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Inactivation of SNF5 Cooperates with p53 Loss to Accelerate Tumor Formation in *Snf5*^{+/-}; *p53*^{+/-} Mice

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Abstract

Malignant rhabdoid tumors (MRTs) are poorly differentiated pediatric cancers that arise in various anatomical locations and have a very poor outcome. The large majority of these malignancies are caused by loss of function of the SNF5/INI1 component of the SWI/SNF chromatin remodeling complex. However, the mechanism of tumor development associated with SNF5 loss remains unclear. Multiple studies have demonstrated a role for SNF5 in the regulation of cyclin D1, p16^{INK4A} and pRb_f activities suggesting it functions through the SWI/SNF complex to affect transcription of genes involved in cell cycle control. Previous studies in genetically engineered mouse models (GEMM) have shown that loss of SNF5 on a p53 null background significantly accelerates tumor development. Here, we use established GEMM to further define the relationship between the SNF5 and p53 tumor suppressor pathways. Combined haploinsufficiency of *p53* and *Snf5* leads to decreased latency for MRTs arising in alternate anatomical locations but not for the standard facial MRTs. We also observed acceleration in the appearance of T-cell lymphomas in the *p53*^{+/-}; *Snf5*^{+/-} mice. Our studies suggest that loss of SNF5 activity does not bestow a selective advantage on the p53 spectrum of tumors in the *p53*^{+/-}; *Snf5*^{+/-} mice. However, reduced p53 expression specifically accelerated the growth of a subset of MRTs in these mice.

Keywords

SNF5; p53; malignant rhabdoid tumor; SWI/SNF

INTRODUCTION

Although representing only a small percentage of pediatric neoplasms, malignant rhabdoid tumors (MRTs) are particularly devastating, killing the majority of affected children before their second birthday. These aggressive tumors, first described in the kidney, were originally defined as “rhabdoid” due to the morphological resemblance of the tumor cells to rhabdomyoblasts [1]. However, further characterization of these tumor cells including the lack of expression of myogenic transcription factors established them as distinct from rhabdomyoblasts [1-3]. Rhabdoid tumors are now recognized to occur in diverse locations including the brain, spinal cord, liver, chest wall, and face [4-6]

MRTs have always presented a diagnostic challenge given the largely undifferentiated although heterogeneous appearance, polyphenotypic immunoprofiles, and similarities to other tumor types. The designation of MRT historically relied on the histological appearance of large epithelioid cells with eccentric eosinophilic cytoplasm, vesicular nuclei, prominent nucleoli, and whorled bundles of intermediate filaments. These characteristic rhabdoid cells, described above, are frequently not the predominant cell in a malignant rhabdoid tumor, even appearing rarely in some tumors. Therefore, false diagnoses were frequent. The discovery of deletions and mutations at 22q11.2 involving *SNF5/INI1* has since facilitated diagnosis and treatment evaluation [7-11]. SNF5 function is now recognized as being lost in almost 100% of MRTs [12]. The finding that genetic alterations in MRTs are usually limited to *SNF5* mutations and deletions, without chromosome- or genome-wide losses or instability, strongly implicates the loss of SNF5 function as the primary cause of these tumors [12-14].

Studies using genetically engineered mouse models (GEMM) have verified SNF5's specific role in tumor suppression. *Snf5* nullizygotes are embryonic lethal, dying at peri-implantation stage while loss of SNF5 activity at birth in conditional *Snf5* mice cause death within 3 weeks due to hematopoietic failure [15-18]. A reversible conditional mutant that causes only partial penetrance of SNF5 loss avoids the bone marrow failure and death experienced with the fully penetrant conditional mutation [16]. However, it shows a fully penetrant phenotype, developing CD8⁺ T cell lymphomas or MRTs by 11 weeks [16]. In comparison, loss of either tumor suppressor genes *p53* or *p16^{INK4A}* leads to tumor development at median ages of 20 weeks and 38 weeks, respectively [19,20]. Therefore, the aggressive phenotype observed in the reversible conditional mice makes *Snf5* the most rapidly lethal cancer mutation observed in GEMM following inactivation of a single gene.

GEMM offer the opportunity to examine the interactions between different oncogenic and tumor suppressive pathways in an in vivo system. For example, Guidi et al. demonstrated that *Snf5* loss may be epistatic to Rb loss [21]. However, a more recent study showed that loss of the entire Rb family accelerated MRT development [22]. These data seem to fit with the etiology of human disease where MRTs do not appear to lose Rb or *p16^{INK4A}* function. Gene expression array analyses have also shown that inactivation of SNF5 function in normal mouse embryo fibroblasts alters the expression of genes regulated by the E2F transcription factor family [23].

As mentioned above, *SNF5* mutations/deletions seem to be the only genetic alterations that appear in MRTs in humans. While most malignancies found in adults harbor inactivating mutations of the *p53* tumor suppressor gene, the mutation frequency in MRTs remains uncharacterized [24,25]. Therefore, several groups examined the effects of *p53* loss on the development of MRT in different *Snf5* GEMM. Isakoff et. al. reported that simultaneous inactivation of *p53* and SNF5 activity in GEM accelerated the development of CD8⁺/CD4⁻ mature T cell lymphomas in 100% of the mice [23]. They further showed an accumulation

of p53 in SNF5 deficient cells in culture displaying growth defects followed by apoptosis. In contrast, the SNF5 deficient cells on the p53-null background showed a reduced level of apoptosis. Yaniv and colleagues further demonstrated that *p53* nullizygosity accelerated tumor formation in *Snf5* heterozygous mice - with complete penetrance within 19 weeks (from 30% penetrant at 60 weeks on wild type p53 background). Neither the tumor spectrum, nor anatomical locations of the resultant MRTs and sarcomas were altered [26]. This implies a true cooperation, not simply additive effect, as all tumors were SNF5-deficient tumors in the same locations found in *Snf5*^{+/-} mice on a wild type *p53* background, but with a much reduced latency and 100% penetrance [26]. Both groups suggested that p53 loss blocked apoptosis associated with SNF5 inactivation in normal cells and accelerated the appearance of the SNF5-deficient tumors [23,26].

While these studies indicate that combined p53 and SNF5 inactivation accelerates the progression of MRT development in GEMM, both studies used p53 nullizygous mice. Therefore, they did not address the potential effects that SNF5 inactivation might have on tumor development in a p53 heterozygous state. To address this question, we characterized tumor development in *p53*^{+/-}, *Snf5*^{+/-} and *p53*^{+/-}; *Snf5*^{+/-} mice. Our results reveal that cooperation between these two important tumor suppressor pathways leads to a reduced latency period but not an apparent increase in tumor penetrance. However, with the possible exception of T cell lymphomas, we do not observe a decrease in the latency period for the spectrum of tumors associated with the *p53*^{+/-} mice. Rather, we find more rapidly arising MRTs in the *p53*^{+/-}; *Snf5*^{+/-} mice developing in anatomical sites infrequently observed in the *Snf5*^{+/-} mice alone.

MATERIAL AND METHODS

Generation of *p53*^{+/-}; *Snf5*^{+/-} mice

The generation, screening and characterization of *p53*^{+/-} [27] and *Snf5*^{+/-} [22] mice were described previously. *Snf5*^{+/-}; *p53*^{+/-} mice were derived by crossing *p53*^{+/-} mice with *Snf5*^{+/-} mice. Mice with resulting genotypes, *Snf5*^{+/+}; *p53*^{+/-}, *Snf5*^{+/-}; *p53*^{+/+}, *Snf5*^{+/-}; *p53*^{+/-} and *Snf5*^{+/-}; *p53*^{+/-} were born with the expected Mendelian frequencies. *p53*^{+/-} mice were maintained on a BDF1 C57BL/6 background and *Snf5*^{+/-} mice were maintained on a C57BL/6 × 129/SV mixed background following procedures approved by the Institutional Animal Care and Use Committees. To minimize potential genetic background effects, all experimental mice used in this study were derived from the same 2 female parental *Snf5*^{+/-} mice. Mice were monitored twice a week for 24 months or until a tumor or evidence of a tumor (paralysis, swelling, extreme lethargy, etc) was observed. Mice were sacrificed by CO₂ asphyxiation followed by cervical dislocation as approved by the Institutional Animal Care and Use Committees.

Histology

Tumor samples and selected tissues were fixed in 10% buffered formalin and sent to the UNC Histopathology Core Facility for paraffin embedding and staining with eosin and hematoxylin. Because of the importance of establishing the identity of each tumor, the histopathology was evaluated independently by 2 senior investigators with previous experience in the area of GEMM for soft tissue sarcomas- Dr. C. Fletcher at Harvard University and Dr. V. Godfrey at University of North Carolina [15,16,22]. Each pathologist evaluated H&E and immunohistochemistry slides in a blinded fashion to further reduce bias. There was initial agreement upon the diagnosis in approximately 90% of the tumors. Cases in disagreement centered SNF5-deficient tumors with scant evidence of typical rhabdoid cell morphology being classified as MRT or sarcoma-non otherwise specified or NOS. Therefore, by agreement, we classified all SNF5-deficient tumors as MRT.

Product	Forward primer	Reverse primer
wild type <i>Snf5</i> allele	CAGGAAAATGGATGCAACTAAGAT	CACCATGCCCCACCTCCCCTACA
mutant <i>Snf5</i> allele	GGCCAGCTCATTCCTCCCACTCAT	CACCATGCCCCACCTCCCCTACA
wild type <i>p53</i> allele	ACAGCGTGGTGGTACCTTAT	TATACTCAGAGCCGCCCT
mutant <i>p53</i> allele	TCCTCGTCGTTTACGGTATC	TATACTCAGAGCCGGGCCT

Genotyping Analyses

DNA was extracted from mouse toes, tumors or organs using the Qiagen DNAeasy Kit for animal tissues. All PCR reactions were carried out as previously described using EasyStart 0.5mL PCR tubes (MolecularBioProducts #6022) [22,27]. The following additions were made to the EasyStart tube: 1-3ul of DNA extracted from mouse toes, Taq DNA polymerase, specific primers for the mutant or wild type alleles, and ddH₂O.

Immunohistochemistry

For histology, sections were stained with hematoxylin and eosin as described [28]. Tumors were fixed in 10% formalin for 16-20 hrs and five μ sections were cut after embedding tissues in paraffin. For IHC analysis, a tissue microarray consisting of triplicate 5 μ m cores of 31 tumors at different locations in the recipient block from *p53*^{+/-}, *Snf5*^{+/-} and *Snf5*^{+/-}; *p53*^{+/-} mice was constructed by the Anatomic Pathology Translational Core Laboratory using a Pathology Devices (Westminster, MD) semi-automated TMArrayer. We also included normal mouse tissues including lung, kidney and lymph nodes as controls. IHC was carried out using the Vectastain *Elite* ABC Kit from Vector Laboratories following manufacturer's directions, as previously described [22]. All sections were quenched in 3% hydrogen peroxide in methanol for 10 min at room temperature, washed in PBS three times and then blocked in 5% goat serum. Sections were incubated with the primary antibody at 4°C overnight. After washing in PBS three times, the sections were incubated with an anti-rabbit antibody (1:333, Vector, Burlingame, CA) at room temperature for 30 min followed by washing in TS-T buffer (50 mM Tris pH 7.6, 150 mM NaCl, and 0.1% Tween-20) three times. Elite ABC reagent (Vector, Burlingame, CA) was then added to the sections at room temperature for 30 min followed by two washes in TS-T buffer. Stains were developed in DAB (diaminobenzidine tetrahydrochloride) (DAKO, Carpinteria, CA) at room temperature for 2-10 minutes, rinsed in PBS, and counter-stained in Light Green Counterstain or hematoxylin (Biomed, Foster City, CA). Sections were rinsed in H₂O and dehydrated before being mounted in Permacent (Fisher Scientific, Pittsburgh, PA). Microscopy was performed with a Zeiss Axioplan 2 Microscope. All antibodies used are included in the table below.

Antibodies

ANTIBODY	Brief Description	Source
SNF5	Mouse monoclonal	Transduction Labs, 612110
Vimentin	Rabbit polyclonal	Biomed V2009
S100B	Rabbit polyclonal	Dako Z0311
p53 (CM5)	Rabbit polyclonal	Novacastra Labs NCL-p53-CM5p
CD3	Rabbit polyclonal	Cell Marque CMC363

ANTIBODY	Brief Description	Source
B220	Mouse monoclonal	BD Pharmingen 550286

RESULTS

SNF5 heterozygosity leads to increased penetrance and decreased latency of tumor formation on $p53^{+/-}$ background

Previous studies have shown combined nullizygosity of SNF5 and p53 leads to increased tumor formation, but this occurs at a rate no longer compatible with life, with 100% lethality within 7 weeks [23]. We crossed $Snf5^{+/-}$ females with $p53^{+/-}$ males to yield the $Snf5^{+/-};p53^{+/-}$ double heterozygous mice, as well as control genotypes: $Snf5^{+/-};p53^{+/+}$, $Snf5^{+/+};p53^{+/-}$, and $Snf5^{+/+};p53^{+/+}$ (see Materials and Methods for background strain and origin of mice). The overall survival of these genotypes can be seen in Figure 1 and Table 1. In the $p53$ heterozygote mice, we observed the expected >50% penetrance and 14.5 month median age (range- 11 to 17.5 months) similar to that observed in previous $p53^{+/-}$ mouse studies [19,27]. Sixty percent of $Snf5^{+/-}$ mice developed tumors with a median age of 15 months (range- 9-20 months), somewhat higher than in the original reports [15,17,18]. The mice heterozygous for both $p53$ and $Snf5$, however, had a median age of tumor formation of only 9.5 months (range- 2 to 17.5 months), and a total penetrance of approximately 80%. Thus, the $Snf5^{+/-};p53^{+/-}$ mice show both an increase in tumor penetrance and an overall reduction in latency similar to previous reports using $Snf5^{+/-};p53^{+/-}$ mice [23,26].

$Snf5^{+/-};p53^{+/-}$ mice develop MRTs, Lymphomas, and Osteosarcomas

As shown by Donehower in 1992 and Harvey in 1993, approximately 50% of $p53^{+/-}$ mice develop tumors by 18 months of age [19,29]. The spectrum of tumors includes primarily lymphomas (~30%) and osteosarcomas (~60%) as well as soft-tissue sarcomas and carcinomas. We also observed a similar spectrum of tumors in our mice with the appearance of 5 lymphomas, 3 osteosarcomas, 1 sarcoma NOS and 1 adenocarcinoma (Table 1). The $Snf5^{+/-}$ mice form primarily malignant rhabdoid tumors of the soft tissues of the head and neck, as well as spinal cord tumors, a pattern that we have consistently found in our $Snf5^{+/-}$ mice. There were 5 facial MRTs and one spinal cord MRT resulting in hind limb paralysis (Table 1).

Approximately 80% of the $Snf5^{+/-};p53^{+/-}$ mice developed tumors by 17.5 months. Twenty-one out of the 32 mice (66%) had tumors that were classified as MRT. The remaining mice had tumors consistent with the $p53^{+/-}$ mouse spectrum including 5 lymphomas, 4 sarcomas NOS, 2 osteosarcomas and 1 fibrosarcoma. Therefore, it appeared the combined $Snf5^{+/-}$ and $p53^{+/-}$ genotypes did not alter the tumor spectrum of either parental genotype as no tumors completely novel to either genotype were observed. However, a significant change in the anatomical sites of MRTs occurred in the $Snf5^{+/-};p53^{+/-}$ mice. While the MRTs arose only in the face and spinal cords of the $Snf5^{+/-}$ mice, MRTs appeared in sites in the $Snf5^{+/-};p53^{+/-}$ mice infrequently or rarely found in the $Snf5^{+/-}$ mice including the limbs, back and intestines.

Loss of SNF5 Expression Occurs in Tumors with Decreased Latency in $Snf5^{+/-};p53^{+/-}$ Mice

Previous studies from several groups using $Snf5^{+/-}$ mouse models have shown virtually 100% LOH in MRTs that arise in these mice [15,17,18]. Due to T-cell infiltration and the high vascularity of these tumors, we could not obtain reliable results by PCR-based assays. Therefore, we generated a tissue array containing 5 tumors from $Snf5^{+/-}$ mice, 6 tumors from $p53^{+/-}$ mice and 18 tumors from $Snf5^{+/-};p53^{+/-}$ mice (Table 2). We also included

normal lung, kidney and lymph node tissue as controls. We then stained and scored each array for expression of SNF5 and p53 protein by IHC. We found that all normal tissues expressed SNF5 protein at high levels (supplemental data).

We first examined the tumors that arose in the $p53^{+/-}$ mice including 3 lymphomas and 3 osteosarcomas. All tumors were positive for SNF5 expression consistent with their wild-type genetic background (Table 2). In contrast, the MRTs that arose in the $Snf5^{+/-}$ mice showed no SNF5 protein expression indicating that inactivation of the remaining wild-type $Snf5$ allele had taken place (Table 2, Figure 2).

We next examined 18 representative tumors from the $Snf5^{+/-};p53^{+/-}$ mice including 10 MRTs, 3 sarcoma NOSs, 2 osteosarcomas and 3 lymphomas. As expected, all MRTs arising on the face of the mice (5/5) did not express SNF5 protein similar to the MRTs from the $Snf5^{+/-}$ mice (Table 2; Figure 2). Furthermore, the ages of onset for these facial tumors did not differ significantly between these groups (Table 2- gray shaded areas). The osteosarcomas retained SNF5 protein expression and also arose in a similar timeframe as the osteosarcomas in the $p53^{+/-}$ mice (Table 2). However, the remaining tumors fell into 2 classes. Lymphomas and sarcoma NOSs appearing on the limbs that arose in a similar timeframe as tumors in the $p53^{+/-}$ mice retained SNF5 expression (Table 2). In contrast, those tumors that had lost SNF5 expression showed decreased latency (<9 months, Table 2- stippled areas). Perhaps, tumor #93 proved most informative (Figure 2). Although this limb sarcoma NOS appeared after 13 months, it contained both an area of SNF5 positive staining and an apparently emerging area of SNF5-negative tumor growth. Interestingly, the H&E shows a more “rhabdoid” appearance in the SNF5-negative section of the tumor suggesting that loss of SNF5 was directing the tumor towards a rhabdoid identity.

We also stained the tissue arrays for p53 expression. Immunohistochemistry analyses for p53 protein expression do not provide clear evidence for loss of expression in tumors because normal tissues show variable levels of expression. Therefore, while we could not determine the status of p53 expression in most of our tumor samples, some of our results were suggestive. Both normal lung and lymph node showed low levels of p53-positive staining cells (supplemental data). Interestingly, 3/5 facial MRTs from both $Snf5^{+/-}$ and $Snf5^{+/-};p53^{+/-}$ mice showed the same low-level p53 staining. In contrast, all 6 tumors from the $p53^{+/-}$ mice and 13/13 of the remaining tumors from the $Snf5^{+/-};p53^{+/-}$ mice showed no p53 staining.

The Immunoprofile of Mouse Malignant Rhabdoid Tumors Recapitulates Their Human Counterparts

One question about the validity of GEMM for human cancers is how accurately they embody the features of their representative human malignancy. The haploinsufficient $Snf5$ mouse has been well characterized as producing MRTs with histologies similar to those observed in humans. We also examined a limited number of immunohistochemical markers to determine whether the MRTs in the $Snf5^{+/-}$ mice showed a similar pattern to their human counterparts. We also looked at the immunoprofile in a representative set of MRTs from the $Snf5^{+/-};p53^{+/-}$ mice to assess whether loss of p53 function affected the phenotype of these tumors. Approximately 60% of the MRTs, from either genotype, were positive for S100B expression, a somewhat lower frequency than our previous report [22] (Table 3). We also observed a low to high level of expression of vimentin, an intermediate filament found in cells of mesenchymal origin, in all MRTs (Table 3). These results appear consistent with those found in human MRTs [30-33]. Furthermore, the immunophenotype of MRTs that arose in the $Snf5^{+/-};p53^{+/-}$ mice did not appear to differ from those found in the $Snf5^{+/-}$ mice.

T-cell Lymphomas Arise in *Snf5^{+/-};p53^{+/-}* mice

We had also previously found that induction of SNF5 inactivation in *Mx⁺;Snf5^{cond/-}* mice led to the rapid induction of T-cell lymphomas, a process accelerated by the co-inactivation of p53 expression [16,23]. Previous reports indicated that lymphomas arising in *p53^{+/-}* mice also appeared to arise primarily from T cells [19,27]. Therefore, we determined the origin of the lymphomas that arose in our mice by IHC staining using the well-established B-cell marker, B220, and T-cell marker, CD3. As shown in Figure 3, all lymphomas stained positive for CD3 but showed little or no staining for B220. Therefore, consistent with the majority of previous results, the inactivation of p53 alone or in combination with SNF5 leads to the development of T-cell lymphomas.

DISCUSSION

Almost 30 years have passed since the formal recognition and diagnosis of MRT came to exist [1,34]. While the underlying genetic defect, *SNF5* loss, associated with MRT development has been identified, this knowledge has not changed the prognosis of individuals diagnosed with this devastating neoplasm. Given the difficulty in translating insights gained from the molecular mechanisms of human cancer initiation and progression to advances in clinical practice, the genetically engineered mouse model has proven invaluable for accelerating this process. However, it has been difficult to find a suitable GEMM for MRT because *Snf5* loss results in embryonic lethality and *Snf5* heterozygosity leads to MRT development with an extended latency. Complicating matters for conditional deletion studies, the cell of origin for MRTs remains unknown.

In the two previous studies examining the interactions between the *p53* and *Snf5* tumor suppressor genes in tumor development in GEMM, loss of SNF5 function took place on a *p53* null background [23,26]. In the current mouse model, cells could lose either one or both of these tumor suppressor pathways in order to progress to tumorigenicity. In the case of the facial MRTs that presumably correspond to those found in *Snf5^{+/-}* mice, we did not observe an increase in penetrance or an apparent decrease in latency for the appearance of these tumors compared to the *Snf5* heterozygous mice on a *p53* wild type background (Table 2). Our data suggest that this lack of difference was associated with retention of wild-type p53 activity in these tumors. In contrast, we found an increase in penetrance and decrease in latency for the spinal cord tumors in the *Snf5^{+/-};p53^{+/-}* mice reminiscent of the dramatic increase in the *TgT121; Snf5^{+/-}* mice (Table 1 & 2) [22]. Therefore, it seems that cooperation between SNF5 loss and reduced p53 expression or pRb_f inactivation accelerates the formation of these neuronally-derived tumors. Recent reports have also shown that *SNF5* mutations are associated with some types of familial schwannomatosis in humans [35-37].

We also found that loss of SNF5 protein expression correlated with accelerated tumor appearance for MRTs that occurred in locations other than the face (Table 2). This association also held true for the time of onset of lymphomas. In contrast, we did not observe any changes on the development of osteosarcomas in the *p53^{+/-};Snf5^{+/-}* mice. Several possibilities may explain the observed increase in tumor formation with the combined haploinsufficiency of *p53* and *Snf5*. First, Vries et al demonstrated in their 2005 study that 10% of MRT cell lines are near tetraploid and over half of the MRT cell lines bear chromosomal aberrations [38]. They went on to show these aberrations could be corrected in the cell population by addition of SNF5 that leads the genetically unstable cells to senesce. Therefore, SNF5 haploinsufficient mice may experience substantial genomic instability leading to secondary genetic events such as loss of p53 activity. This model could account for the acceleration of development of MRTs in *Snf5^{+/-};p53^{+/-}* mice. However, molecular and cytogenetic studies of human MRTs suggest that genomic instability occurs infrequently, with chromosomal aberrations localized mainly to region surrounding the

SNF5 gene [7,13]. Furthermore, a recent report by McKenna et al. demonstrated that genomic instability does not occur after SNF5 inactivation in genetically engineered mice [14]. Thus, this may not provide the most likely mechanism.

A second explanation for the increase in tumor formation in the double heterozygous mice is that p53 null cells allow genetic instability, increasing the frequency of *Snf5* LOH. The role of p53 loss in generating genetic instability in GEMM remains unresolved [39]. Some studies have shown an increase in genetic instability after loss of p53 function while others, including some of our own reports, suggest that its loss does not lead directly to global chromosomal instability [39,40]. Furthermore, loss of p53 has not been found in primary MRTs of children presenting with this disease (J. Biegel, personal communication). In fact these tumors often initially respond well to chemotherapy and radiation and are sometimes ablated. Unfortunately, MRTs frequently return months to years later in a chemotherapy-resistant form in which the p53 status has not been established. Therefore, we do not favor this paradigm to account for our findings.

We prefer a third explanation for observations in human MRT development as well as the increase in MRTs in the double heterozygous mice. *Snf5* LOH is likely occurring in the *Snf5*^{+/-}; *p53*^{+/-} mice at an equivalent rate and locations as it does in the *p53*^{+/+} mice. However, on a *p53* wild type background, these *Snf5* null cells are arrested or undergo apoptosis via induction of p21 and subsequent cell cycle arrest via the RB pathway. On the *p53*^{+/-} background, most cells possess enough functional *p53* to undergo these protective processes if they lose SNF5 function. However, a few cells are able to escape them due to low p53 levels causing a slight increase in tumor formation. Since tumors developing following *Snf5* loss have a shorter latency than those that arise following *p53* loss (as determined in previous mouse studies), an increase in *Snf5*^{+/-} mouse spectrum tumors (spinal cord and possibly limb MRTs) as well as those associated with p53 heterozygosity (lymphomas) occurs.

Whether secondary genetic events drive the progression of malignant rhabdoid tumors remains unresolved. Given the extensive loss of p53 in most human tumors, this study may lead to a deeper understanding of its potential inactivation in MRT patients. The dramatic increase in spinal cord MRTs in the *Snf5*^{+/-}; *p53*^{+/-} mice may implicate p53 loss in the progression or reoccurrence of atypical teratoid/rhabdoid tumors in the CNS or AT/RTs, in human patients. While primary MRTs rarely show evidence of p53 inactivation, tumors recurring after radiation and chemotherapeutic treatment have not been analyzed. Indeed, several reports have demonstrated a role for *p53* in survival and in the control of death checkpoints in central nervous system neurons [41,42]. Further insight into the relationship of classical tumor suppressor gene pathways like p53 in the etiology of MRT and its specific interactions with SNF5 will inevitably allow for more successful treatment avenues for this devastating childhood neoplasm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Kaplan-Meier Survival Curve of *Snf5*^{+/-}, *p53*^{+/-} and *Snf5*^{+/-}; *p53*^{+/-} Mice

Mice were sacrificed when tumor, paralysis or physical distress was observed. Healthy mice at 25 months were sacrificed and recorded as non-events. N values are noted on the figure. Wild type mice experienced 100% survival at 25 months and are not included here.

Figure 2. Representative IHC Analysis of SNF5 and p53 Expression in Primary Malignant Rhabdoid Tumors

The tumor array was stained for p53 or SNF5 expression using the appropriate antibody and counterstained with hematoxylin as described in the Materials and Methods. Representative cores were visualized using a Nikon FXA microscope and captured using Q-Image software on an Apple G4 computer. All images are 100X.

Figure 3. Representative IHC Analysis of CD3 and B220 Expression in Primary Lymphomas
 The tumor array was stained for CD3 or B220 expression using the appropriate antibody and counterstained with Light Green or hematoxylin as described in the Materials and Methods. Representative cores were visualized using a Nikon FXA microscope and captured using Q-Image software on an Apple G4 computer. All images are 100X.

Table 1
Tumor Incidence in $p53^{+/-}$, $Snf5^{+/-}$ and $Snf5^{+/-};p53^{+/-}$ mice

Genotype	No. with Tumors (Total No.)	Median Age (Months)	Penetrance	Tumor Histology (# of tumors)
$Snf5^{+/-}$	6 (n=10)	15.0	60%	MRT (5 f, 1 s)
$p53^{+/-}$	10 (n=15)	14.5	63%	Soft Tissue Sarcoma (4) Lymphoma (5) Carcinoma (1)
$Snf5^{+/-};p53^{+/-}$	32(n=43)	9.5	74%	MRT [*] (5f, 13s, 4other) Soft Tissue Sarcoma (5) Osteosarcomas [@] (2) Lymphoma [@] (5)

* - one mouse had 2 MRTs, one located in the spinal cord and one located on a limb.

@ - one mouse had a lymphoma and a osteosarcoma. f-facial; s-spinal cord; other sites included limb, back, shoulder and brain.

Table 2
Loss of Heterozygosity (LOH) Analysis of Mouse Tumors

Genotype	Mouse #	Age at Tumor Onset	Histologic Diagnosis	p53 IHC Score	SNF5 IHC Score
<i>p53^{+/+}</i>	130	11.5	Lymphoma	-	+
<i>p53^{+/+}</i>	113	12.5	Lymphoma	-	+
<i>p53^{+/+}</i>	103	14	Lymphoma	-	+
<i>p53^{+/+}</i>	133	11.5	Osteosarcoma	-	+
<i>p53^{+/+}</i>	B19	15.25	Osteosarcoma	-	+
<i>p53^{+/+}</i>	1401	17.5	Osteosarcoma	-	+
<i>Snf5^{+/+}</i>	B47	9	Rhabdoid (face)	-	-
<i>Snf5^{+/+}</i>	B73	10	Rhabdoid (face)	+/-	-
<i>Snf5^{+/+}</i>	B29	16	Rhabdoid (face)	+/-	-
<i>Snf5^{+/+}</i>	B56	17	Rhabdoid (face)	-	-
<i>Snf5^{+/+}</i>	B27	20	Rhabdoid (face)	+/-	-
<i>p53^{+/+};Snf5^{+/+}</i>	72	4	Rhabdoid (limb)	-	+/-
<i>p53^{+/+};Snf5^{+/+}</i>	141	5.5	Rhabdoid (spine)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	1407	6.2	Lymphoma	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	1507	6.5	Rhabdoid (spine)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	151	7.5	Rhabdoid (limb)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	86	8.3	Rhabdoid (brain)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	161	9	Rhabdoid (face)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	B34	12.5	Rhabdoid (face)	+/-	-
<i>p53^{+/+};Snf5^{+/+}</i>	85	13	Rhabdoid (face)	-	-
<i>p53^{+/+};Snf5^{+/+}</i>	81	14	Rhabdoid (face)	+/-	-
<i>p53^{+/+};Snf5^{+/+}</i>	1400	17	Rhabdoid (face)	+/-	-
<i>p53^{+/+};Snf5^{+/+}</i>	111	12.5	Osteosarcoma	-	+
<i>p53^{+/+};Snf5^{+/+}</i>	93	13	Sarcoma NOS (limb)	-	+/-
<i>p53^{+/+};Snf5^{+/+}</i>	106	13	Lymphoma	-	+
<i>p53^{+/+};Snf5^{+/+}</i>	146	16	Lymphoma	-	+
<i>p53^{+/+};Snf5^{+/+}</i>	146	16	Osteosarcoma	-	+
<i>p53^{+/+};Snf5^{+/+}</i>	143	16	Sarcoma NOS (limb)	-	+
<i>p53^{+/+};Snf5^{+/+}</i>	B42	17	Sarcoma NOS (limb)	-	+

Tissue arrays were stained for p53 and SNF5 expression as described in the Material and Methods. Each core was then scored for average signal strength of positive tumor cells and for percentage of tumor cells demonstrating some positivity by 2 independent pathologists. These two values were multiplied for each score and averaged for each tumor. Total scores were then assessed for significance by plotting average signal strength versus average % positive. Bases on this analysis, we considered less than <70 as negative, 70-160 as +/- and >160 as positive.

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Table 3
Immunophenotype of Tumors from *Snf5^{+/-}*, *p53^{+/-}* and *Snf5^{+/-};p53^{+/-}* Mice

Malignant Rhabdoid Tumors	Vimentin			S100β		
	<10	10-70	>70	<10	10-70	>70
<i>Snf5^{+/-}</i> mice	0	2	3	2	0	3
<i>Snf5^{+/-};p53^{+/-}</i> mice	0	2	16	1	7	10

Each core was scored for average signal strength of positive tumor cells and for percentage of tumor cells demonstrating some positivity by 2 independent pathologists. These two values were multiplied for each score and averaged for the total number of scores. The values are an average of the scores for at least 3 cores.