Tumor-Specific Cooperation of Retinoblastoma Protein Family and Snf5 Inactivation

Jingjing Chai, Xiadongdong Lu, Virginia Godfrey, Christopher Fletcher, Charles W.M. Roberts, Terry Van Dyke, and Bernard E. Weissman

Departments of Pathology and Laboratory Medicine, Genetics, and Pediatrics and UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina; Department of Pathology, Brigham and Women's Hospital; Department of Pediatric Oncology, Dana-Farber Cancer Institute, Children's Hospital Boston and Harvard University, Boston, Massachusetts

Abstract
Malignant rhabdoid tumors (MRT) are rare aggressive cancers that occur in young children. Seventy-five percent of sporadic MRTs harbor inactivating SNF5 mutations, and mice heterozygous for an Snf5-null allele develop MRTs with partial penetrance. The diagnosis of choroid plexus carcinomas (CPC) in addition to MRTs in families with a single mutant SNF5 allele prompted us to assess the role of SNF5 loss in CPC in genetically engineered mice. With high frequency, TgT121 mice develop CPCs that are initiated by inactivation of retinoblastoma protein (pRb) and related proteins p107 and p130. However, CPC penetrance and latency were not significantly affected by Snf5 heterozygosity, consistent with recent evidence that CPCs in SNF5 families were, in many cases, misdiagnosed MRTs. Surprisingly, although the CPC phenotype was unaffected, TgT121Snf5+/− mice developed MRTs with increased penetrance and decreased latency compared with TgT121Snf5−/− littermates. MRTs expressed the T121 protein with a concomitant increase in mitotic activity. The predominant appearance of TgT121Snf5−/− MRTs in the spinal cord led to the discovery that these tumors likely arose from a subset of spinal cord neural progenitor cells expressing T121 rather than from transdifferentiation of CPC. Significantly, the target cell type(s) for MRT is unknown. Hence, this study not only shows that pRb and SNF5 inactivation cooperate to induce MRTs but also provides new insight into the MRT target population. [Cancer Res 2007;67(7):3002–9]

Introduction
Malignant rhabdoid tumors (MRT) are highly malignant pediatric renal and extrarenal tumors with unique cellular characteristics. Rhabdoid cells have large vesicular nuclei, a prominent single nucleolus, and globular eosinophilic cytoplasmic inclusions. The most frequent extrarenal site of MRTs is the central nervous system (CNS), and approximately 10% to 15% of patients with renal MRTs develop independent primary tumors of the CNS (1, 2). MRTs are extremely aggressive, with most patients perishing within 1 year after diagnosis (3, 4). Therefore, new treatment approaches for this disease remain a high priority.

Because MRTs typically consist of poorly differentiated cells with a few admixed rhabdoid cells, diagnosis based on histology alone proves difficult. However, recent studies have shown that virtually all MRTs lose expression of the SNF5/INI1/BAF47 gene, providing a definitive diagnostic marker (5–7). The SNF5 gene, which encodes the smallest member of the SWI/SNF chromatin remodeling complex, localizes to 22q11.2. The SNF5 protein interacts with many transcription regulators and viral proteins, such as c-MYC, p53, and HIV-1 (8–10). The SNF5 protein is highly conserved among different species with complete identity between mouse and human proteins (11).

In mice, Snf5 deficiency results in embryonic lethality by E6.5, whereas ~15% of Snf5−/− mice develop rhabdoid-like tumors at 8 to 10 months of age (12–14). Conditional Snf5 inactivation results in complete bone marrow aplasia, leading to anemia, hemorrhage, and death of most mice 1 to 3 weeks after induction (14). Furthermore, SNF5 seems essential for hepatocyte differentiation based on the phenotype of hepatocyte-specific Snf5 deletion in vivo (15). These data show that Snf5 not only is a MRT tumor suppressor but also plays a critical role in both organ and cell differentiation.

Reexpression of SNF5 in SNF5-deficient human MRT cell lines reduces phosphorylation of the retinoblastoma protein (pRb) through activation of p16 (INK4a) transcription and/or inhibition of cyclin D1 expression, leading to suppression of activating E2Fs (16–20). The Rb/E2F pathway regulates cell cycle progression and plays an important role in a wide array of human cancers (21–25). A recent in vivo study also suggests that Rb is epistatic to SNF5 in tumor suppression (26).

Previous studies suggest that SNF5 loss might also contribute to the development of choroid plexus carcinoma (CPC; refs. 27–30), a rare pediatric tumor of the active transport epithelium located in the brain ventricles. However, because MRTs often develop in similar locations and can be difficult to diagnose based on histology alone, there is some debate as to whether MRTs and CPCs are distinct tumors both affected by SNF5 inactivation, tumor types that share a common origin, or unrelated tumors with histologic similarities that cloud diagnosis (see Discussion). Therefore, we used a genetically engineered mouse (GEM) model, TgT121, in which CPCs develop with high frequency, to investigate whether loss of SNF5 contributes to CPC progression.

TgT121 mice express a truncated SV40 large T antigen (T121) that inactivates pRb and related proteins p107 and p130 (but not p53) under the control of the lymphotropic papovavirus (LPV) promoter (31). Transgene expression is robust in the choroid plexus epithelium (CPE), predisposing to CPC, and in B and T lymphoid cells without consequence (31, 32). Dominant interference of pRb, p107, and p130 by T121 is an effective strategy for complete inactivation of pRb function in the mouse due to compensation of pRb inactivation by p107 and/or p130 (33–35). TgT121 mice develop CPCs upon spontaneous CPE p53 inactivation and become terminal ~7 months of age (36, 37).
Here, to examine the combined effects of pRb f and SNF5 inactivation and to determine the relationship (if any) between CPC and MRT, we analyzed the development of these tumor types in TgT121;Snf5+/- mice. We report interesting tumor-specific differences in the cooperativity of these events. The results further provide insight into the MRT target cell type and the relationship between CPCs and MRTs.

Materials and Methods

**Generation of TgT121;Snf5+/- mice.** The generation, screening, and characterization of TgT121 transgenic (31, 36) and Snf5+/- (14) mice were described previously. TgT121;Snf5+/- mice were derived by crossing TgT121 mice with Snf5+/- mice. Mice with resulting genotypes, TgT121, Snf5+/-, TgT121;Snf5+/-, and TgT121;Snf5+/+, were born with the expected Mendelian frequencies. TgT121 mice were maintained on a BDF1 (The Jackson Laboratory, Bar Harbor, ME) background, and Snf5+/- mice were maintained on a C57BL/6/C2129/SV mixed background following procedures approved by the Institutional Animal Care and Use Committees.

**Genotyping.** Mice were genotyped by PCR amplification of genomic DNA from either mouse digits or postimplantation embryos (E9.5–E12.5). Mouse genomic DNA was extracted by incubating tissues with buffer A [0.2 mmol/L EDTA (pH 8.0), 25 mmol/L NaOH] at 95°C for 1 h. The lysate was neutralized by the same volume of buffer B (40 mmol/L Tris-HCl) followed by vigorous vortexing. Debris was pelleted and the supernatant was used in PCR amplification. PCR was done using the EasyStart 50 PCR kit (Molecular BioProducts, Inc., San Diego, CA). The wild-type Snf5 allele was detected using primers against a sequence before exon 1 (Snf5-01 5′-CAC-CATGCCCCCACCTCCCTACA-3′) and exon 1 (Snf5-02 5′-CAGGAAAATG-GATGCAACTAAGAT-3′), whereas the Snf5-null allele was amplified using primers against the neo insert (in exons 1 and 2; 5′-GGCCAGCTCA-TTCTCCTCCACTCAT-3′ and Snf5-01. T121-positive animals were detected by using the primers 5′-GAATCTTTGCAGCTAATGGACC-3′ and 5′-GCATCC-CAGAAGCTCCAAGG-3′. PCR conditions were as follows: 94°C for 1 min, 61°C or 59°C for 2 min, and 72°C for 1 min for 35 cycles. Agarose gel electrophoresis was used to detect the PCR products.

**Genomic DNA isolation and loss of heterozygosity analysis of Snf5.** Tumor genomic DNA was isolated from frozen tumor samples or from paraffin-embedded tissue slices by using the DNeasy tissue kit (Qiagen, Valencia, CA). For primary tumors, samples were crushed into a fine powder under liquid nitrogen before DNA isolation. Amplification of the Snf5 wild-type and null alleles by PCR was done using the EasyStart 50 PCR kit using the conditions described above.

**Western blot analysis.** Protein concentration was quantified by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Protein (35 μg) was separated by electrophoresis on 4% to 20% SDS-polyacrylamide gels (Cambrex, East Rutherford, NJ) and electrotransferred onto Immobilon-P membranes (Millipore, Billerica, MA) as per the manufacturers’ directions. Western blot analyses of proteins were carried out by using 1:500 anti-p16INK4a (G175-1239; BD PharMingen, San Diego, CA), 1:1,000 anti–poly(ADP-ribose) polymerase (Roche, Indianapolis, IN), 1:1,000 anti–phosphorylated Rb (Ser780; Cell Signaling, Danvers, MA), 1:1,000 anti–phosphorylated p53 (Ser15; Cell Signaling), and 1:2,000 horseradish peroxidase–conjugated anti-rabbit or anti-mouse IgG (Amersham, Piscataway, NJ). Individual proteins were detected with enhanced chemiluminescence reagent (Amersham) on Biomax ML film (Kodak, Rochester, NY).

Figure 1. TgT121;Snf5+/- mice develop CPCs and MRTs in brain, soft tissues, and spinal cord. A, survival curves for TgT121, Snf5+/-, and TgT121;Snf5+/- mice. B, H&E staining of two types of tumors at different locations in the TgT121;Snf5+/- mice. Tu, tumor.
**Immunohistochemistry.** Brain and facial tumors were fixed in 10% formalin for 16 to 20 h, whereas embryos were fixed for <1 h. Tissues were washed in running water for 3 min and saved in 70% ethanol. Bone surrounding the spinal cord was removed under a dissecting microscope 3 to 4 days after fixation in 10% formalin. Sections (5 μm) were cut after embedding tissues in paraffin. For histology, sections were stained with phenylindole buffer and stored at 4°C. Three washes in PBS. The sections were sealed in 4°C.

Probes, Carlsbad, CA) at room temperature in the dark for 1 h followed by incubation with Alexa Fluor 594 goat anti-guinea pig IgG (1:200; Molecular

Developmental Studies Hybridoma Bank, Iowa City, IA), or anti-tubulin-β-III (1:200; Chemicon, Temecula, CA) at 4°C overnight. After washing in PBS thrice, the sections were incubated with anti-mouse or anti-rabbit antibody (1:333; Vector, Burlingame, CA) at room temperature for 30 min followed by two washes in TS-T buffer. Stains were developed in 3,3′-diaminobenzidine (DAKO) at room temperature for 2 to 10 min, rinsed in PBS, and counterstained in Light Green Counterstain (Biomeda). Sections were rinsed in H2O and dehydrated before being mounted in Permount (Fisher Scientific, Pittsburgh, PA). Microscopy was done with a Zeiss Axioplan 2 microscope (Thornwood, NY).

**Immunofluorescence.** Tissues were fixed and processed as above. For immunofluorescence, sections were boiled in citrate buffer for 15 min, cooled to room temperature, and then washed twice with PBS. Sections were incubated with anti-cytokeratin (CK) 8/18 (1:450; Progen, Toowong, Australia) at 4°C overnight. After washing in PBS thrice, the sections were incubated with anti-mouse or anti-rabbit antibody (1:333; Vector, Burlingame, CA) at room temperature for 30 min followed by two washes in TS-T buffer. Stains were developed in 3,3′-diaminobenzidine (DAKO) at room temperature for 2 to 10 min, rinsed in PBS, and counterstained in Light Green Counterstain (Biomeda). Sections were rinsed in H2O and dehydrated before being mounted in Permount (Fisher Scientific, Pittsburgh, PA). Microscopy was done with a Zeiss Axioplan 2 microscope (Thornwood, NY).

**Apoptosis assay.** Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling staining was done following the manufacturer’s instructions. In brief, formalin-fixed, paraffin-embedded tissues were deparaffinized and pretreated with proteinase K and quenched in 3% hydrogen peroxide. Strength TdT Enzyme was applied to the section for 30 min. The reaction was then stopped by stop/wash buffer for 10 min. An aliquot of anti-digoxigenin conjugate was added to the sections, washed with PBS, and stained with peroxidase substrate. After three washes of H2O, the slices were counterstained with Light Green Counterstain and washed in 100% n-butanol. Slices were then mounted with Permount after dehydration in xylene. Immunohistochemical microscopy was done under a Zeiss Axioplan 2 microscope.

**Quantification of mitotic figures.** Formalin-fixed, paraffin-embedded tissues were stained with H&E. At least 10 different fields of view were chosen, evenly distributed within the tumors. Total cell numbers and mitotic figures were counted using a Zeiss Axioplan 2 microscope. The percentage of cells in mitosis was calculated and graphed by GraphPad Prism 4.0 (GraphPad Software, San Diego, CA).

**Histology.** The tumor histopathology was evaluated independently by C.F. and V.G.

**Results**

*TgT121;Snf5+/−* mice develop both MRTs and CPCs. TgT121;Snf5−/− and TgT121;Snf5−− cohorts were derived from matings between TgT121 and Snf5−/− mice (see Materials and Methods). TgT121;Snf5−/− and TgT121;Snf5−− offspring were generated at expected Mendelian ratios, indicating no deleterious effects during embryogenesis. TgT121;Snf5−/− and TgT121;Snf5−− mice seemed healthy until CPCs, MRTs, or both arose. Based on histologic assessment, TgT121;Snf5−/− mice developed exclusively CPCs, whereas TgT121;Snf5−− mice developed both CPCs and MRTs (Fig. 1B; Table 1). Thus, the tumor spectrum was unaffected by the compound genotype.

**No effect of Snf5 heterozygosity on CPC tumorigenesis.** All TgT121;Snf5−/− mice developed CPC with a long latency as previously shown for TgT121 (37). The penetrance and latency were not significantly affected by the Snf5−/− background, although earlier development of MRTs in many TgT121;Snf5−/− mice precluded determination of the CPC end point in a subset of animals (Fig. 1A; Table 1). Nonetheless, the stage of CPC development at termination from MRTs was comparable with that of TgT121 mice of the same age (data not shown); hence, CPC penetrance was 100%. The median age of TgT121;Snf5−/− and TgT121;Snf5−− mice terminal because of CPCs did not differ (9.0 months; range, 4–14 months versus 9.0 months; range, 2.5–13 months; Table 1). No statistical difference was noted based on Wilcoxon (P = 0.67) or log-rank (P > 0.88) analyses. The apparent lack of influence on CPC development by Snf5−− heterozygosity was consistent with the retention and expression of the wild-type Snf5 allele in the CPCs from the TgT121;Snf5−/− mice (Fig. 2).

In TgT121 mice, inactivation of pRbf function in CPC induces both proliferation and apoptosis, with most apoptosis dependent on p53 function (36). CPC is dysplastic in all mice until ~6 weeks of age. Subsequent to this time and at variable ages, focal tumors develop that fall into two classes. Type I tumors (~40%) with classic CPC histology spontaneously lose p53 and grow aggressively, whereas type II tumors (~60%) retain p53 function, are histologically distinct, and grow slowly (37). Both subtypes arose in TgT121;Snf5−/− mice with similar frequencies (data not shown). To determine whether Snf5−− haploinsufficiency affected CPC tumor cell proliferation in either class, mitotic indexes were calculated based on the percentage of mitotic figures. No significant difference in proliferation was detected between.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tumor/location</th>
<th>No. with tumors (total no.)</th>
<th>Median age (mo)</th>
<th>Penetrance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>TgT121;Snf5−/−</em></td>
<td>CPC</td>
<td>32 (n = 71)</td>
<td>9.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MRT</td>
<td>17 (n = 71)</td>
<td>5.2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Soft tissue</td>
<td>22 (n = 71)</td>
<td>4.7</td>
<td>31</td>
</tr>
<tr>
<td><em>TgT121;Snf5−/−</em></td>
<td>CPC</td>
<td>36 (n = 36)</td>
<td>9.0</td>
<td>100</td>
</tr>
<tr>
<td><em>Snf5−/−</em></td>
<td>MRT</td>
<td>2 (n = 45)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Soft tissue</td>
<td>9 (n = 45)</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table 1. Tumor incidence in TgT121;Snf5−/− and TgT121;Snf5−/− mice**

Snf5 haploinsufficiency seems to have no detectable effect on choroid plexus tumor initiation or progression when pRb function is compromised. The retention of Snf5 in terminal choroid plexus tumors also indicates no role for Snf5 inactivation in tumor progression. These results indicate that SNF5 tumor suppression either functions through pRb inactivation or has no role in CPE (see Discussion).

**pRb inactivation reduces latency and increases frequency of MRTs.** Consistent with previous reports (12–14), only 24% of the Snf5+/− mice (11 of 45) became terminal with MRTs within 8 to 10 months of age at a median age of 8.2 months (range, 4–13 months; Fig. 1A; Table 1). These MRTs presented as soft tissue tumors mostly on the face and limbs (20%) with occasional paravertebral sites close to the distal end of spinal cord (4%). Due to the large size of the tumor, we could not determine whether the tumor originated within the spinal cord or the surrounding tissue. In combination with the T121 transgene, MRTs developed with increased frequency (55%) and reduced latency (median, 4.9 months; range, 2–9 months; Fig. 1A). Both soft tissue and CNS tumor frequencies increased (31% and 24%, respectively; Table 1). Forty-four percent (17 of 39) of the TgT121;Snf5+/− MRTs were located around the dorsal roots or spinal nerves within the spinal cord, most frequently near the thoracolumbar junction (Fig. 1B), with a median age of 5.2 months (range, 2.5–8.7 months). Because choroid plexus tumors develop in these mice with complete penetrance and overlapping latencies, the frequency of MRTs detected represents a minimum value.

The dramatic increase in penetrance and decrease in latency of MRTs in TgT121;Snf5+/− compared with Snf5+/− mice indicates...
cooperativity of pRb and SNF5 in MRT tumor suppression. Indeed, $T_{121}:Snf5^{+/+}$, but not $Snf5^{+/+}$, MRTs expressed $T_{121}$ (Fig. 2B and C). Moreover, all MRTs examined had selectively lost the wild-type $Snf5$ allele (Fig. 2A) and failed to express SNF5 (Fig. 2B), further indicating that $Snf5$ and $pRb$ inactivation cooperate in MRT development. Significantly, $T_{121}:Snf5^{+/+}$ MRTs (both soft tissue and CNS) had a >4-fold increased mitotic index compared with their $Snf5^{+/+}$ counterparts (Fig. 3). Increased proliferation rates could account for the increased frequency and decreased latency of $T_{121}:Snf5^{+/+}$ MRTs. However, because the target cell population for MRTs is unknown and tumors arise in varied locations, we could not examine preneoplastic and early neoplastic proliferation rates.

Characterization of differentiation markers in MRT and CPC. Both CPC and MRT tumors have been diagnosed in SNF5 families (38), and SNF5 loss has been noted in sporadic tumors with CPC diagnosis (see Discussion). Given that only choroid plexus tumors arise in $T_{121}$ mice, it was possible that $Snf5$ inactivation in $T_{121}$-induced CPC cells facilitated conversion to MRT. Although such a hypothesis would not explain the increase in soft tissue MRTs, CPC tumors do metastasize to the spinal cord where most $T_{121}:Snf5^{+/+}$ CNS MRTs were observed. Thus, we characterized both CPCs and MRTs for expression of differentiation markers for evidence of a CPC to MRT conversion. Human MRTs are highly positive for both vimentin and CK intermediate filaments and less commonly positive for $S100\beta$ (1, 39–43). CPCs also stain positive for CK and vimentin (44, 45). A significant number of $T_{121}:Snf5^{+/+}$ MRTs stained positive for vimentin (8 of 8) and $S100\beta$ (9 of 10) by immunohistochemistry, whereas only some of the tumors were focally positive for CK8/18 (3 of 8) by immunofluorescence (Fig. 4). No difference in staining patterns was detected between soft tissue and spinal cord MRTs (Fig. 4). In contrast, all CPCs were negative for both vimentin and $S100\beta$ expression, whereas the endothelium (mesenchymal origin) was positive for vimentin and the surrounding brain matter was positive for $S100\beta$ (Fig. 4). Most CPCs were focally positive for CK8/18 (Fig. 4). Although CK8/18 was diffusely expressed in the cytoplasm of CPC cells, it was found in occasional agglomerates in MRT cells (Fig. 4). These findings confirm the similarity of MRTs in $Snf5^{+/+}$ mice and in human patients (46). Moreover, the distinct staining patterns of CPCs

Figure 3. Mitotic rates in mouse CPC and MRT. Mitotic figures were counted in representative tumors at each time as described in Materials and Methods. Bars, SE.

Figure 4. Differentiation marker expression in CPC and MRT. Representative CPCs and rhabdoid tumors from $T_{121}:Snf5^{+/+}$ mice were assessed for vimentin, $S100\beta$, and CK8/18 expression by immunohistochemistry as described in Materials and Methods.
and MRTs within the same mice, with no evidence of conversion between types within any independent tumor analyzed, support the interpretation that these tumor types have independent and distinct origins.

**T121 expression in non-CPE CNS cells identifies a potential MRT target population.** Previous analysis of \( TgT121 \) tissues indicated that \( T_{121} \) expression is robust only in CPE and lymphoid cells. However, this study did not include spinal cord or embryonic tissues and would miss a minor subpopulation of \( T_{121} \)-expressing cells. Because the target cell population for MRTs is unknown, it is possible that \( T_{121} \) expression in previously unidentified non-CPE cells is responsible for SNF5 and pRb cooperation. It is also possible that \( Snf5 \) inactivation, which is selected for in rhabdoid tumorigenesis, ectopically induces \( T_{121} \) expression from the LPV promoter. To determine whether SNF5 loss can result in nonspecific activation of the LPV promoter, \( TgT_{121};Snf5^{+/+} \) mouse embryo fibroblasts, which do not express the transgene, were infected with a retrovirus expressing

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**Figure 5.** \( T_{121} \) expression in E12.5 mice. E12.5 embryos from \( TgT_{121} \) mice were fixed and stained for the indicated markers as detailed in Materials and Methods. Three different embryos were examined to confirm the expression patterns.
Snf5-specific short hairpin RNA or a dominant-negative SNF5 mutant. Despite shown reduction in SNF5 protein expression, we saw no induction of T121 (data not shown). Furthermore, transfection of a LPVpro-reporter vector into SNF5-deficient human MRT cell lines failed to induce luciferase activity (data not shown).

Because T121 expression in TgT121 mice may identify the MRT target cell, we analyzed TgT121 mouse embryos at E12.5 and E15.5 by immunohistochemistry for T121 expression. By E12.5, several cell types throughout the embryo were positive, including the expected CPE precursors as well as lymphoid precursors in fetal liver (Fig. 5). Importantly, a cluster of T121-positive cells was located within the spinal cord near the thoracolumbar junction (the location of most TgT121;Snf5+/−/− CNS MRTs). These cells also expressed the neural precursor markers nestin and tubulin-β-III, but not islet-1, a neuroectoderm motor neuron progenitor marker (Fig. 5). These results raise the exciting possibility that MRTs in the TgT121;Snf5+ mice arise from T121+positive neural progenitor cells.

Discussion

MRTs are rare, poorly understood, aggressive, embryonic neoplasms. One common feature of almost all MRTs is an alteration of SNF5 resulting in protein loss. Loss of expression mutations of SNF5 has also been reported at a lower frequency in CPCs (29, 30, 47). Biegel et al. (48) screened 28 tumors with a submitted diagnosis of CPC by immunohistochemistry and found that seven tumors showed loss of SNF5 expression. However, reexamination of the histopathology of these tumors resulted in a revision of the diagnosis from CPC to MRT (48). Our study provides evidence to support this notion. Snf5 haploinsufficiency did not accelerate CPC growth nor did loss of heterozygosity (LOH) of Snf5 occur in these tumors. In contrast, MRTs in the TgT121;Snf5+/− mice selectively lost the wild-type Snf5 allele. Thus, our findings support the conclusion from the recent human study that loss of SNF5 appears consistently in MRTs but not in CPCs (48).

The Rb family plays an important role in regulating eukaryotic cell cycle progression, apoptosis, and terminal differentiation (49). In MRTs, loss of SNF5 seems closely related to oncogenesis through the inactivation of p16INK4a function (17, 18, 20, 50). The MRTs arising in the TgT121;Snf5+/− and Snf5+/− mice also show a similar pattern of protein expression. They were 100% positive for vimentin, a developmentally regulated intermediate filament protein found in cells of mesenchymal origin, and 90% positive for S100β, a marker widely distributed in peripheral and CNS tissues but not in epithelial cells. Thirty-eight percent of the MRTs stained positive for CK8/18, a nonsquamous epithelia marker. Interestingly, the protein appeared mainly in the classic rhabdoid cells within the cytoplasmic inclusion bodies that contain whirls of intermediate filaments. The histologic similarities between the human and mouse MRTs further validate the TgT121;Snf5+/− and Snf5+/− mice as appropriate models for the human disease.

Several cellular origins have been proposed for MRTs, including neuroectodermal, myogenic, histiocytic, neural, mesenchymal, and epithelial (56). Ota et al. (56) suggested that these tumors arise from primitive pluripotent cells, such as neural crest cells or the equivalent, expressing a neuroectodermal or ectomesenchymal phenotype. We found that a few neural stem cells localized at a site close to the thoracolumbar junction within the spinal cord at E12.5 expressed the T121 transgene, the most frequent location of the spinal MRTs in the adult mice. Considering the unexpected T121 expression in all MRTs, we hypothesize that the MRTs originate from a neural stem cell with an active LPV promoter. Therefore, the TgT121;Snf5+/− mice may provide the first opportunity to identify the precursor cell for MRT in vivo during early development. Detailed characterization of the TgT121+positive cells with additional differentiation markers will help define the correct origin of these precursor cells. Furthermore, whereas other cell types express the T121 transgene at E12.5 in the TgT121;Snf5+/− mice, MRTs only appear in the same sites as the Snf5+/− mice (Table 1; data not shown). Therefore, our results imply that loss of SNF5 function leads to tumor development in a tissue-specific context. Further characterization of these mouse models, such as targeted disruption of SNF5 in neural progenitor cells, will determine how SNF5 inactivation plays different roles in different cellular environments.

In summary, our results show that the loss of the Rb family increases MRT progression and occurrence within the CNS, especially the spinal cord. These findings indicate that SNF5 loss in these tumors does not equal the inactivation of all pRb function. Therefore, the TgT121;Snf5+/− mouse model provides an in vivo system to evaluate the interaction between inactivation of SNF5 and pRb functions to further understand the mechanism of the tumor suppressor function of SNF5. Our results also emphasize that SNF5 loss apparently drives tumor development in a tissue-dependent context. Finally, due to the high penetrance of MRTs in this GEM, the TgT121;Snf5+/− mice should prove useful for the development of new strategies for the treatment of this deadly pediatric cancer.

Acknowledgments

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Cooperation of pRB Family and Snf5 Inactivation
Tumor-Specific Cooperation of Retinoblastoma Protein Family and Snf5 Inactivation

Jingjing Chai, Xiangdong Lu, Virginia Godfrey, et al.


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