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Mutation of the INI1 gene in composite rhabdoid tumor of the endometrium

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Abstract

Composite rhabdoid tumors are typically adult tumors that contain a component of rhabdoid cells, which are characteristic of the aggressive childhood malignant rhabdoid tumor. Pediatric rhabdoid tumors are characterized by the inactivation of the hSNF5/INI1/SMARCB1 gene, with subsequent loss of expression of the protein. In contrast, only a single composite rhabdoid tumor has demonstrated involvement of the INI1 gene. In our study, INI1 protein expression was studied in two uterine carcinosarcomas with rhabdoid components (composite rhabdoid tumors). The rhabdoid component of one tumor showed lack of immunoreactivity for the INI1 protein and strong positivity for cyclin D1, while the adenocarcinomatous component of the tumor and both components of the second tumor were immunoreactive for the INI1 protein and negative for cyclin D1. Loss of one INI1 allele and a mutation in exon 7 of the remaining allele were detected in the first tumor, consistent with the immunohistochemistry results. Our results demonstrate that deletions and mutations of the INI1 gene can occur also in rare composite rhabdoid tumors of adulthood. Further studies are necessary, however, to determine the prognostic significance of this finding.

Keywords

INI1 gene; composite rhabdoid tumor; uterine carcinosarcoma; cyclin D1

Introduction

The INI1/SMARCB1 gene at chromosomal band 22q11.2 encodes a member of the SWI/SNF chromatin remodeling complex. This complex is a negative regulator of the cell cycle, modulates cytoskeleton organization and functions as a tumor suppressor gene (1-6).

Homozygous deletion of both alleles of the INI1 gene or deletion of one allele and mutation of the second allele result in the development of rhabdoid tumors of the kidney, central nervous system, soft tissue or liver in infants and young children (2,6-9). The majority of mutations are

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single base pair nonsense mutations, and frameshifts that introduce a novel stop codon, and predict a prematurely truncated protein. (9). In practice however, the INI1 protein is not detectable, and a virtually diagnostic feature of these pediatric rhabdoid tumors is their lack of immunoreactivity with monoclonal antibodies against the INI1 protein (10-12).

Tumors that are morphologically similar to pediatric rhabdoid tumors can also develop in adult patients, often in tumors of diverse histogenesis (carcinomas, sarcomas, melanomas, gliomas, meningiomas) (13-21). These tumors, in contrast to pediatric rhabdoid tumors, typically retain the INI1 gene, and are immunoreactive with antibodies to BAF47 (22). The molecular pathogenesis of these tumors is not known. Neither deletion nor mutation of the INI1 gene was present in a disseminated uterine rhabdoid tumor that was likely of endometrial origin (15). In a recent study of 40 composite rhabdoid tumors, the majority of them arising in adult patients, loss of immunoreactivity for the INI1 protein was found only in a single tumor, a rhabdoid component of a leiomyosarcoma (22). For technical reasons, it was not possible to determine whether the INI1 gene was mutated but no deletion of 22q was detected in the tumor by fluorescence in situ hybridization with a probe to the BCR gene, which is proximal to INI1 within 22q11.2 (21,22).

We present a case of endometrial composite rhabdoid tumor with deletion of one copy of the INI1 gene, mutation of the second allele, and lack of immunoreactivity with the BAF47 antibody, the features typical for rhabdoid tumors. In contrast, the second endometrial tumor was immunopositive with antibody BAF47, and thus has presumptively a distinct molecular etiology.

Report of Cases

Case 1—A 51-year-old woman presented with a 12.0 × 7.5 × 2.5 cm hemorrhagic and partially necrotic fleshy tan endometrial mass with pushing border invading 80% of the myometrial thickness. Hysterectomy and bilateral salpingo-oophorectomy were followed by radiation therapy. She is alive, free of disease, 14 months after surgery.

Case 2—A 71-year-old woman presented with a 7.0 × 5.0 × 1.5 cm hemorrhagic pale tan endometrial mass that invaded 50% of myometrial thickness, extended to the external cervical os and the right fallopian tube. The tumor recurred in the upper portion of the vagina six weeks after hysterectomy and bilateral salpingo-oophorectomy and was treated by radiation therapy. A 6.0 × 5.5 × 3.1 cm lobulated, hemorrhagic tan metastatic mass to the incision site of the abdominal wall was noted two months after surgery. She died two months later in an outside hospital, reportedly free of tumor.

Pathological Findings

Case 1—The tumor was composed of three distinct components – an adenocarcinoma of endometrium, a keratinizing squamous cell carcinoma and a rhabdoid component. The adenocarcinoma was poorly differentiated, formed solid sheets but also contained glandular areas. The nests of squamous cells were surrounded by sheets of poorly differentiated adenocarcinoma, a fact indicative of their metaplastic origin. The rhabdoid cells were positive for vimentin, focally for epithelial membrane antigen and negative for keratin (AE-1/AE-3), desmin and INI1 protein, while both carcinomatous components were INI1 protein positive (Figure 1). While 5% of the adenocarcinoma cells were moderately positive for cyclin D1, 60-70% of the rhabdoid cells and all metaplastic squamous cells were strongly positive.

Case 2—The tumor was composed of two distinct components – an adenocarcinoma of endometrium and a rhabdoid component. The adenocarcinoma was also poorly differentiated, formed solid sheets, but contained glandular areas as well. The abdominal wall metastasis was

composed solely of the rhabdoid cells. The rhabdoid cells were positive for vimentin, CD10 and were, together with the adenocarcinomatous component, positive for INI1 protein. They were negative for keratin (AE-1/AE-3, CK7, CK 20), epithelial membrane antigen, epithelial cell adhesion molecule BER EP4, desmin, smooth muscle actin, S100 protein and glial fibrillary acidic protein. Approximately 10-30% of the adenocarcinomatous cells and 5% of the rhabdoid cells were weakly or moderately positive for cyclin D1 (Figure 2). They contained characteristic paranuclear whorls of intermediate filaments when examined by electron microscopy.

Interphase fluorescence in situ hybridization

Sections from a paraffin block containing the rhabdoid component of case 1 were used for interphase FISH according to established protocols (9). Loss of one copy of the INI1 locus as well as the EWS locus was detected in 22% of cells, while both loci were retained in the remaining cells (Figure 3). The findings are consistent with monosomy 22 in a population of the tumor.

Molecular genetics

Genomic DNA was extracted from a paraffin block of case 1 containing the rhabdoid component using the Puregene kit (Gentra Systems, Minneapolis, MN). Exons 1-9 of the INI1 gene were amplified by polymerase chain reaction as previously reported (7). Sequencing was performed on an automated ABI sequencer and the sequences were compared to the normal control sequence for the INI1 gene (1). A single guanine deletion at one of four bases (947-950) in either codon 316 or 317 in exon 7 was identified. This mutation is predicted to result in a frameshift and a novel stop codon after codon 318 (Figure 4).

Discussion

Our results show a similar mechanism of tumorigenesis between a minority of composite rhabdoid tumors of adulthood and classic pediatric rhabdoid tumors. Loss of one INI1 allele and mutation of the remaining allele were followed by the development of the rhabdoid tumor component. The mechanisms of tumorigenesis in the majority of composite rhabdoid tumors remain poorly elucidated. The rhabdoid components in these tumors are, in contrast to pediatric rhabdoid tumors, typically immunoreactive with the BAF47 antibody. Deletions of 22q were detected in 10/16 rhabdoid meningiomas (22) but deletions or losses of 22q are characteristic for meningiomas regardless of their histologic appearance (23). Deletion of 22q was detected in a case of rhabdoid carcinoma of the small intestine, but this tumor remained immunoreactive for the INI1 protein (22). Composite rhabdoid tumors of endometrium (carcinosarcomas with rhabdoid tumor components) are rare and clinically aggressive. Only four cases have been reported (24-26). The epithelial derivation of the rhabdoid components was demonstrable in two tumors by immunohistochemistry. The remaining two tumors expressed only vimentin. None of the four tumors was studied for the expression of the INI1 gene.

Recent studies have shown that cyclin D1 is overexpressed in rhabdoid tumors with inactivated INI1 (3,27). Overexpression of cyclin D1 in the composite endometrial rhabdoid tumor with an inactivated INI1 gene, in contrast to low expression of cyclin D1 in the tumor with expressed INI1, was also consistent with the molecular etiology of this tumor. Cyclin D1 plays a key role in cell cycle regulation during the G1 to S phase transition by binding to cyclin-dependent kinases 4 and 6, resulting in phosphorylation and inactivation of the tumor suppressing retinoblastoma protein RB and entrance of cells to S phase (28-30). The role of cyclin D1 as a key mediator in the genesis of rhabdoid tumors was supported by a study of mice with disrupted *Ini1* and cyclin D1 genes (31). *Ini1*^{+/-} mice with cyclin D1 deficiency did not develop rhabdoid tumors, in contrast to the parental *Ini1*^{+/-} mice, supporting the hypothesis that cyclin D1 is necessary for tumorigenesis. Therapeutic targeting of cyclin D1 induced G1 arrest and

apoptosis in rhabdoid cell lines and resulted in growth inhibition of xenografted rhabdoid tumors (31). Although specific targeting of cyclin D1 in human tumor cells is not yet feasible, this approach may offer a new strategy for treatment of pediatric rhabdoid tumors and for composite rhabdoid tumors with inactivated INI1 genes.

Based on the limited number of cases studied, it is not possible to predict prognosis based on the INI1 status of composite rhabdoid tumors. Further studies of a variety of pediatric and adult tumors with rhabdoid histology will be required to determine if inactivation of INI1 is always associated with an aggressive clinical course.

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Figure 1.
Case 1 -- Note abrupt transition of adenocarcinoma and rhabdoid tumor components (A).
Rhabdoid tumor component (B), its negativity for INI1 protein (C) and strong positivity for cyclin D1 (D).

Figure 2.
Case 2 -- Note abrupt transition between adenocarcinoma and rhabdoid tumor components (A). Rhabdoid component (B), its expression of INI1 protein (C) and lack of overexpression of cyclin D1 (D).

Figure 3. Interphase FISH of the rhabdoid component from case 1. The INI1 probe is labeled in red, and is present in one copy. The control probe (green) from the EWS locus in 22q12 is also deleted, consistent with monosomy 22.

Exon 7 mutation in the INI1 gene

Figure 4.

Mutation of the INI1 gene in case 1. A deletion of one guanine in either codon 316 or 317 causes a frameshift, and the introduction of a novel stop codon after amino acid 318.