Inactivation of the Retinoblastoma Tumor Suppressor Induces Apoptosis Protease-activating Factor-1 Dependent and Independent Apoptotic Pathways during Embryogenesis

Zhong Guo, Shi Yikang, Hiroki Yoshida, Tak W. Mak, and Eldad Zacksenhaus

Division of Cell and Molecular Biology, Toronto General Research Institute–University Health Network, Departments of Medicine, Laboratory Medicine, and Pathobiology and Medical Biophysics, University of Toronto, Toronto, Ontario, Canada MS2M21 (Z. G., S. Y., E. Z.), and The Amgen Institute, Ontario Cancer Institute, and the Department of Medical Biophysics and Immunology, University of Toronto, Toronto, Ontario, Canada MS2C1 (H. Y., T. W. M.) Canada

Abstract

Inactivation of the retinoblastoma (Rb) tumor suppressor in the mouse induces mid-gestational death accompanied by massive apoptosis in certain tissues. Herein, we analyzed the role of the apoptosis protease-activating factor Apaf-1, an essential component of the apoptosome, in mediating apoptosis in Rb-deficient mice. Analysis of compound mutant embryos lacking Rb and Apaf-1 revealed that Apaf-1 was absolutely required for apoptosis in the central nervous system and lens. In contrast, apoptosis in the peripheral nervous system and skeletal muscles only partly depended on Apaf-1 function. The dependency on Apaf-1 coincided with the requirement documented previously for E2F1 and p53 in the respective tissues. Loss of Apaf-1 specifically suppressed apoptosis but not the proliferation and differentiation defects in Rb-mutant embryos. We also show that the Apaf-1+ but not the Rb+ allele is retained in pituitary tumors arising in Rb+/−:Apaf-1+/+ double heterozygous mice. Our results indicate that Apaf-1 plays a critical role in apoptosis in a subset of tissues and that both E2F1:p53:Apaf-1-dependent and -independent apoptotic pathways operate downstream of Rb.

Introduction

The Rb tumor suppressor exerts its effects on cell growth by modulating the activity of transcription factors such as E2F1 (1). Disruption of Rb leads to both cell proliferation and apoptosis and is an obligatory step in the progression of most, perhaps all of the human cancers (2). In animal models, germ-line mutations in Rb predispose mice to intermediate lobe pituitary tumors (3). Homozygous mutations induce mid-gestational death accompanied by ectopic proliferation, apoptosis, and incomplete differentiation in the PNS, CNS, skeletal muscles, and lens where Rb is highly expressed during embryogenesis (3, 4). Genetic analysis revealed that loss of p53 accelerates tumorigenicity in Rb+/−:p53 double mutant mice (5, 6). Indeed, Rb and p53 are often inactivated in human cancer, and both are targeted by distinct viral oncoproteins, such as SV40 large T antigen, as a prerequisite for neoplastic transformation. Apoptosis in Rb mutant embryos is mediated by E2F1 and p53 in the CNS and lens but by E2F1- and p53-independent mechanisms in peripheral neurons and skeletal muscles (7–11). Detailed analysis of these p53-dependent and -independent apoptotic pathways downstream of Rb is important for understanding cancer progression and may lead to the identification of novel therapeutic targets to treat p53-negative tumors.

Two major apoptotic pathways have been recognized in cells: cell surface death receptor- and mitochondrial-mediated apoptosis (12–14). Activation of cell surface death receptors leads to processing of procaspase 8 and subsequent activation of procaspase 3, a downstream effector caspase. In contrast, apoptotic signals to the mitochondria induce the release of proapoptotic factors such as cytochrome c. In the presence of ATP, cytochrome c oligomerizes for Apaf-1/Ced-4 with procaspase 9, an apical/signalizing caspase, and the formation of the apoptosome (12–14). The apoptosome induces cleavage of procaspase 9; in a coupled reaction procaspase 3 is activated and apoptosis ensues. Gene disruption experiments of individual components of the apoptosome have illustrated their essential role in mitochondrial but not death receptor-dependent apoptosis (14). Specifically, Apaf-1−/− mice die preferentially at E16.5–E18.5 with various defects associated with the inability to execute normal apoptotic programs (15–17).

To delineate the apoptotic pathways downstream of Rb, particularly in tissues where E2F1 and p53 are dispensable (i.e., PNS and skeletal muscles), we addressed the role of Apaf-1 in compound mutant mice lacking both Rb and Apaf-1. Here we show that Apaf-1 is required for apoptosis only in a subset of tissues in Rb mutant embryos. Moreover, the dependency on Apaf-1 coincides well with the requirement for E2F1 and p53, hence, defining E2F1/p53/Apaf-1-dependent and -independent apoptotic pathways downstream of Rb.

Materials and Methods

Intercrossing and Genotyping. The mgRb:Rb+/− and Apaf-1+− mice were described previously (15, 18). Animals were maintained in compliance with the Canadian Council on Animal Care. E13.5 Rb−/−:Apaf-1−/− and E16.5 mgRb:Rb−/+:Apaf-1−/− composite mutant embryos and control litters were recovered by intercrossing Rb−/−:Apaf-1−/− and mgRb:Rb+/−:Apaf-1−/− double heterozygous mice. The morning of vaginal plug observation was considered as E0.5. Each embryo was genotyped in duplicates by PCR using two independent tissue biopsies. The PCR primers for mgRb, Rb+−, and Rb−− alleles were described previously (18). Apaf-1 was genotyped using the following primers:

Mutant (neo) allele

EZ149 5′-GGGCCAGCTCATTCTCTCC and EZ150 5′-CACCTATGGTCCAGGCTATC, and

EZ152 5′-AACACGGAGCCGCTCTTT.

PCR conditions included 30 cycles of 1 min each at 94°C, 58°C, and 72°C. RT-PCR Analysis. RNA was extracted from the indicated tissues using Trizol reagent (Life Technologies, Inc.). RNA (1 μg) was reverse transcribed into single-stranded cDNA and used as template for PCR amplification of Apaf-1. The following primers that flank intronic DNA sequences in Apaf-1 were used: EZ247 5′-CTCTGCACACTTTAGCTGAGGCT and EZ152 5′-ATACTTGGAGAGGTTGAGTAC and EZ248 5′-ATACTTGGAGAGGTTGAGTAC.

Received 5/31/01; accepted 10/12/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by a Canadian Institute of Health Research Grant MOP-44036 (to E. Z.). E. Z. is a scholar of the Cancer Research Society/Canadian Institute of Health Research.

2 To whom requests for reprints should addressed, at Division of Cell & Molecular Biology, Toronto General Research Institute–University Health Network, Toronto General Research Institute–University Health Network, 67 College Street, Room 407, Toronto, Ontario, Canada MS2M21. Phone: (416) 340-3453; E-mail: eldad.zacksenhaus@utoronto.ca.

3 The abbreviations used are: Rb, retinoblastoma; Apaf-1, apoptosis protease-activating factor; E, embryonic days; CNS, central nervous system; DRG, dorsal root ganglia; RT-PCR, reverse transcription-PCR; PNS, peripheral nervous system; mgRb, Rb mini-gene; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated nick end labeling.
DIFFERENTIAL ROLES OF Apaf-1 DOWNSTREAM OF Rb

Analysis of Pituitary Tumors. Rb+/−:Apaf1+/− mice (10–12 months old) that appeared wasted were sacrificed, and pituitary tumors were identified and resected. The tumors were dissected into 3–4 pieces and used to extract DNA for PCR analysis or RNA for RT-PCR.

Histology, in situ Hybridization, and TUNEL. Embryos were fixed overnight in 4% paraformaldehyde, embedded in paraffin, sectioned at 8 μm, and stained with H&E. In situ hybridization with 35S-labeled filensin riboprobe was performed as described elsewhere (11). Apoptosis was detected in situ using modification of the TUNEL method exactly as described (9). For Hoehct staining, sections were deparaffinated, hydrated, and immersed in Hoechst 33258 (Sigma Chemical Co.; 0.5 μg/ml in water) for 30 min, washed, and analyzed by confocal microscopy.

Results

Apaf-1 Is Expressed during Neuronal and Skeletal Muscle Development. Loss of Rb induces p53-dependent and -independent apoptosis during neuronal and skeletal muscle development, respectively (8, 9). Previous analysis has established that Apaf-1 is highly expressed during early neurogenesis (E9.5; Ref. 15). Using RT-PCR analysis, we detected Apaf-1 transcripts in the nervous system and skeletal muscles in older embryos (E13.5 and E16.5, respectively; Fig. 1A). Thus, Apaf-1 is expressed at E13.5–16.5 in tissues that undergo both p53-dependent and -independent apoptosis in Rb-mutant mice.

Apaf-1 Expression Is Retained during the Progression of Pituitary Tumors in Rb+/−:Apaf1+/− Mice. To address the role of Apaf-1 downstream of Rb, we crossed the Apaf-1 and Rb mutant mice. Double heterozygote Rb+/−:Apaf1+/− mice appeared indistinguishable from their Rb+/− or Apaf1+/− littermates and succumbed to pituitary tumors at ~1 year of age, like Rb+−/− mice (3). Loss of Apaf-1 has been shown to accelerate tumorigenicity induced by myc (19) and be associated with malignant melanoma (20). Therefore, we asked whether the progression of pituitary tumors in Rb+/−:Apaf1+/− mice involved loss of heterozygosity at the Apaf-1 locus. PCR analysis of four pituitary tumors derived from Rb+/−:Apaf1+/− mice revealed that the Rb+ allele was invariably lost (Fig. 1B). In contrast, the Apaf1+ allele was retained in all four of the cases (Fig. 1B and data not shown). Notably, Apaf-1 transcripts were still detected by RT-PCR in these pituitary tumors (Fig. 1A). These results suggest that inactivation of Apaf-1 may not provide a growth advantage during the progression of pituitary adenocarcinomas in Rb-mutant mice.

Apaf-1 Dependent and Independent Apoptosis in the Nervous System. Homozygote Apaf1+/− mice die preferentially at E16.5-E18.5 (15–17), whereas Rb−/− null embryos die at ~E13.5–14.5 (3, 21, 22). Therefore, we attempted to recover Rb−/−:Apaf1+/− embryos at E13.5 by mating Rb+/−:Apaf1+/− mutant mice. Double mutant embryos were obtained at a frequency of ~5% (8 of 163; Table 1), less than half the expected value (12.5%; ~20 of 163). This low percentage could imply that the combined loss of Rb and Apaf-1 had an adverse effect on embryonic development. Alternatively, our rigorous PCR analysis on duplicate biopsies might have inadvertently eliminated some double mutants. Indeed, the recovery of partially rescued E15.5 and E16.5 mgRb:Rb−/−:Apaf1+/− compound mutant fetuses (see below), which were readily identified by their abnormal shape in addition to the PCR analysis, was relatively higher (6/125 = 4.8%; expected 6.25%; Table 1).

To determine the role of Apaf-1 in neuronal cell death downstream of Rb, the E13.5 Rb−/−:Apaf1+/− mutants and control littermates were subject to TUNEL analysis, which detects in situ nicked and fragmented DNA, the hallmark of apoptosis. Rb−/− mutant embryos exhibited massive apoptosis in the CNS, particularly around the ventricles where neurons attempt to permanently exit the cell cycle and migrate to the marginal zone (Fig. 2A). In contrast, compound mutant embryos lacking both Rb and Apaf-1 showed very little neuronal cell death (Fig. 2A), indicating a required role for Apaf-1 in the CNS downstream of Rb.

Intriguingly, as opposed to the CNS, many TUNEL-positive nuclei were detected in the PNS in the same Rb−/−:Apaf1+/− mutant embryos (and the same sections). Apoptosis was evident in DRG (Fig. 2B), trigeminal, and other ganglia (data not shown). To quantify apoptosis, TUNEL-positive nuclei were counted in four sets of mutant and control embryos and plotted against the level of apoptosis in Rb−/− mutant embryos. As shown in Fig. 3B, apoptosis in the CNS of the double mutant embryos was reduced to background levels. In contrast, the level of apoptosis in Rb−/−:Apaf1−/− DRGs was reduced on average to only ~52% relative to Rb−/− embryos (Fig. 3D). We conclude that in the CNS, Apaf-1 as well as p53 (7–11) are both required to execute apoptotic programs in response to Rb loss; in the PNS, Apaf-1 is partly required, whereas p53 is not.

Apaf-1 Is Essential for Apoptosis in the Lens. Next, we sought to examine the role of Apaf-1 in apoptosis induced by loss of Rb in lens and skeletal muscles, which undergo E2F1/p53-dependent and -independent apoptosis, respectively, in Rb-deficient mice (7–11). We showed previously that mgRb is exclusively expressed in the nervous system (23) and specifically rescues the neurogenic defect of Rb−/− embryos. Partially rescued mgRb:Rb−/− fetuses survive to birth and

<table>
<thead>
<tr>
<th>Stage</th>
<th>Heterozygote</th>
<th>Rb mutant</th>
<th>Apaf1 mutant</th>
<th>Double mutant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13.5</td>
<td>Rb+/−:Apaf1+/−</td>
<td>Rb−/−</td>
<td>Apaf1−/−</td>
<td>Rb−/−:Apaf1+/−</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>105 (64)</td>
<td>24 (15)</td>
<td>26 (16)</td>
<td>8 (5)</td>
<td></td>
</tr>
<tr>
<td>E15.5</td>
<td>mgRb:Rb+/−:Apaf1+/−</td>
<td>mgRb:Rb−/−</td>
<td>mgRb:Apaf1−/−</td>
<td>mgRb:Rb−/−:Apaf1+/−</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>15 (70)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>E16.5</td>
<td>mgRb:Rb+/−:Apaf1+/−</td>
<td>mgRb:Rb−/−</td>
<td>mgRb:Apaf1−/−</td>
<td>mgRb:Rb−/−:Apaf1+/−</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>67 (65)</td>
<td>18 (17)</td>
<td>15 (14)</td>
<td>4 (4)</td>
<td></td>
</tr>
</tbody>
</table>

* One E15.5 compound mutant embryo had a cone-shaped head, the other embryo appeared normal.

* One E16.5 compound fetus had a forehead mass; one had a midline facial cleft palate; other two mutants had no obvious anomalies.
display severe defects in lens (11) and skeletal myogenesis (9, 18), both of which are accompanied by massive apoptosis and failure to undergo permanent cell cycle exit and terminal differentiation. TUNEL analysis of E16.5 mgRb:Rb+/+H11002:H11002:Apafl+/+H11002:H11002 mutant fetuses revealed a complete suppression of apoptosis in the lens, indicating an essential role for Apaf-1 downstream of Rb during lens development (Figs. 2C, top, and 3C). A similar inhibition of apoptosis was observed in the lens of E13.5 Rb−/−:Apafl−/− embryos (data not shown) or when cell death was monitored by Hoechst staining to reveal nuclear condensation (Fig. 2C, bottom). The level of apoptosis

A

B

C

D

E

Fig. 2. TUNEL analysis of Rb−/−:Apafl−/− mutant embryos and partially rescued mgRb:Rb−/−:Apafl−/− mutant fetuses. A–B, sections through the lateral ventricles (CNS) and DRG (PNS) of E13.5 Rb−/−:Apafl−/− and control littermates were analyzed by the TUNEL method to detect nicked and fragmented DNA in situ. Apoptotic nuclei are stained brown (arrowheads). C–D, sections through the lens and back muscles from E16.5 mgRb:Rb−/−:Apafl−/− mutant fetuses were analyzed by TUNEL and Hoechst staining (blue nuclei). The level of apoptosis in skeletal muscles was heterogeneous; the sections shown represent areas with high levels of apoptosis. Note the presence of large nuclei (+), indicative of endoreduplication, in both mgRb:Rb−/− and mgRb:Rb−/−:Apafl−/− mutant muscles. Original magnification: TUNEL, ×400; Hoechst staining, ×200.

Fig. 3. Differential effects of Apaf-1 on apoptosis in Rb-deficient mice. A–D, relative apoptosis was determined by counting TUNEL-positive apoptotic nuclei and normalizing for the respective areas using two to three sections for each embryo. The levels of apoptosis in the different embryos in four groups of mutant and control littermates were plotted relative to the Rb mutant in the group, which was assigned the value of 100%, and the results shown represent the average from the four groups; bars, ± SD. E, relative apoptosis was determined by counting TUNEL-positive nuclei in multiple, random areas of skeletal muscles under ×400 magnification from four groups of mutant and control littermates.
in the lens and CNS in the Rb+/−:Apaf1−/− double mutant embryos (Figs. 2 and 3) was lower than that observed in Rb−/−:E2F1−/− or Rb+/−:p53−/− composite mutants (8, 10, 11). Thus, in these tissues both E2F1/p53-dependent and -independent apoptotic pathways appear to converge on the mitochondria and require Apaf-1.

The near-complete suppression of apoptosis in the lens of mgRb:Rb+/−:Apaf1−/− fetuses prompted us to test whether this was accompanied by reversal of the differentiation defects associated with Rb loss. Histology analysis revealed that as in mgRb:Rb+/− embryos, the mgRb:Rb+/−:Apaf1−/− lens fibers failed to polarize and elongate properly (Fig. 2C and not shown). In addition, expression of filensin, a lens-specific filament protein, though slightly elevated relative to mgRb:Rb+/− embryos, was greatly reduced in the mgRb:Rb+/−:Apaf1−/− lens compared with wild-type control (Fig. 4A). Thus, the differentiation defect associated with loss of Rb cannot be completely corrected by suppressing Apaf-1-mediated apoptosis.

Apaf-1 is Only Partly Required for Apoptosis during Skeletal Myogenesis. In marked contrariety to the lens, the level of apoptosis in some areas of skeletal muscles appeared similarly high in mgRb:Rb+/− and mgRb:Rb+/−:Apaf1−/− fetuses, though overall there were slightly less areas with apoptotic nuclei in the double mutants (Fig. 2D). Exact quantification of cell death was complicated by the fact that whereas apoptosis in the PNS, CNS, and lens was localized, the distribution of apoptotic nuclei in skeletal muscles was dispersed and heterogeneous. Therefore, we used two methods to measure apoptosis in skeletal muscles. Measurements were performed on 2–3 TUNEL-stained sections from four sets of double-mutant fetuses and control littersmates. In one approach, TUNEL-positive nuclei were counted in highly apoptotic regions in each section and normalized for the respective areas (Fig. 3D). This analysis indicated that the level of apoptosis in mgRb:Rb+/−:Apaf1−/− muscles was only slightly reduced (to 80%) relative to Rb-mutant muscles with intact Apaf-1. In a second approach, TUNEL-positive nuclei were scored in multiple, random areas of skeletal muscles under ×400 magnification (Fig. 4E). This analysis showed that the loss of Apaf-1 reduced apoptosis in Rb-mutant skeletal muscles to ~45% relative to mgRb:Rb+/− mutants. Similar results were obtained with the two E15.5 mgRb:Rb+/−:Apaf1−/− mutant fetuses (Table 1 and data not shown). Although the different methods of assessing cell death produced somewhat different results, both revealed that Apaf-1 is only partly required for apoptosis downstream of Rb during skeletal myogenesis.

We have reported previously that the reduced muscle mass in mgRb:Rb+/−:Apaf1−/− mutant fetuses resulted in lack of cervical curvature and severe hunchback (Ref. 18; Fig. 4B). In accord with the partial inhibition of apoptosis in the mgRb:Rb+/−:Apaf1−/− muscles, more than half of the compound mutant fetuses (4 of 7) exhibited improved posture (Fig. 4B). However, the level of cell death was similar irrespective of the posture of the double-mutant fetuses. Whereas loss of Apaf-1 partially suppressed apoptosis in Rb-mutant skeletal muscle, it did not affect endoreduplication within myotubes, which was observed in both mgRb:Rb+/− (9, 18) and mgRb:Rb+/−:Apaf1−/− mutant fetuses (indicated by stars in Fig. 2D and data not shown).

Discussion

We provide evidence that Apaf-1 is required for apoptosis downstream of Rb in a subset of tissues where it is highly expressed, particularly in the developing CNS. However, other tissues such as the PNS and skeletal muscles undergo apoptosis in response to Rb loss via a pathway(s), which is at least in part independent of Apaf-1. The analysis presented herein and elsewhere (7–11) indicates that inactivation of Rb elicits two independent apoptotic pathways (Fig. 4C). In the lens and CNS, apoptosis is mediated by a relatively well-understood E2F1/p53/Apaf1-dependent mechanism. Intermediate steps in this pathway may include the transcriptional activation of p14ARF by E2F1, the sequestration of MDM2 by p14ARF, and the stabilization of p53 (5). In turn, p53 transcriptionally activates a number of pro-apoptotic factors such as Bax and Pid that may act in concert to induce the release of cytochrome c from the mitochondria, the assembly of the apoptosome with Apaf-1, and activation of effector caspases (12–14). In addition to p14ARF, E2F1 transcriptionally activates Apaf-1 and several other apoptotic factors (24).

Our results indicate that in skeletal muscles and PNS, the loss of Rb induces an as yet to be defined E2F1/p53/Apaf1-independent apoptotic pathway (Fig. 4C). We note that Apaf-1-independent
necrotic cell death, which involves “mottled” chromat, mitochondria and membrane lesions without nuclear condensation, and DNA degradation, and, hence, is TUNEL-negative, has been demonstrated in the interdigital space of Apaf-1 null embryos (25). Similarly, in response to cytotoxic drugs, Apaf1−/− embryonic stem cells undergo delayed cell death in vitro, which is also thought to involve necrosis rather than apoptosis (26). In contrast, both DNA degradation (TUNEL-positive) and chromosomal condensation (Hoechst staining) accompany the cell death observed in the PNS and muscles in RbxApaf-1 double mutant embryos (Figs. 2–4) indicating bona fide apoptosis.

Pituitary tumors in Rb+/− mice are preceded by ectopic cell proliferation and apoptosis (27), and the inhibition of apoptosis likely contributes to tumor progression. Herein, we show that Apaf-1 is retained in pituitary tumors developed in Rb+/−/Apaf-1+/− double-mutant mice (Fig. 1). The tumor suppressor p53 is also retained in pituitary tumors arising in Rb−/−:p53+/− mutant mice, and the incidence of pituitary tumors is not increased in Rb−/−:p53−/− mice (6). Together, these results suggest that apoptosis in the Rb−/− pituitary gland is mediated by a pathway yet to be defined, which is independent of p53 and Apaf-1.

A critical question at this juncture is whether procaspase 9 is cleaved in the PNS and skeletal muscles of Rb−/−:Apaf-1−/− mutant mice. Using two commercially available antibodies, we have found that caspase 9 is expressed in skeletal muscle and that the level of the processed caspase 9 is either the same or only marginally elevated in Rb-mutant embryos compared with wild-type tissues (data not shown). Nonspecific cleavage was also detected in lysates prepared from Apaf1−/− embryos but not from cultured cells. Caspase 9 may be processed during the preparation of the protein lysate, for example by self-cleavage induced by protein aggregation. Alternatively, a cross-reacting band migrating like caspase 9 may be expressed in the mouse tissues. Thus, we are presently unable to determine whether processing of caspase 9 occurs in mgRb:Rb−/−:Apaf-1−/− skeletal muscles. This issue will be resolved by using improved caspase 9 antibodies and by analyzing primary Apaf1−/− myoblasts in culture or mgRb:Rb−/−:casp9−/− compound mutant fetuses lacking both Rb and caspase 9 (28).

The apoptosis observed in the PNS and muscles of Rb−/−: Apaf-1−/− double mutants might be orchestrated by the mitochondria via Apaf-1-like factors. Several factors with structural homology to Apaf-1, such as Nod1/CARD4, Nod2, and Nac/Defcap, have been reported (29). However, Nod proteins are involved in modulating nuclear factor κB activity rather than promoting caspase 9 activation and are dispensable for the level of the processed caspase 9 is either the same or only marginally increased in Rb-mutant embryos compared with wild-type tissues (data not shown). Nonspecific cleavage was also detected in lysates prepared from Apaf1−/− embryos but not from cultured cells. Caspase 9 may be processed during the preparation of the protein lysate, for example by self-cleavage induced by protein aggregation. Alternatively, a cross-reacting band migrating like caspase 9 may be expressed in the mouse tissues. Thus, we are presently unable to determine whether processing of caspase 9 occurs in mgRb:Rb−/−:casp9−/− compound mutant fetuses lacking both Rb and caspase 9 (28).

We thank Zhe Ji for help with the analysis of pituitary tumors and TUNEL, Andrew Ho for confocal microscopy, and Yule Liu for in situ hybridization.

Acknowledgments

References


Inactivation of the Retinoblastoma Tumor Suppressor Induces Apoptosis Protease-activating Factor-1 Dependent and Independent Apoptotic Pathways during Embryogenesis


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/61/23/8395

Cited articles This article cites 33 articles, 10 of which you can access for free at: http://cancerres.aacrjournals.org/content/61/23/8395.full#ref-list-1

Citing articles This article has been cited by 12 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/61/23/8395.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.