Chromosome Aberrations in Uterine Smooth Muscle Tumors: Potential Diagnostic Relevance of Cytogenetic Instability

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ABSTRACT

Cytogenetic studies were carried out on a low-grade metastatic uterine leiomyosarcoma and on a large degenerating uterine leiomyoma. The leiomyosarcoma and leiomyoma were hyperdiploid and hypodiploid, respectively, and both tumors contained multiple consistent chromosome aberrations. In the patient with leiomyosarcoma, flow cytometric studies of proliferative foci from a previously resected uterine leiomyoma revealed near triploidy, suggesting that the leiomyosarcoma was metastatic from an unrecognized malignant uterine primary lesion. The leiomyosarcoma was characterized by extreme cytogenetic instability, whereas the leiomyoma demonstrated cytogenetic stability. The present cases and review of the literature on leiomyosarcomas and leiomyomas reveal cytogenetic instability to be very common in leiomyosarcomas (present in 8 of 10 cases) and uncommon in leiomyomas (present in 1 of 25 cases). A grading system is described which might be useful in evaluating the diagnostic and prognostic relevance of cytogenetic instability in uterine, and other, malignancies.

INTRODUCTION

Uterine leiomyomas are extremely common neoplasms, having an incidence of nearly 40% in women older than 50 years (1); these benign tumors rarely undergo malignant transformation and in general are easily distinguished from low-grade leiomyosarcomas. It is widely accepted that uterine smooth muscle neoplasms with fewer than 5 mitoses/10 high-power fields behave in a benign fashion, whereas those with greater than 10 mitoses/10 high-power fields will often pursue a malignant course (2). In the absence of cellular atypia, however, it can be difficult to predict the behavior of uterine smooth muscle tumors having intermediate mitotic activity (5 to 10 mitoses/10 high-power fields). Accordingly, it has been suggested that these neoplasms be designated “smooth muscle tumors of unknown malignant potential” (1-3). Particularly difficult to categorize are the so-called “benign metastasizing leiomyomas” which are histologically benign neoplasms having little or no mitotic activity. It is uncertain whether such cases might represent malignant tumors which are misclassified due to inadequate sampling or malignant tumors in which metastatic phenotype is dissociated from mitotic rate and cellular atypia; alternatively, these tumors might represent multicentric benign neoplasms or benign neoplasms which have been disseminated by mechanical means, such as surgical implantation (4-6). Herein, we describe a pelvic leiomyosarcoma in which karyotypic analysis revealed aneuploidy, multiple consistent structural chromosome aberrations, and extreme cytogenetic instability. Correlation of the cytogenetic findings with flow cytometry data suggests that this leiomyosarcoma is metastatic from a uterine smooth muscle neoplasm that had been resected 5 years earlier. The karyotypic aberrations and cytogenetic instability in this leiomyosarcoma are contrasted with those in a large degenerating uterine leiomyoma from another patient, and with those in previously reported uterine leiomyomas and leiomyosarcomas.

MATERIALS AND METHODS

Cytogenetic Analysis. Tissues were minced with scalpels, and disaggregated for 12 h in a 200 units/ml collagenase (Gibco) solution, according to the method of Limon et al. (7). The disaggregated cell clusters were cultured in T25 flasks, using RPMI 1640 (Gibco) with 16% fetal calf serum, 1% l-glutamine, and 1% penicillin-streptomycin in a 5% CO2 incubator at 37°C. After 5 to 8 days, the adherent cells were exposed to colcemid (0.01 µg/ml) for 1 h, lifted from the flasks with trypsin, treated in a 0.075 M KCl hypotonic solution for 10 min, and then fixed with two changes of methanol:acetic acid (3:1). Slides were made by conventional techniques, using stem to assist in metaphase spreading. After 2 to 3 days of incubation on a slide warmer at 60°C, the chromosomes were banded by the GTG method (8).

Cytogenetic Stability Grading. In order to assess cytogenetic instability in a standardized manner, we have devised a “cytogenetic stability” grading system. This stability grading system is patterned after the conventional histopathological grading systems in which Grade I denotes a low grade and Grade II or IV a high-grade malignancy. In the cytogenetic context, grade refers to the percentage of cells within a given tumor population for which additional and unique cytogenetic events, beyond those in the stem line, are observed. These unique events may include both structural and numerical aberrations. However, numerical aberrations that likely result from technical artifact, e.g., random loss of whole chromosomes in overspread metaphases, are not counted as unique events. Also, groups of identical metaphases that have arisen from the stem line by clonal evolution are counted as only one event. The stability grade is applied only when a minimum of 10 metaphases from a given stem line are available for analysis, and is included in the karyotypic description of a given tumor. Stability Grades I, II, III, and IV indicate unique cytogenetic events in 0-10%, 11-49%, 50-90%, and greater than 90% of metaphases, respectively. As a hypothetical example, “45XX,−20 [stability grade I]” would be the cytogenetic designation for a tumor in which each of at least 10 metaphases was monosomic for chromosome 20 in the absence of additional chromosome aberrations. “46,XX,del(1p)(p32) [stability grade II]” would be the designation for a tumor in which 5 of 10 metaphases had the del(1p) only (stem line karyotype), 4 metaphases had del(1p) and trisomy 8 (one additional event), and 1 metaphase had del(1p), trisomy 6, and trisomy 11 (a second additional event).

Flow Cytometry. For fixed tissues, paraffin-embedded tissue blocks were deparaffinized, rehydrated, and pepsin digested by using modifications of the Hedley technique (9, 10). Adjacent normal myometrium served as an internal control. For fresh tissues, nuclei were mechanically dissociated by using a modification of a method described by Thornwaite et al. (11); normal peripheral lymphocytes were added to one-half of the specimen to provide an internal control (12). Nuclei were stained with propidium iodide and analyzed on a FACS Scan (Becton Dickinson, Mountain View, CA). DNA histograms were generated from analysis of 5000 nuclei and were displayed as linear fluorescence. The DI was calculated as the ratio of the aneuploid G0/G1 peak channel to that of the normal internal control G0/G1 peak channel (13). A DI

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The abbreviation used is: DI, DNA index.
UTERINE SMOOTH MUSCLE TUMOR CYTOGENETICS

of 1.00 was assigned, in either fixed or frozen tissues, if no $G_0/G_1$ peak distinctly different from control cells was detected. The coefficient of variation of the normal $G_0/G_1$ peak was 9.1% for fixed tissues and 3.2% for fresh tissues.

CASES AND RESULTS

Case 1. In October 1988, a 68-year-old woman was noted to have a lower abdominal mass. At laparotomy, a 14- x 11-x 6-cm partially encapsulated and multicystic tumor was resected from the pelvic side wall. Microscopically, the tumor contained short, ill-defined fascicles composed of round to slightly pleomorphic cells with perinuclear halos (Fig. 1A). Mitotic activity varied considerably throughout the tumor, with the most cellular areas containing 6 mitoses/10 high-power fields, and an overall average of 3 mitoses/10 high-power fields. Immunoperoxidase studies revealed diffuse staining for desmin, and electron microscopy demonstrated abundant microfilaments, pinocytic vesicles, subplasmalemmal linear densities, and stretches of external lamina. There was no evidence of epithelial differentiation and a diagnosis of low-grade leiomyosarcoma was made.

Cytogenetic analysis of the leiomyosarcoma yielded 28 metaphases, of which 16 were diploid (46,XX) and 12 were aneuploid. Diploid metaphases derived presumably from nonneoplastic stromal and vascular elements. Aneuploid metaphases showed several consistent chromosome rearrangements and striking cytogenetic instability (Figs. 2 and 3). Counts in aneuploid metaphases ranged from 37 to 156 chromosomes (median and modal chromosome counts of 78 and 80, respectively), and each aneuploid metaphase contained unique chromosome abnormalities, in addition to consistent aberrations. The karyotypic description, including only structural aberrations shared by all metaphases, was 37–156,XX, cx,der(1)(:1cen→1q42::1q32→1qter),der(1)(5pter→5p11::1cen→1q42::1q32→1qter),der(4)(?:p12→qter),der(6)(6pter→6q21::1q31→1qter),del(6)(q15q25),del(7)(q11),t(10;17)(q22;12 or 21)[stability grade IV].

Four years previously, this patient had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy following a clinical diagnosis of “22-week fibroid uterus.” The pelvic side walls were unremarkable and the resected uterus, fallopian tubes, and ovaries had a combined weight of 1470 g. The posterior uterine wall was distended by a 20-x 12-x 9.5-cm partially cystic transmural mass which was consistent with a degenerating leiomyoma. Inspection of the uterine cavity revealed diffuse thickening of the endometrium and two endometrial polyps. Microscopy revealed a clinically unsuspected well-differentiated (Grade I, International Federation of Gynecology and Obstetrics) endometrial adenocarcinoma with minimal myometrial invasion (less than 1 mm). The entire endometrial tumor was examined histologically and no evidence of sarcoma was identified. Postoperatively, the patient received adjuvant radiation therapy via a vaginal cylinder which delivered 5000 cGy to the mucosa.

When the pelvic leiomyosarcoma was resected, microscopic sections of the antecedent hysterectomy specimen were reex-

Fig. 1. A, Case 1, leiomyosarcoma involving pelvic sidewall. Broad fascicles of proliferating smooth muscle are interspersed between paucicellular foci. H&E; × 550. B, Case 1, previously resected uterine smooth muscle neoplasm. Histology is virtually identical to pelvic sidewall recurrence. H&E; × 550.
Fig. 2. Karyotype of pelvic leiomyosarcoma (Case 1). This metaphase contains 80 chromosomes, of which 20 are structurally abnormal. Arrows indicate consistent chromosome rearrangements observed in all tumor metaphases; these consistent rearrangements include der(1)(cen-\(\rightleftharpoons\)1q42::1q32-\(\rightleftharpoons\)qter), der(1)(5pter-\(\rightleftharpoons\)5pl1::cen-\(\rightleftharpoons\)1q42::1q32-\(\rightleftharpoons\)qter), der(4)(?::4pter-\(\rightleftharpoons\)4qter), der(6)(6pter-\(\rightleftharpoons\)6qter), del(6)(q15q25), del(7)(q11), and t(10;17)(q22;q12 or 21). Structural rearrangements not designated by arrows (e.g., the 9q+ chromosome) were not present consistently and reflect the genetic instability of this neoplasm.

We have presented cytogenetic and flow cytometric data for a low-grade metastatic uterine leiomyosarcoma (Case 1) and for a large, degenerating, uterine leiomyoma (Case 2). Those data demonstrate that both benign and malignant uterine smooth muscle tumors may have complex cytogenetic aberrations; the data also suggest that certain malignancies may have striking genetic instability despite a low-grade histopathological appearance.
leiomyosarcoma are controversial, several factors, including mitotic rate, histological grade, nuclear atypia, and vascular invasion, can be useful in distinguishing uterine leiomyomas from leiomyosarcomas (1–3, 14, 15). Nonetheless, a minority of uterine smooth muscle tumors appear histologically to be “borderline” lesions which are of uncertain malignant potential. A recent flow cytometric study demonstrated that nuclei of uterine leiomyosarcoma characteristically have aneuploid or polyploid DNA patterns (16). In that study aneuploid/polyploid patterns were also found in several uterine leiomyomas, and 18% of the leiomyosarcomas were diploid. Based on those data, it appears that DNA ploidy alone does not reliably predict biological behavior in uterine smooth muscle tumors. Cytogenetic findings in the low-grade leiomyosarcoma reported here, included aneuploidy, multiple consistent chromosome rearrangements, and a striking component of genetic instability. Those findings, in aggregate, are characteristic of aggressively malignant neoplasms, whereas benign tumors typically have normal karyotypes or noncomplex cytogenetic aberrations (17, 18). The discovery of pronounced genetic instability in the leiomyosarcoma suggested that this tumor was a fully malignant lesion. That suspicion was confirmed by microscopic reassessment of a smooth muscle tumor in a previously resected uterine specimen. Although that uterine tumor was inadequately sampled (three histological sections) originally, the mitotically active foci present on the same slide as the leiomyoma are consistent with a leiomyosarcoma arising within a leiomyoma (15). Had the foci of increased mitotic activity been recognized in the leiomyoma at the time of original diagnosis, further sampling might have yielded diagnostic leiomyosarcoma based on mitotic counts (2). Flow cytometric analysis of nuclear DNA content in this leiomyoma demonstrated a hyperdiploid DNA content comparable to that in the subsequent pelvic leiomyosarcoma.

Cytogenetic aberrations have been described previously in 10 cases of leiomyosarcoma (19–27). The most consistent aberrations, identified in four of those leiomyosarcomas, are rearrangements involving chromosome bands 1p12-13 (19, 22, 24, 27); those rearrangements result in monosomy for the distal portion of 1p. Of interest, the present case contained two distinct derivative chromosomes 1 of this nature. Because del(1p) has been observed in 50% of cytogenetically studied leiomyosarcomas, it is possible that a tumor suppressor gene(s), located on chromosome 1p, might be critical to the genesis of leiomyosarcomas.
and/or progression of certain of these tumors. The prognostic significance of del(1p) in leiomyosarcoma has not been investigated previously, but of the nine leiomyosarcomas with cytogenetic aberrations for which clinical information has been published, all five, including the present case, with del(1p) developed metastases. In contrast, two of the four patients without del(1p) were free of metastatic disease (20, 21). Although those findings suggest that del(1p) might be associated with a poor prognosis in leiomyosarcoma, it must be emphasized that the data have been culled from separate case reports and that the data have been culled from separate case reports.

Only 1 of 10 previously described leiomyosarcomas was a uterine neoplasm (21), and that tumor had a single cytogenetic aberration, 46,XX,t(10;17)(q22.1;p13). Rearrangements involving chromosome bands 10q22 or 17p13 were not seen in any of the nonuterine leiomyosarcomas, but a consistent rearrangement involving 10q22, t(10;17)(q22;q21), was also observed in the metastatic uterine leiomyosarcoma described in this report. Although it would be premature to conclude that 10q22 rearrangements characterize uterine leiomyosarcomas, additional cytogenetic studies of uterine and nonuterine leiomyosarcomas might test this interesting observation.

Several characteristic cytogenetic aberrations have been described recently in uterine leiomyomas. Those events include rearrangements at 12q14-15 and 14q22-24, as well as deletions involving the long arm of chromosome 7 (29-38). The leiomyoma reported herein was characterized by monosomy for the entire long arm of chromosome 7; cytogenetic aberrations were not detected, however, in either copy of chromosomes 12 and 14.

Cytogenetic instability has not been assessed previously in leiomyosarcomas, and the prognostic implications of this finding are unclear. In other varieties of malignancy, however, genetic instability is believed to promote successive mutations which facilitate the acquisition of malignant phenotypic and clinical features (39-41). In this light, it is interesting that the low-grade leiomyosarcoma (Case 1) had “high-grade” cytogenetic instability, whereas the large degenerating leiomyoma (Case 2) did not exhibit instability. It is possible that radiation exposure, 5 years earlier, contributed to the marked cytogenetic instability in the metastatic leiomyosarcoma. It is unlikely, however, that the leiomyosarcoma represented a de novo radiation-induced primary tumor, because radiation-associated leiomyosarcomas are extremely uncommon (42, 43). A literature review revealed cytogenetic studies of 24 additional uterine leiomyomas in which clonal aberrations were detected and for which available data were sufficient for us to assign a cytogenetic stability grade (29-38). Twenty-three of those leiomyomas were stability grade I (29-38), whereas the remaining tumor appeared to be stability grade II or III (36). Of note, this last tumor was a cellular leiomyoma with slight nuclear atypia in which some areas showed 3 to 4 mitoses/10 high-power fields. Accordingly, that tumor might have had borderline malignant potential. In contrast, among nine leiomyosarcomas (19-27, 35-38), two are assigned stability grade I (19, 21), one grade II (22), and one grade III (27). The remaining five cases are assigned grades II-IV, although exact grade cannot be determined from data provided (20, 23, 24-26). These data suggest that smooth muscle tumors of unknown or borderline malignant potential having high-grade cytogenetic instability might manifest aggressive clinical behavior. Accordingly, it is possible that cytogenetic instability will prove to have diagnostic and prognostic utility among uterine smooth muscle tumors.

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