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Original contribution

Use of X-chromosome inactivation pattern to determine the clonal origins of uterine leiomyoma and leiomyosarcoma

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Summary Uterine leiomyomas (LMs) and leiomyosarcomas (LMSs), both of smooth muscle origin, sometimes coexist in the same uterus. Little genetic evidence exists concerning the developmental relationship between LM and LMS. Using the X-chromosome inactivation pattern of the human androgen receptor gene, we examined the clonality of LM and LMS. Of the 24 patients with LM, 21 had multiple neoplasms; all were clonal and individual LMs derived from separate clones. Of the 20 patients with LMS, 6 exhibited multiple tumors in the uterus, and 4 of these individuals also harbored coexisting uterine LMs. We found all LMSs to be clonal. Separate tumors showed identical pattern of X inactivation in 4 patients, and in 2 other individuals, multiple LMSs developed from independent clones. Among the 4 patients with LMS and coexisting LM, 3 showed the same pattern of X inactivation in LMS and the adjacent LM. In 2 of the 3 patients, the tumor also exhibited a morphological transition between benign cells in LM and malignant cells in LMS, supporting the possibility of transformation from LM to LMS. One patient displayed different clones in LMS and the coexisting LM, indicating their independent origins. We conclude that (i) both LM and LMS are clonal; (ii) different nodules in multiple LM are of independent origins; (iii) multiple lesions of LMS may be either monoclonal or multiclonal; (iv) most LMSs are solitary lesions and are most likely de novo, but an individual LM may undergo "malignant transformation" to a LMS; and (v) some LMSs and coexisting LMs are of independent origins.

1. Introduction

Uterine smooth muscle tumors are the most common neoplasms of the female genital tract. Most of them are leiomyomas (LMs), and only 1 in 800 tumors is leiomyosarcoma (LMS) [1]. Most LMSs are regarded as arising de novo rather than from preexisting LM; however, so-called malignant transformation of LM to LMS has been reported [2-5]. Despite these few reports, little genetic evidence exists to support either the de novo development of LMS directly from the myometrium or the transformation of pre-existing LM to LMS.

Clonal overgrowth represents the hallmark of neoplastic proliferations and theorizes that acquired somatic mutations

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afford a survival advantage to a clonal cell population [6]. Clonality has been widely studied by analysis of karyotypic abnormalities, loss of heterozygosity, gene rearrangements, and patterns of oncogene activation. The most consistently informative marker of clonal composition of neoplastic and preneoplastic disorders in females is probably the nonrandom pattern of X-chromosome inactivation, which randomly occurs in either the maternally or the paternally derived allele [7-10]. Functional inactivation of 1 of the 2 X chromosomes takes place in all female somatic cells during early embryogenesis and represents a mechanism of dosage compensation that ensures equal expression of X-linked genes in both sexes of the mammalian species [11,12]. The established pattern of X-chromosome inactivation is passed on from a given cell to its progeny. A polyclonal tissue is represented by a mosaic of random inactivation of the paternal and the maternal X chromosomes. In contrast, a group of cells resulting from clonal expansion of a single progenitor cell exhibits an identical pattern of X-chromosome inactivation.

Previous studies in women heterozygous for the isoenzymic forms of the X chromosome–linked phosphoglycerokinase genes [13,14] and the glucose-6-phosphate dehydrogenase genes [15,16] have shown not only that LMs are the result of monoclonal proliferations of smooth muscle cells but also that multiple nodules in LM are independently generated.

An alternative method of studying clonality makes use of the differences in DNA methylation between active and inactive X chromosomes. The methylation of HhaII and HhaI sites near the polymorphic tandem trinucleotide CAG repeats in exon 1 of the human androgen receptor gene (HUMARA) on chromosome Xq11-12 correlates with X-chromosome inactivation. Approximately 90% of the female population is heterozygous at this polymorphic site [7], making HUMARA useful in clonality analysis. The combination of microdissection techniques and the use of polymerase chain reaction (PCR) assay allows for clonality determination in virtually all female tissue samples. Discrimination between active and inactive alleles is made by digestion of DNA with HhaII or HhaI. The enzyme breaks up the active X chromosome, preventing its amplification during PCR. Because of the heavy methylation of the inactive X chromosome, it resists digestion by the enzyme and serves as a template for amplification during the PCR. The HUMARA method has been used to investigate clonality in several tumors including uterine LMs [17-19]. However, to our knowledge, no information is available concerning its application in clonality studies of uterine LMSs.

In this study, we used the HUMARA technique to examine uterine smooth muscle tumors in several aspects: (i) the clonality in LM and LMS, (ii) the clonal relationship among individual lesions in multiple LM or LMS, and (iii) the clonal association between LMS and coexisting synchronous LM in the same uterus.

2. Materials and methods

2.1. Samples

We studied 30 consecutive cases of LM treated by hysterectomy during 1 month at our institution (July 2003). Twenty-two cases of LMS were also retrieved from our institutional archival files for the period between January 1992 and June 2005. Eighteen specimens of LMS resulted from total abdominal hysterectomy with bilateral salpingo-oophorectomy. Four were derived from myomectomy specimens performed for the presumed clinical diagnosis of "uterine leiomyomas" and were followed by total hysterectomy with bilateral salpingo-oophorectomy. Eight patients with LMS underwent pelvic lymph node sampling. Patients were also subjected to excision or biopsy of other clinically visible metastatic lesions in the abdominal or pelvic cavity.

Paraffin-embedded blocks were sectioned at 10-μm thickness. The sections were hydrated with a gradient of alcohol, stained briefly with hematoxylin, and immersed in 10% glycerol. Approximately 1000 cells from each area of

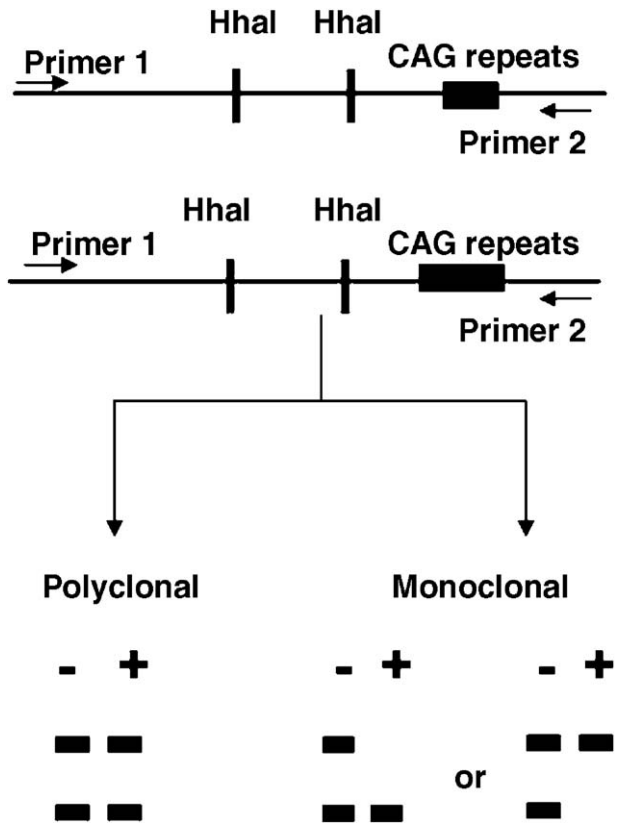

Fig. 1 Schematic presentation of clonality analysis by using the HUMARA method. The primers flank the polymorphic tandem of CAG repeats near the HhaI sites. In the absence of HhaI, 2 electrophoretic bands are formed in samples from either random (polyclonal) or nonrandom (monoclonal) X-chromosome inactivation. With the addition of HhaI, tissue with a polyclonal cell population shows 2 bands, whereas monoclonal tumor exhibits 1 band of either maternal or paternal origin. NOTE. −, without HhaI digestion; +, with HhaI digestion.