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Disruption of In11 Leads to Peri-Implantation Lethality and Tumorigenesis in Mice

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SNF5/INI1 is a component of the ATP-dependent chromatin remodeling enzyme family SWI/SNF. Germ line mutations of INI1 have been identified in children with brain and renal rhabdoid tumors, indicating that INI1 is a tumor suppressor. Here we report that disruption of In11 expression in mice results in early embryonic lethality. In11-null embryos die between 3.5 and 5.5 days postcoitum, and In11-null blastocysts fail to hatch.

The role of SWI/SNF enzymes in whole organisms is unclear. While homozygous disruption of Brm1 in mouse embryonic carcinoma cells resulted in lethality, disruption of Brm expression in mice produced only mild proliferative effects (35, 46). The upregulation of Brml in the Brm-deficient mice may provide a compensatory effect; however, one cannot rule out the possibility that these differences are due to distinct functions of Brm- or Brml-containing complexes.

SNF5/INI1 is a member of both Brg1- and Brm-containing SWI/SNF complexes (29, 51). INI1 was shown to interact with ALL-1, translocations of which are associated with several types of human acute leukemias (37). Furthermore, INI1 has been found to be altered in malignant rhabdoid tumors, chordoid plexus carcinomas, medulloblastomas, and central primitive neuroectodermal tumors (2, 9, 39, 40, 49). Identification of constitutional mutations in a subset of these tumors indicates that INI1 is a tumor suppressor (2, 40). In an attempt to generate a mouse model that would allow further characterization of the mechanisms of In11 in tumorigenesis and to determine the role of the mammalian SWI/SNF complexes in development, we generated mice deficient for In11 expression.
We show that *Ini1*-deficient mice die early in embryogenesis, likely due to an inability of the blastocysts to hatch, implant in the uterus, and continue development. In addition, we report that a subset of the *Ini1*-heterozygous mice present with a variety of tumors in the soft tissues of the head and neck and that loss of heterozygosity at the *Ini1* locus is correlated with tumor formation.

**MATERIALS AND METHODS**

*Ini1* targeting. Embryonic stem (ES) cells (Omnibank no. OST32815) bearing a retroviral promoter trap that functionally inactivates one allele of *Ini1* were generated as described previously (55). Analysis by rapid amplification of cDNA ends also is described. The site of insertion was determined using sequence analysis.

**Creation of *Ini1*-null mice.** *Ini1*-targeted ES cells were injected into 3.5 days postcoitum (d.p.c.) C57BL/6 blastocysts. Male chimeric mice were mated with wild-type C57BL/6 or 129 females. Germ line transmission of the mutant allele was determined by PCR analysis of tail genomic DNA using the following primers: for wild-type C57BL/6 or 129 females 3.5 d.p.c. with M15 media (Dulbecco’s minimum essential medium, 15% fetal calf serum, 100 μM β-mercaptoethanol, 2 mM glutamine, and 1 × penicillin-streptomycin) and cultured in tissue culture plates for 96 h. Embryo cultures were genotyped as described above.

**Blastocyst culture.** Blastocysts from heterozygous intercrosses were flushed to generate *Ini1*-null mice. Embryos 6.5 d.p.c. and younger were genotyped by nested PCR. The size and position of wild-type and targeted bands are indicated.

**Materials and Methods**

*Ini1*-targeted cells stained positive for *Ini1* protein was performed as described previously (10).

**RESULTS AND DISCUSSION**

Mouse ES cells bearing a retroviral promoter trap that functionally inactivates one allele of *Ini1* were constructed as described previously (55). Sequence analysis revealed that the promoter trap was inserted within intron 3 of *Ini1* (Fig. 1A). The beta-galactosidase-neomycin (β-geo) gene fusion cassette within the retroviral insertion has a 5′ splice acceptor site; thus, β-geo expression is regulated by the native *Ini1* promoter. We were able to utilize the β-geo gene cassette in a colorimetric assay to determine if *Ini1* is normally expressed in ES cells. *Ini1*-targeted cells stained positive for β-galactosidase activity, indicating that *Ini1* is expressed in ES cells (Fig. 2A). Northern analysis of ES cell total RNA confirmed *Ini1* expression (data not shown).
not shown). Furthermore, sequence data obtained from 5’ rapid amplification of cDNA ends analysis of the Ini1-β-galactosidase fusion mRNA revealed that transcripts utilizing either splice donor site in exon 2 spliced into the trap, indicating that both splice variants of Ini1 were inactivated (5).

To determine the role of Ini1 in mammalian development and tumorigenesis, we used the targeted ES cells in blastocyst injection experiments to generate Ini1-heterozygous (Ini1<sup>in3</sup>/1) mice. In order to monitor expression of Ini1 during embryogenesis, we performed whole mount staining for β-galactosidase activity in embryos harvested from Ini1<sup>in3</sup>/1 matings at various times during development. We found that Ini1<sup>in3</sup>/1 embryos stained positive in all tissues at all time points examined, including 6.5, 8.5, 9.5, and 10.5 d.p.c., indicating that Ini1 is ubiquitously expressed during embryogenesis (Fig. 2B). Ini1 expression was also detected by Northern analysis in a wide range of adult tissues (35) (data not shown).

Chimeric mice generated from C57BL/6 strain blastocyst injections of the 129 strain-derived ES cells were bred to wild-type C57BL/6 or 129 mice in order to obtain Ini1<sup>in3</sup>/1 mice on either a mixed (C57BL/6 × 129) or pure (129) background. Intercrosses of Ini1<sup>in3</sup>/1 mice in both backgrounds yielded Ini1<sup>in3</sup>/1 offspring and wild-type offspring at a 2:1 ratio (63:26 in the mixed background, 34:17 in the pure background) and no Ini1-null offspring, indicating that disruption of Ini1 induces embryonic lethality (Fig. 1B). Timed matings of Ini1<sup>in3</sup>/1 mice were performed, and embryos were harvested at various time points in gestation for genotyping via PCR. Ini1-null embryos could be isolated at 3.5 d.p.c. and were normal in appearance (Fig. 1C and 3). However, no Ini1<sup>in3</sup>/in3 embryos were detected at 6.5 d.p.c. or later (Table 1). Dissection of maternal deciduae at 6.0 to 6.5 d.p.c. revealed no significant increase in the number of embryo reabsorptions, suggesting that Ini1<sup>in3</sup>/in3 lethality occurred between days 3.5 and 5.5 of gestation. These results

![FIG. 2. Ini1 is expressed in ES cells and ubiquitously throughout development. (A) β-galactosidase staining of targeted ES cells showing expression of Ini1. Wild-type (WT) AB2.2 ES cells were used as a control. (B) Whole mount staining of Ini1<sup>in3</sup>/1 embryos showing ubiquitous expression of Ini1 at indicated time points. Wild-type embryos at 6.5 and 10.5 d.p.c. are shown as controls.](image)

![FIG. 3. Ini1-null mice are early embryonic lethal and fail to hatch in vitro. Blastocysts were harvested from C57BL/6 Ini1<sup>in3</sup>/1 females and plated in culture for 96 h, at which time outgrowths were processed for PCR. Blastocysts are shown before and after culturing. TE, trophectoderm; ICM, inner cell mass.](image)

<table>
<thead>
<tr>
<th>Embryo category</th>
<th>WT</th>
<th>Het</th>
<th>Null</th>
</tr>
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<tr>
<td>3.5 d.p.c.</td>
<td>7</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>In vitro culture</td>
<td>11</td>
<td>14</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.5 d.p.c.</td>
<td>9</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>8.5 d.p.c.</td>
<td>4</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>13.5 d.p.c.</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> WT, wild type; Het, heterozygous.
<sup>b</sup> The embryo was dead.

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Age (wk)</th>
<th>Tumor site</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ini 139</td>
<td>36</td>
<td>Face</td>
<td>Undifferentiated sarcoma</td>
</tr>
<tr>
<td>Ini 44</td>
<td>32</td>
<td>Face</td>
<td>Undifferentiated sarcoma</td>
</tr>
<tr>
<td>Ini 138</td>
<td>23</td>
<td>Face</td>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Ini 100</td>
<td>23</td>
<td>Face</td>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Ini 180</td>
<td>31</td>
<td>Face</td>
<td>Undifferentiated sarcoma</td>
</tr>
<tr>
<td>Ini 26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>Face</td>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Ini 262</td>
<td>16</td>
<td>Face</td>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Ini 29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41</td>
<td>Face</td>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Ini 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59</td>
<td>Face/eye</td>
<td>Liposarcoma</td>
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<td>Ini 56</td>
<td>52</td>
<td>Neck mass</td>
<td>Lymphoma</td>
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<tr>
<td>Ini 322</td>
<td>21</td>
<td>Brain</td>
<td>Undifferentiated sarcoma</td>
</tr>
<tr>
<td>Ini 10</td>
<td>32</td>
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<td>Undifferentiated sarcoma</td>
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<tr>
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<td>22</td>
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<tr>
<td>Ini 127</td>
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<td>Neck mass</td>
<td>Lymphoproliferative disorder</td>
</tr>
<tr>
<td>Ini 328</td>
<td>25</td>
<td>Eye</td>
<td>Undifferentiated sarcoma</td>
</tr>
</tbody>
</table>

<sup>a</sup> 129 strain.
FIG. 4. Ini1-heterozygous mice present with various tumors. Microscopic features of different tumors from Ini1<sup>lnh<sup>1</sup></sup> mice. Parafin-embedded tumors were sectioned, stained with hematoxylin and eosin, and examined under a microscope at magnification ×75. Mouse numbers corresponding to those presented in Table 2 are indicated in each panel.
indicate that Ini1-null embryos either failed to be implanted into the uterine wall or were implanted and were reabsorbed shortly thereafter. In order to examine further the developmental defect of Ini1<sup>+/−</sup> embryos, we analyzed the ability of blastocysts from Ini1<sup>+/−</sup> intercrosses to expand in vitro. When 3.5-d.p.c. blastocysts were plated in culture, wild-type and Ini1<sup>+/−</sup> blastocysts hatched from the zona pellucida and were implanted onto the tissue culture plastic. Both wild-type and Ini1<sup>+/−</sup>-implanted embryos formed the trophectoderm and expanded their inner cell mass (ICM). In contrast, no Ini1<sup>+/+</sup> blastocysts hatched and were implanted in culture (Fig. 3). The results of these experiments suggest that the peri-implantation embryonic lethality of Ini1-null mice may be due to a defect in the hatching of the blastocyst from the zona pellucida, an obligate step for implantation of the embryo into the wall of the uterus during normal development. Manual disruption of the zona pellucida of 19 (C57BL/6 × 129) blastocysts harvested from Ini1<sup>+/−</sup> intercrosses did not result in expansion of the Ini1-null trophectoderm or ICM during in vitro culture, suggesting that growth of these tissues also is compromised (data not shown). Expression of Ini1 in ES cells, which are derived from the ICM of 3.5 d.p.c. blastocysts, is consistent with a gene crucial to the peri-implantation or preimplantation stage of embryogenesis.

In humans, loss of Ini1 is correlated with a variety of tumors, the vast majority of which are neuronal or renal in nature. To date, most human malignant rhabdoid tumors and choroid plexus carcinomas examined have deletions and/or mutations in INI1, as do a subset of central primitive neuroectodermal tumors and medulloblastomas (39). In mice, we found that approximately 15% of Ini1 heterozygotes in both the mixed F1 (C57BL/6 × 129) or pure 129 backgrounds presented with tumors. All of these tumors arose in the head or neck regions of the mice, particularly in the soft tissue of the face (Table 2). While 2 of the 15 mouse tumors analyzed thus far had varying degrees of rhabdoid-like cells, none had the characteristic, monomorphous appearance of human rhabdoid tumors. Two Ini1<sup>+/−</sup> mice were found to have a lymphoproliferative disorder or lymphoma originating in an ill-defined region on the neck (Fig. 4). Two-thirds of the tumors originated on the faces of the mice. Interestingly, expression of Ini1 appears to be elevated during development in the branchial arch and in the frontonasal and maxillary processes (Fig. 2B), structures which contribute to formation of the face. While the majority of the facial tumors were poorly differentiated or undifferentiated sarcomas and not neuronal in origin, it is possible that the tumors arose in cells derived from neural crest progenitors, since neural crest cells, along with mesodermal cells, coordinate to form the facial primordia (12, 38).

We have analyzed tumors in three representative mice. Northern analysis of total RNA harvested from tumor tissue indicated the presence of wild-type-length Ini1 message (data not shown). However, Western blot analysis of proteins harvested from these tumors revealed the absence of Ini1 protein in all three samples (Fig. 5). This indicates that loss of heterozygosity at the Ini1 locus is responsible for tumor formation in the Ini1<sup>+/−</sup>- mice.

The mechanism of Ini1-mediated tumor suppression is unclear. Other subunits of the human and mouse SWI/SNF chromatin remodeling complexes have been reported to associate with known tumor suppressors, including Rb and Brca1 (3, 11, 48, 56), and several of the SWI/SNF subunits appear to be molecular targets of viral regulators of cell proliferation (18, 27, 28, 53). In addition, one of these subunits, BRG1, recently has been reported to be missing or mutated in a variety of human tumor cell lines, and reintroduction of BRG1 into these tumor cells reverses their transformed morphology (52). These findings suggest a role for chromatin remodeling in regulation of cell growth and/or in tumor suppression.

While this report was in preparation, Roberts et al. and Klochendler-Yeivin et al. published data consistent with our findings (21, 36). The fact that these results are reproducible in knockout lines generated by different targeting strategies confirms the importance of Ini1 in development and tumorigenesis. Klochendler-Yeivin et al. (21) further report in their study that Ini1-deficient embryos can induce the formation of maternal decidua, suggesting that Ini1-deficient embryos undergo hatching and implantation prior to their demise. In contrast, Ini1<sup>+/−</sup> embryos fail to hatch from the zona pellucida, suggesting that subtle strain variations may influence the precise timing of embryonic lethality. In agreement with these other groups, a percentage of the Ini1-heterozygous mice in our colony presented with tumors that contained variable numbers of rhabdoid cells. However, we are hesitant to classify these undifferentiated sarcomas as true rhabdoid tumors, which are described as monomorphous tumors in the human population. Discrepancies between tumor types associated with disruption of Ini1 in humans and in mice may be due to differences in species-specific differentiation pathways. Regardless, the Ini1-heterozygous mice should provide a useful model for studying the general mechanisms involved in tumor suppression by Ini1.

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