Analysis of p53 and ras Gene Mutations in Endometriosis

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Abstract
No activating mutations in codons 12, 13 and 61 of ras genes nor inactivating mutations in exons 5–9 of the p53 tumor suppressor gene were detected by polymerase chain reaction and single-strand conformation polymorphism methods in either eutopic or ectopic endometrium from 10 women with severe endometriosis.

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Introduction
Endometriosis sometimes behaves as a progressive disease, affecting multiple organs and infiltrating the surrounding tissue. Factors involved in the development and spread of the implants are still unclear. Protooncogenes and tumor suppressor genes regulate normal cell growth and differentiation. Alterations of these cellular genes have been found associated with many types of human tumors. Recent immunocytochemical studies have demonstrated that both endometrial and endometriotic tissue may express relatively high levels of proteins encoded by various oncogenes such as c-myc, c-fms, c-erbB-i and -2 and ras [1,2]. Activation of ras genes and inactivation of the p53 tumor suppressor gene represent the most common genetic lesions in human neoplasms including endometrial and ovarian cancers. To investigate the potential role of these alterations in patients with invasive and aggressive forms of endometriosis, we used polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) methods to detect activating mutations in codons 12, 13 and 61 of the H-, K- and N-ras genes and inactivating mutations in exons 5-9 of the p53 gene.

Materials, Methods and Results
Biopsy specimens were taken from 10 women with a median age of 29 years (range 23-38) undergoing conservative surgery at laparotomy for severe endometriosis. All the patients had ovarian endometriomas, obliteration of the pouch of Douglas and infiltration of the rectovaginal septum. Eutopic and ectopic endometrial tissue samples were quick-frozen in liquid nitrogen and stored at -80°C. The specimens were also processed routinely, and histology confirmed the
diagnosis of endometriosis in all cases. Frozen endometriotic and endometrial tissue samples were minced on dry ice, and DNA was purified by protease K digestion, extraction with phenol-chloroform and ethanol precipitation. DNA was amplified in vitro by PCR for exons 5-9 of the p53 gene and for sequences across codons 12, 13 and 61 of the three ras genes. To detect mutations, the amplified products were evaluated by SSCP analysis with polyacrylamide gel electrophoresis under nondenaturing conditions. Briefly, PCRs were performed using 100 ng of genomic DNA, 5 pmol of each primer, 2.5 µM dNTPs, 1 µCi of [α32P]dCTP, 10 × Taq polymerase buffer and 0.5 U Taq polymerase in a final volume of 10 µl. Thirty cycles of denaturation, annealing

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Fig. 1. SSCP analysis of representative exons 6, 7 and 8 of p53 gene in endometriosis. ND = Nondenatured normal control; N = normal control; M = positive control harboring a known mutation; arrows indicate abnormal migrating bands. Endometriotic samples are indicated by numbers.

No activating ras gene mutations were observed in either endometrial or endometriotic tissue from any of the patients (data not shown). Neither were alterations of the normal migrating patterns of exons 5-9 of the p53 gene detected by SSCP analysis (see fig. 1 for representative exons).

Comment

The aggressive and infiltrating tendency of some endometriotic forms might suggest an anomaly in ectopic endometrial cell behavior. In this context, alterations of normal cell differentiation in endometriotic tissue and an association between endometriosis and endometrioid and clear-cell ovarian cancer have been reported repeatedly [3]. Schenken et al. [1] evaluated c-myc protooncogene poly-peptide expression in endometrial and endometriotic tissue using an immunocytochemical technique and found that ectopic specimens demonstrated less intense nuclear and cytoplasmic staining and a greater variability in staining among individual glands. Bergqvist et al. [2], employing the same technique, observed that expression of protooncogenes c-fms, z-erbB-1 and -2 and ras is more pronounced in ectopic than eutopic endometrium. This suggests that protooncogene expression may be an important regulator of cell proliferation in endometriotic tissue [1]. In our study we showed that mutations activating ras genes or inactivating the p53 tumor suppressor gene seem not to be involved in abnormal growth and infiltrating behavior of ectopic endometrial tissue in patients with particularly severe and progressive endometriosis. Our data should be considered reasonably accurate because the PCR and SSCP methods have been reported to be highly sensitive and specific in the detection of nucleotidic sequence variability. Further studies evaluating ras and p53 protein expression are needed to investigate whether functional rather than structural abnormalities of these genes play a role in endometriosis development and spread.

and extension were carried out on an automated heat-block. The reaction mixture (4 µl) was mixed 1:1 with a sequencing stop solution containing 20 mM NaOH. Samples were heated at 95
°C for 5 min, chilled on ice and immediately loaded onto a 6% acrylamide-TBE gel containing 10% glycerol. Gels were run at 8 W for 12-15 h at room temperature, fixed in 10% acetic acid and air dried. Autoradiography was performed overnight at -80°C using an intensifying screen.

References