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Case report

Cytogenetic study of botryoid rhabdomyosarcoma of the uterine cervix

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Summary. We report a case of sarcoma botryoides of the uterine cervix occurring in a 19-year-old woman. By light microscopy the tumor showed round and spindle cells with hyperchromatic nuclei and, focally, a cambium layer subjacent to the surface epithelium and surrounding endocervical glands. Strap-shaped cells with and without cross-striations and small foci of immature cartilage were also present. Immunohistochemical studies showed positive staining within the tumor cells for myoglobin, desmin, vimentin, muscle-specific actin and CD56. By electron microscopy, tumor cells showed cytoplasmic filaments in an alternating pattern of thick and thin filaments. Chromosomal analysis demonstrated deletion of the short arm of chromosome 1, and trisomies 13 and 18. To our knowledge, this is the first reported case of sarcoma botryoides of the endocervix with chromosomal analysis.

Key words: Botryoid rhabdomyosarcoma – Uterine cervix – Immunohistochemistry – Ultrastructure – Chromosomal analysis

Introduction

Sarcoma botryoides, a subtype of embryonal rhabdomyosarcoma, affects the uterine cervix in young women. It is rare and few cases have been reported in the literature (Montag et al. 1986; Brand et al. 1987; Daya and Scully 1988). Like their counterparts which occur in the vagina of infants, these tumors are characteristically composed of polypoid masses resembling bunches of grapes. Microscopically they show a cambium layer subjacent to the surface epithelium, and primitive rhabdomyoblasts including strap-shaped cells with cross striations.

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To our knowledge, the botryoid type of embryonal rhabdomyosarcoma has not been characterized cytogenetically, while cytogenetic analysis of alveolar rhabdomyosarcoma (AR) has demonstrated a characteristic translocation t(2; 13) in the majority of cases (Turc-Carel et al. 1986; Douglass et al. 1987). Embryonal rhabdomyosarcoma (ER) shows various chromosomal changes but lacks the t(2; 13) which appears to be restricted to the alveolar type (Wang-Wuu et al. 1988).

In this report, we have characterized sarcoma botryoides using immunohistochemistry and electron microscopy. We also describe the cytogenetic findings from cultured tumor cells. Several chromosomal alterations were seen including deletion of the short arm of chromosome 1, and trisomies 13 and 18. There was no evidence of the t(2; 13) translocation observed in alveolar rhabdomyosarcoma.

Case report

The patient, a 19-year-old (gravida I; para O) black female, presented with menorrhagia over several weeks. Her past history was significant for a currettage for an incomplete abortion. Family history revealed a sister diagnosed with ovarian carcinoma at the age of 22 years. Physical examination was remarkable only for a large 2×2 cm, friable, necrotic cervical mass. A biopsy demonstrated sarcoma botryoides. A computed tomography scan showed no adenopathy or intra-abdominal disease. A magnetic resonance imaging scan revealed an exophytic, enhancing mass arising from the inferior surface of the cervix. Cystoscopy, proctoscopy and examination under anesthesia confirmed stage IB disease. The clinical impression was that cervical conization would result in inadequate resection, and the patient underwent total abdominal hysterectomy and pelvic lymphadenectomy. She is currently receiving vincristine, actinomycin D and cyclophosphamide chemotherapy.

Pathology

The uterus weighed 72 g. On gross examination, there was an endocervical pedunculated polypoid mass protruding through the cervical os and firmly attached to

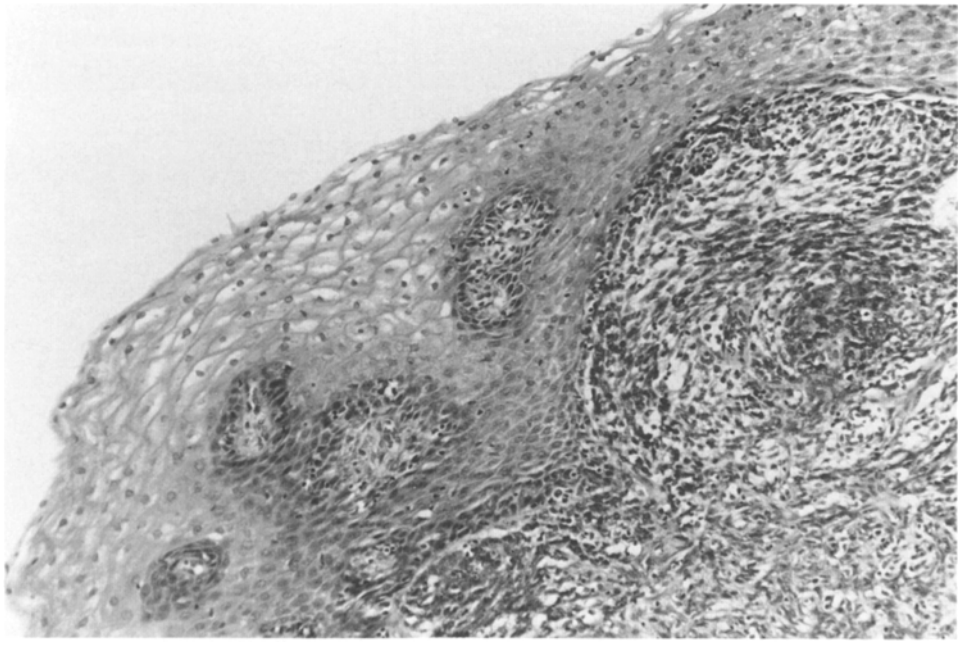

Fig. 1. Squamous cervical epithelium overlying hypercellular areas with a focally prominent cambium layer composed of spindle cells. H&E, ×160

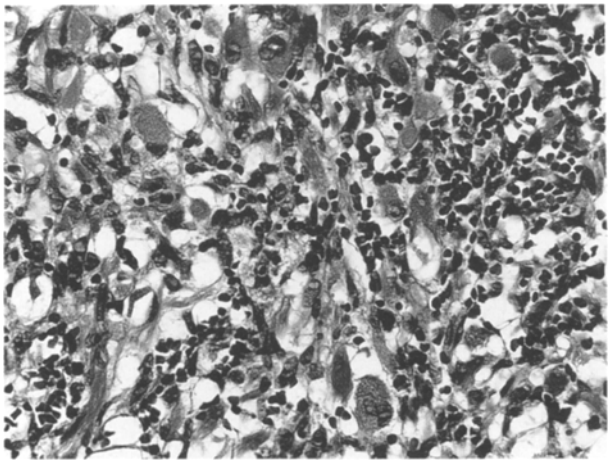

Fig. 2. Rhabdomyoblasts showing ample cytoplasm, some with cross striations. H&E, ×200

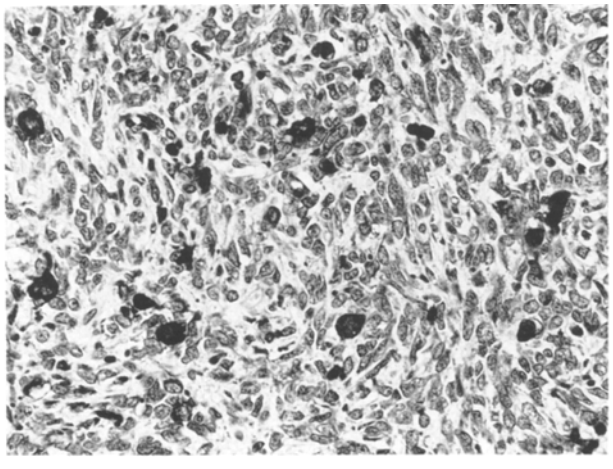

Fig. 3. Immunoperoxidase stain for myoglobin showing cytoplasmic reactivity in neoplastic cells. ×200

the endocervical wall. The mass measured 3.0 × 1.8 cm and was soft, focally ulcerated and hemorrhagic. The macroscopic examination of the uterus was otherwise unremarkable. By light microscopy, the tumor was covered by benign stratified squamous epithelium and normal endocervical lining epithelium, except where ulcerated. Focally evidence of a cambium layer was seen, composed of spindle cells with an edematous stroma underneath the surface epithelium (Fig. 1), but the cambium layer was best seen around endocervical glands and blood vessels. Tumor cells were seen between entrapped endocervical glands and occasional foci of immature cartilage were present. The tumor cells were round with scanty cytoplasm. In places strap-shaped cells with eosinophilic cytoplasm and cross striations were also identified (Fig. 2). The mitotic count in the more cellular areas ranged from two to five per ten high-power fields. Metastatic tumor was present in one of five iliac lymph nodes excised. Immunohistochemical studies using the standard three-step indirect avidin-biotin-peroxidase method (Hsu et al. 1981) showed that the tumor cells stained positively for myoglobin (Fig. 3), desmin (Dakopatts, Carpinteria, Calif., USA), muscle-specific actin (Enzo Diagnostics, New York, N.Y., USA), and CD56 (Becton-Dickinson, San Jose, Calif., USA). The cells were negative for cytokeratin (Becton-Dickinson), S-100 protein, alpha smooth-muscle actin and neurone-specific enolase (Dakopatts). The vimentin was focally weakly positive. By electron microscopy, cultured tumor cells showed alternating thick and thin filaments and cytoplasmic glycogen granules.

Chromosome analysis

Portions of the tumor and normal myometrium were placed in a sterile culture medium for chromosomal stu-

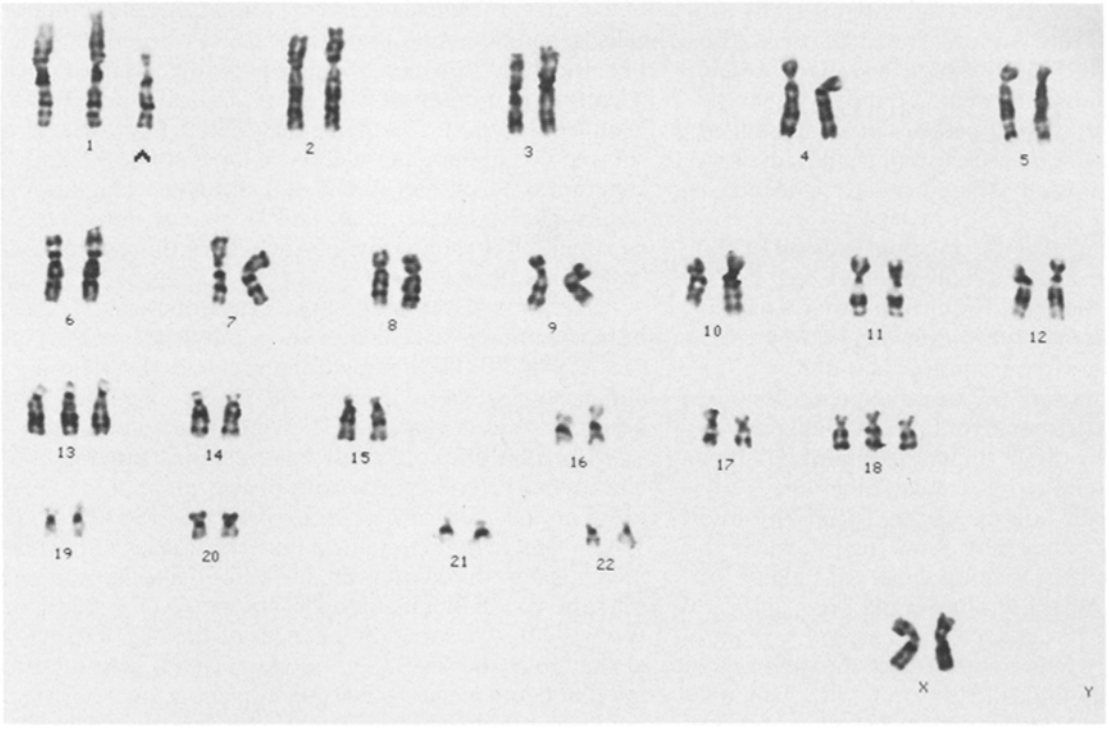

Fig. 4. G-banded karyotype of the stem-line showing trisomies for chromosomes 13 and 18. The extra copy of chromosome 1 with a short arm deletion is indicated by the *arrow*

dies. Tissue was minced with small curved scissors on a Petri dish and transferred into centrifuge tubes containing 0.8% solution of collagenase II (Sigma, St. Louis, Mo., USA) in complete culture medium. The tubes were placed on a Hema-Tek mixer at 37° C and rocked gently for 3 h. The resulting cell suspension was spun down at 1000 rpm, the supernatant discarded and the cells re-suspended in RPMI 1640 medium substituted with 20% fetal calf serum, 100 μg/ml gentamycin and L-glutamine. The cells were seeded on 2 × 2 cm coverslips in 32 mm Petri dishes, as described for amniocyte cultures (Verma and Babu 1989). All cultures were harvested after 4–5 days by standard methods described for amniocyte cultures. A hypotonic treatment was performed using 0.5% sodium citrate at room temperature followed by in situ fixation with a mixture of methanol and acetic acid (3:1). Chromosome spreading was facilitated by a brief exposure to hot steam. G-banding was performed by conventional methods using trypsin pretreatment and Giemsa staining. The karyotypes were prepared on a Cytoscan automatic karyotyping system (Image Recognition Systems, Pittsburgh, Pa., USA) and described according to the International System for Human Cytogenetic Nomenclature (1985). The modal chromosome number of the tumor was 49. All metaphases showed a pericentric inversion of chromosome 9, trisomies for chromosomes 13 and 18, and an extra copy of chromosome 1 with a large deletion of the short arm (Fig. 4). A side-line showing trisomies for chromosomes 2 and 8, in addition to the above abnormalities, was also identified. The pericentric inversion of chromosome 9 was of the type commonly observed in normal individuals and was interpreted as a constitutional polymorphism rather than an acquired, tumor-associated abnormality. All abnormalities were observed in multiple culture vessels. No normal metaphases were identified in cultures of the tumor. The karyotype of the tumor was interpreted as 49, XX, inv(9) (*p*11*q*13), +del(1) (*p*12), +13, +18 [18]/51, XX, in(9) (*p*11*q*13), +del(1) (*p*12), +2, +8, +13, +18 [5]. Cytogenetic analysis of cultures established from the normal myometrium demonstrated a 46, XX, inv(9) (*p*11*q*13) karyotype, consistent with phenotypically normal female.

Flow cytometry for DNA content was performed on a Becton-Dickinson FAscan analyser (Becton-Dickinson, Sunnyvale, Calif., USA). The nuclei were stained with propidium iodide, and normal human lymphocytes were used as a diploidy reference. The histogram showed a single G_1 peak with a DNA index of 1.15 when compared with the reference value. A small G_2/M peak was also present. The coefficient of variation of the G_1 peak was 4.2%.

Discussion

The present case showed the typical macroscopic and light microscopic features of ER of the sarcoma botryoides subtype. This tumor has been classically described as originating below an epithelial surface and differentiating toward skeletal muscle; its immunohistochemical profile is characteristic of ER. The ultrastructural findings are supportive of a striated muscle origin and the presence of positive staining for myoglobin and

alternating thin and thick filaments ultrastructurally are also characteristic of this tumor. The cells were also strongly positive for CD56, a cell surface glycoprotein expressed in all human natural killer cells (Mechtersheimer et al. 1991). It is also present in fetal striated muscle, regenerating skeletal muscle and rhabdomyosarcoma but is absent in adult striated muscle (Mechtersheimer et al. 1991).

The differential diagnosis of polypoid masses of the endocervix includes benign and malignant lesions. Within the benign group, the most important differential diagnosis is pseudo-sarcoma botryoides, which can grossly appear very similar to sarcoma botryoides and is often associated with pregnancy. Of the malignant lesions, adenosarcoma, carcinosarcoma and mixed müllerian tumor (MMT) have to be differentiated from sarcoma botryoides. The former tend to occur in an older age group and usually involve the uterine corpus primarily and the cervix secondarily. They may show foci of cartilaginous tissue but an epithelial component is a feature of MMT and adenosarcoma, not of sarcoma botryoides.

A recent review of 13 cases of endocervical sarcoma botryoides in young women stated that these patients had a better prognosis than did children with sarcoma botryoides of the vagina (Daya et al. 1988). One of these women, with deep myometrial invasion of the uterine wall, died. In another report of endocervical sarcoma botryoides in a young woman the outcome was fatal despite surgery and chemotherapy (Perrone et al. 1990).

A non-random chromosomal translocation t(2; 13) ($q35$; $q14$) is known to occur in the majority of cases of AR (Seidel et al. 1982; Turc-Carel et al. 1986; Douglass et al. 1987). Initial reports of similar translocation in ER have not been substantiated by later reports, and it now appears that t(2; 13) is a specific marker for AR (Wang-Wuu et al. 1989). Variant translocations involving chromosome 13 have been recently documented in several cases, suggesting that loci at band $13q14$ are critical in the development of AR (Douglass et al. 1991). Much less is known about chromosomal rearrangements associated with ER. No consistent structural abnormality has been documented so far in this tumor type. However, Wang-Wuu et al. (1988), observed several non-random numerical abnormalities, including trisomies for chromosomes 2, 8, 13 and 20. A combination of trisomies 2 and 13 was observed in the majority of ER cases in this series. Trisomy 2 was also observed in a case of AR which was t(2; 13) negative. In this context it is of interest that trisomy 2 is a frequently observed abnormality in hepatoblastoma, another type of embryonal malignancy which occasionally shows rhabdomyoblastic differentiation (Fletcher et al. 1991). There are only a few cytogenetic studies of MMTs of the uterus in the literature and no recurring chromosomal changes have been reported. These tumors are typically characterized by complex karyotypic changes with both numerical and structural rearrangements (Milatovitch et al. 1990; Nilbert et al. 1990; Emoto et al. 1992). However in some tumors simple structural and numerical abnormalities have been observed (Harker et al. 1983; Emoto et al. 1992). In the case reported by Emoto et al. (1992) trisomy 8 was the sole abnormality present in the tumor cells. It is interesting that trisomy 8 is also common in ER and has been described in hepatoblastoma, albeit usually in combination with other abnormalities (Wang-Wuu et al. 1988; Fletcher et al. 1991). Our case also showed a side-line characterized by trisomies 2 and 8 superimposed on the stem-line karyotype. The suggestion made by Emoto et al. (1992), that trisomy 8 may be a marker of rhabdomyoblastic differentiation certainly deserves further study.

Deletions of the short arm of chromosome 1 have been previously described in rhabdomyosarcoma (Trent et al. 1985). Both trisomy 13 present in the stem-line, and trisomies 2 and 8 observed in the side-line, have been observed in cases of ER (Wang-Wuu et al. 1988).

Molecular studies of ER have demonstrated consistent loss of heterozygosity with probes mapping to band $p15.5$ on the short arm of chromosome 11 (Scrable et al. 1987). This region contains a cluster of genes important in myogenic differentiation including MyoD1 and the M subunit of lactate dehydrogenase (LDH) (Scrable et al. 1990). AR shows no changes of the $11p15.5$ region at the molecular level (Scrable et al. 1989). It is interesting that cytogenetic studies failed to show any consistent involvement of the short arm of chromosome 11 in ER. Clearly, chromosomal changes are only one facet of the complex genetic changes leading to the development of rhabdomyosarcoma.

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