Clonal Chromosomal Aberrations in a Leiomyosarcoma of the Sinonasal Tract

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ABSTRACT: We report the first cytogenetic analysis of a leiomyosarcoma of the sinonasal tract, a rare neoplasm. Karyotypic analysis showed near-triploid and near-tetraploid modal chromosome numbers with extensive structural and numerical aberrations. Three consistent structural changes, including i(6p), der(10)ins(10;1)(q26;q23q44), and der(12)t(1;12)(q11;q24) were observed in most cells. A der(11)t(11;?) (p15;?) was observed in 14 of 20 cells. Clonal structural rearrangements, including i(1q), del(2)(q37), der(3)t(3;?) (p25;?), del(4)(q31), del(7)(q32), der(12)t(12;?) (p12;?), der(15), del(21)(q22), and der(X) were each observed in a few cells. Numerical changes, including trisomies for chromosomes 2-5, 7, 9, 11, 15, 17, 18, and 20 and monosomies 10 and 12 were observed. Comparison of our findings to those of leiomyosarcomas at different sites showed trisomies 7 and 20 and rearrangements of 11p12-p15 and 21q22.

INTRODUCTION
Leiomyosarcomas account for approximately 7% of all soft tissue sarcomas and can occur in many organs [1]. They are most frequent in the gastrointestinal tract and uterus and are less common in somatic soft tissues and skin. To date, cytogenetic findings have been reported in 27 cases of leiomyosarcoma from various anatomic sites, including the small bowel, prostate, uterus, and retroperitoneum [2-9]. Although leiomyosarcomas of these different sites are histologically similar, no consistent cytogenetic abnormalities have been reported [5]. Rearrangements of three chromosomal regions, 1p12-13 [3-5, 9], 11p12-15 [5, 6, 9-11], and 21q22 [2, 11-13], monosomies for chromosomes 9, 14, 18, and/or 22 and trisomies 7 and 20 have been reported in other cases [2-6, 9, 10]. We present the first cytogenetic analysis of a leiomyosarcoma of the sinonasal tract.

MATERIALS AND METHODS
Clinical Summary
T. G. was a 32-year-old white woman in whom in April 1990 blurred vision developed in the left eye. She was evaluated by magnetic resonance imaging (MRI) in July 1990 and diagnosed as having a tumor of the sphenoid sinus and clivus. In July 1990, she underwent nasal endoscopic biopsy and partial tumor removal followed by a 5 1/2-week course of external beam radiotherapy that ended in August 1990. MRI showed no change in tumor appearance.

We first saw the patient in November 1990. She was alert, oriented, and moving all extremities well. Her cranial nerve deficit was limited to decreased vision in the left eye (light perception only). Her left pupil responded to light, and corneal sensation was intact. There was no limitation to globe movement.

In December 1990, she underwent resection of the sphenoid sinus and clival tumor as well as left optic nerve decompression. Grossly, the tumor was soft, friable, and brownish. Postoperatively, she did well and was scheduled to receive chemotherapy from her local physician. In June 1991 (6 months after last operation), she died suddenly of a suprasellar recurrence.

Histopathology
Microscopically, the tumor was cellular and composed of interlacing fascicles of spindle-shaped cells with pink cytoplasm and elongated, vesicular to hyperchromatic nuclei with blunt ends (Fig. 1). Mitoses were prominent and averaged 10 in each high-power field (× 400). On immunostaining, the tumor cells were positive for muscle-specific actin and desmin and negative for cytokeratin and S-100 protein.

Cell Culture and Cytogenetics
The tumor specimen was collected under semisterile conditions in the surgical pathology laboratory and placed in α-minimum essential medium (α-MEM, described below). The tissue was then washed in initial wash medium, consisting of 250 U/ml penicillin, 250 μg/ml gentamicin, 50
Figure 1  (A) Medium power view of the leiomyosarcoma showing interlacing bundles of smooth muscle cells. (Hematoxylin-eosin, original magnification ×315.)  (B) High-power view showing cells with elongated nuclei with blunt ends; mitotic activity is evident. (Hematoxylin-eosin, original magnification ×500.)

μg/ml amphotericin B, 100 μg/ml clindamycin, and 50 μg/ml chloramphenicol in Hanks' balanced salt solution (HBSS, Irvine Scientific, Santa Ana, CA) [14], and finely minced aseptically in a Petri dish. The cultures, labeled PCI:SG467, were initiated in 25-cm² flasks containing α-MEM (Earle's salts) with nucleosides supplemented with 15% fetal bovine serum, 43 μg/ml gentamicin, and 2 mM L-glutamine (Irvine Scientific). Cultures were periodically subcultured on reaching confluency by detaching the cells with trypsin/EDTA. The first successful cytogenetic harvest of PCI:SG467 was achieved at passage 3, 30 days after culture initiation. The cells were treated with 0.1 μg/ml Colcemid (GIBCO/BRL) and 10 μg/ml ethidium bromide (Fisher Biotech, Pittsburgh, PA) for the last hour before harvest. The cells were then subse-
quently trypsinized with trypsin/EDTA solution (0.25 g/0.1 g/L) and treated with prewarmed hypotonic solution KCl/HEPES/EDTA [15] for 20 minutes at 37°C. Air-dried chromosome preparations were prepared according to routine procedures, baked for 20 minutes at 90°C, and trypsin-Giemsa banded. Twenty metaphases were analyzed, photographed, and karyotyped. Clonal aberrations are defined as numerical gains or structural abnormalities observed in two or more cells and/or numerical losses observed in three or more cells. Consensus findings are those present in at least 50% of cells analyzed.

RESULTS

A detailed karyotypic analysis of 20 metaphase cells from passage 3 showed 14 cells with near-triploid (range 50–82) and six cells with near-tetraploid (range 97–149) chromosome numbers. No karyotypically normal cells were observed. The consensus karyotype was 69,XX, + 2, + 3, + 4, + 5, + i(6p)x 2, + 7, + 7, + 9, + 9, - 10, + der(10)ins(10;1)(q26;q3q44)x 2, + 11, + der(11)t(?;11)(?;p15), - 12, + der(12)t(1;12)(q11;q24)x 2, + 15, + 17, + 18, + 20, + 20, + 20, + mar1, + mar. Three major clonal structural findings, including i(6p), der(10)ins(10;1)(q26;q3q44), and der(12)t(1;12)(q11;q24) were observed in 17 of 20 metaphases (Figs. 2 and 3). A der(11)t(?)(p15;?) was observed in 14 of 20 cells. In addition to these arrangements, clonal structural aberrations, including i(1q), del(2)(q37), der(3)t(3;?)(p25), del(4)(q31), del(7)(q32), r(8), der(12)t(12;?)(p12;?), der(15), del(21)(q22), and der(X) were observed (Fig. 4). These findings were observed in less than 50% of cells and therefore were not included in the consensus karyotype.

DISCUSSION

Of the 27 cases of leiomyosarcoma characterized cytogenetically, seven tumors showed normal karyotypes [3–5] whereas clonal abnormalities were reported in 16 cases [3–7, 11] and
Figure 3  Partial karyotypes from nine different metaphase cells showing the three major clonal abnormalities, i(6p), der(10)ins(10;1)(q25;q23q44), and der(12)t(1;12)(q11;q24).

four cases expressed nonclonal abnormalities. Although most cytogenetic abnormalities observed in these tumors were tumor-specific, several findings were noted in multiple tumors. One such abnormality was monosomy for the distal short arm of chromosome 1 resulting from rearrangements involving breakpoint 1p12-13 [3–5, 9]. Fletcher et al. [8] suggested that since the del(1p) is observed in many cases, a tumor suppressor gene(s) located in this region may be responsible for development of these tumors. We observed an i(1q) in 3 of 20 cells. Therefore, because most cells had two apparently normal chromosomes 1, chromosomal loss of heterozygosity for 1p does not adequately explain tumor formation in our case. Nilbert et al. [6] proposed that a rearrangement of chromosome 11 involving 11p15 may also be responsible for tumor growth by an inactivation mutation or loss of heterozygosity. Other investigators report rearrange-
Figure 4 Representative karyotype showing some of the less frequent clonal abnormalities. 71,XX, + 2, + 3, + 3, + der[5], i(6p), + i(6p), + 7, + 7; der[10ins(10;1)[q26;q23][q44]] × 2, + der[11][t(11;?)[p15;?]] × 2, - 12, + der(12)[t(12;?)[q11;q24]] × 2, + der(13)[p12], + der(15)[t(15;?)[p11;?]] × 2, - 17, - 18, + del(20)[t(20;?)][q13;?] × 2, - r, + mar2 × 4, + mar. Arrowheads indicate abnormal chromosomes. U, unique nonclonal marker chromosomes; r, ring chromosome; m2, clonal marker chromosome.

ments of 11p12-p15 [5, 6, 9-11]. We observed a rearrangement at 11p15 in our case. Other findings in common with cases in the literature include trisomy 7 in a leiomyosarcoma of the small bowel [5] and trisomy 20 in leiomyosarcomas of the knee [12] and stomach [5, 10]. In addition, Pathak and Dhaliwal [13] suggested that a deletion of the long arm of chromosome 21 is associated with a subgroup of leiomyosarcomas [2, 11-13]. The observation of five of 20 cells in our case with del(21)[q22] supports this suggestion. Boghosian et al. [1] classified leiomyosarcomas into three subgroups based on cytogenetic features, including 1) a pseudodiploid chromosome number, 2) a hypodiploid chromosome number with consistent chromosomal losses, and 3) miscellaneous findings. Although the case we report is characterized by chromosomal losses described in their category 2, its hyperdiploid nature places it in category 3. Consistent cytogenetic abnormalities, including del(1)[p13], rearrangements at 11p15 and 21q22, and trisomies 7 and 20 appear to be emerging as key findings in leiomyosarcoma, although clear-cut definition of their significance requires examination of a larger population of tumors at each site and correlation with disease stage, response to therapy, and clinical outcome.

This project was funded by the Pittsburgh Cancer Institute. Suguna Sankary was supported by a Government of India Postdoctoral Fellowship. The authors thank Drs. Robert E. Ferrell and Ronald B. Herberman for continued support.

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