at increased risk of developing both gastrointestinal and nongastrointestinal cancers. Giardiello et al. (6) estimated that these patients have an 18-fold higher risk of malignancy than the general population. AM is one of the nongastrointestinal cancers associated with PJS. Since the first description of PJS-associated AM in 1969 (7), Srivatsa et al. (8) have reported that up to 10% of AM cases occur in patients with PJS.

Recently, Hemminki et al. reported localization of a putative tumor suppressor gene responsible for PJS to the short arm of chromosome 19 by way of comparative genomic hybridization of PJS polyps and demonstrated the presence of a high-penetration locus in distal 19p with a multipoint lod score of 7.0 at marker D19S886 by using targeted genetic linkage analysis of 12 PJS families (9, 10). Rare tumor syndromes with Mendelian inheritance have been invaluable indicators of the genes involved in tumorigenesis, and the study of such syndromes has led to the identification of tumor suppressor genes that are also important targets for somatic mutation in sporadic cancers. Therefore, the above-mentioned findings suggest that the putative locus responsible for PJS might also be associated with development of the sporadic form of AM.

In the present study, tumor DNA and corresponding normal DNA from H&E-stained histological sections of 9 sporadic cases of AM were obtained using our microdissection technique (11), and high-density LOH analyses were performed using 8 markers, including D19S886, spanning 19p13.2–13.3; additionally, we analyzed 10 conventional endocervical adenocarcinomas and 3 cases of endocervical type A tunnel clusters, pseudoneoplastic lesions mimicking the histomorphology of AM. Our results indicate that a putative tumor suppressor gene for AM is located at D19S216 in band 19p13.3 and plays an important role in AM tumorigenesis, although not in conventional adenocarcinomas or tunnel clusters of the uterine cervix.

Materials and Methods

Materials. Paraffin-embedded histological sections of 9 AMs were obtained from Sungkyunkwan University Medical College (Seoul, Korea); not one patient had a family history of PJS. The histological diagnosis was made based on the established criteria, including the presence of markedly irregular and angular-shaped glands, deep cervical invasion, foci of obvious adenocarcinoma, vascular invasion, perineural invasion, or a desmoplastic or loose edematous stromal reaction (12). All nine cases were diagnosed from hysterectomy specimens. Additionally, 10 cases of conventional endocervical adenocarcinomas and 3 cases of type A tunnel clusters were collected from the Catholic University Medical College-affiliated hospital (Seoul, Korea).

Microdissection. Malignant glandular epithelium was selectively procured from H&E-stained slides without any normal cell contamination using a 30G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator, as described previously (11). We also obtained inflammatory cells or squamous epithelium for corresponding normal DNA from the same slide in all cases.

DNA Extraction. DNA extraction was done using a modified single-step DNA extraction method (13). Procured cells (50 cells/μl) in 20 μl of DNA extraction buffer containing 100 mM Tris-HCl (pH 8.0), 0.1% Tween 20, and 2 mg/ml proteinase K were incubated at 52°C for 1 or 2 days. The mixture was boiled for 10 min to inactivate proteinase K, and 1 μl of this solution was used as the DNA template for PCR amplification.

LOH Analysis. Tumor DNA and corresponding normal DNA from each slide were amplified by thermal cycler (MJ Research, Inc., Watertown, MA) with eight microsatellite markers (Research Genetic, Huntsville, AL), including D19S886, D19S883, D19S565, D19S894, D19S216, D19S901, and D19S873 in the 19p13.3 region and D19S413 in the 19p13.2 region. Each PCR reaction was generally performed under standard conditions in a 10-μl reaction mixture containing 1 μl of template DNA, 0.4 μM each primer, 1.25 μM each

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3 The abbreviations used are: AM, adenoma malignum; PJS, Peutz-Jeghers syndrome; LOH, loss of heterozygosity.
deoxynucleotide triphosphate, 1.5 mM MgCl\textsubscript{2}, 0.4 unit of Taq polymerase, 0.5 mCi of \(^{32}\)PdCTP (Amersham, Buckinghamshire, United Kingdom), and 1 \(\mu\)l of 10\(\times\) buffer. The reaction mixture was denatured for 5 min at 95°C and incubated for 35 cycles (denaturing at 95°C for 50 s, annealing at 57°C for 90 s, and extending at 72°C for 90 s), with some variations in the annealing temperature. Final extension was continued for 10 min. Reaction products (2 \(\mu\)l) were then denatured and electrophoresed in 6% polyacrylamide gels containing 7 M urea. After electrophoresis, the gels were transferred to 3 MM Whatman paper, dried, and subjected to autoradiography using Kodak X-OMAT film (Eastman Kodak, Rochester, NY).

**Results**

Through microdissection, we successfully procured malignant glandular epithelial cells without normal cell contamination, as shown in Fig. 1. Nine AMs were analyzed for allelic loss within 19p13.2–13.3 using eight pairs of unique primers corresponding to the marker loci shown in Fig. 2. The fixed marker order, female genetic distances, and physical map information for the eight marker loci were obtained from the genetic location database (http://cedar.genetic.soton.ac.uk/public.html) at the University of Southampton (Southampton, England, United Kingdom; Ref. 14).

Patients heterozygous for a given marker were considered informative. All nine AMs were informative and showed allelic loss of at least one informative marker within this chromosomal region. Cases 4 and 7 showed allelic loss at every informative marker tested. Cases 3 and 8 (and perhaps case 5) revealed terminal deletion, with all informative markers distal to the D19S901 locus. The other four tumors showed interstitial deletions. Among these, cases 2 and 9 revealed allelic loss at only one marker, D19S216; this single region, marked distally by D19S894 and proximally by D19S901, revealed allelic loss in all informative tumor cases (100%; six of six cases). Constitutional heterozygosity at D19S901, 4 cM proximal to D19S216, was retained in cases 3 and 5. And two of six tumors, namely, cases 1 and 2, retained heterozygosity at D19S894, which is about 2 cM distal to D19S216. With the exclusion of cases 4 and 7, which showed allelic loss at every informative marker, the two most proximal markers, D19S873 and D19S413, showed retention of heterozygosity in all informative tumors. These results indicate that a locus at D19S216 in chromosomal band 19p13.3 may harbor a tumor suppressor gene in an area less than 4.3 Mb in physical map distance defined by marker D19S873 and D19S894.

The autoradiograms of two selected cases showing LOH are displayed in Fig. 3. The almost complete absence of signal in deleted alleles of tumor DNA (Fig. 3, arrowheads) suggests that tumor samples are nearly devoid of normal cell contamination. Ten endocervical adenocarcinomas and three tunnel clusters showed no allelic loss with eight polymorphic markers spanning 19p13.2–13.3.

**Discussion**

AM is known to be one of the malignant tumors commonly associated with PJS (8, 15). Recently, Hemminki et al. (9) described the localization of the gene for PJS to the short arm of chromosome 19 by comparative genomic hybridization and target linkage analysis combined with LOH study. They demonstrated a high-penetrate locus in distal 19p with a multipoint lod score of 7.00 at marker D19S886 in band 19p13.3, without evidence of gender heterogeneity (9). These results have been supported by others (10) and led us to focus our attention on chromosomal region 19p13 in the sporadic form of AM. For finely detailed deletion mapping, we performed a high-density LOH study with eight polymorphic microsatellite markers including D19S886; seven of these markers were positioned approximately every 3–5 cM throughout chromosomal band 19p13.3, and a last proximal marker was located at 19p13.2. Our deletion mapping data revealed a distinct region with 100% LOH frequency at marker D19S216 that was flanked by loci D19S873 and D19S894, measuring 4.3 Mb in physical distance. Allelic deletion at specific chromosomal loci in tumor DNA, detected by a polymorphic DNA marker, can identify specific regions associated with certain tumors and, in turn, suggest that the presence of a tumor suppressor gene in that region may have been inactivated by such loss. The results reported here indicate that a locus at D19S216 in chromosomal band 19p13.3 may harbor a tumor suppressor gene that may be involved in the genesis and/or progression of the sporadic form of AM. To our knowledge, this is the first report documenting the observation of specific allele loss in the sporadic form of AM and is an important step toward the
eventual isolation of a putative tumor suppressor gene associated with AM by positional cloning.

Hemminki et al. (9) pinpointed the locus of the PJS gene to D19S886, which lies 13 cM distal to marker D19S216, which showed 100% LOH in all informative AMs in the present study. One of three informative tumors (case 1) retained heterozygosity at D19S886, and the remaining two cases (cases 7 and 8) showed LOH at D19S886. These two cases, however, showed a broad range of terminal deletion; case 7 revealed LOH at all informative markers, including D19S413 positioned at 19p13.2. In case 8, LOH was observed at all informative markers distal to D19S901. The question of whether the PJS and AM loci are the same was unclear at the time of this study, but during the submission of this manuscript, serine threonine kinase, STK11, was identified as a PJS-responsible gene, residing at a distance 190 kb proximal to D19S886 (16, 17); this clearly shows that the AM and PJS loci are different. Patients with PJS accompanied by AM would have a broad range of terminal deletion, including the locus at D19S216, because the physical distance between the two corresponding genetic

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CASE 7

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loci is only 2.2 Mb. Additional detailed LOH analysis on the sporadic form of sex cord tumors and ovarian epithelial cancers, which are also commonly associated with PJS (8), will provide more information on this subject matter. In addition, positional cloning and characterization of a putative tumor suppressor gene locus at D19S216 should not only help to provide a better understanding of AM tumorigenesis but should also elucidate the relationship between PJS and AM.

At present, there is sparse information regarding the etiology and pathogenesis of endocervical adenocarcinomas (1). After having additionally analyzed 10 conventional endocervical adenocarcinomas, no LOH was observed in all 8 polymorphic markers at 19p13.2-13.3. These findings suggest that AM is a distinct tumor with a different molecular genetic background, rather than what was conventionally believed to be a morphological variant of endocervical adenocarcinomas. In view of its innocent histological appearance and rarity, AM is prone to misinterpretation as nonmalignant glandular lesions of the cervix such as type A tunnel clusters (18) or florid deep glands, especially in small biopsied specimens (19). In this study, three cases of endocervical type A tunnel clusters revealed no LOH at 19p13.2-13.3. Thus, the detection of 19p13.3 genetic changes through LOH analysis might well be a useful approach to differentially diagnose these benign mimickers or well-differentiated conventional endocervical adenocarcinomas.

In summary, we have been able to detect a distinct region for a putative tumor suppressor gene for AM with 100% LOH frequency at marker D19S216, measuring 4.3 Mb in physical distance. The narrow LOH region reported in this study should provide the impetus to further decipher the relationship between AM and PJS. Once the gene is known and isolated, it will help to further decipher the relationship between PJS and AM.

References

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